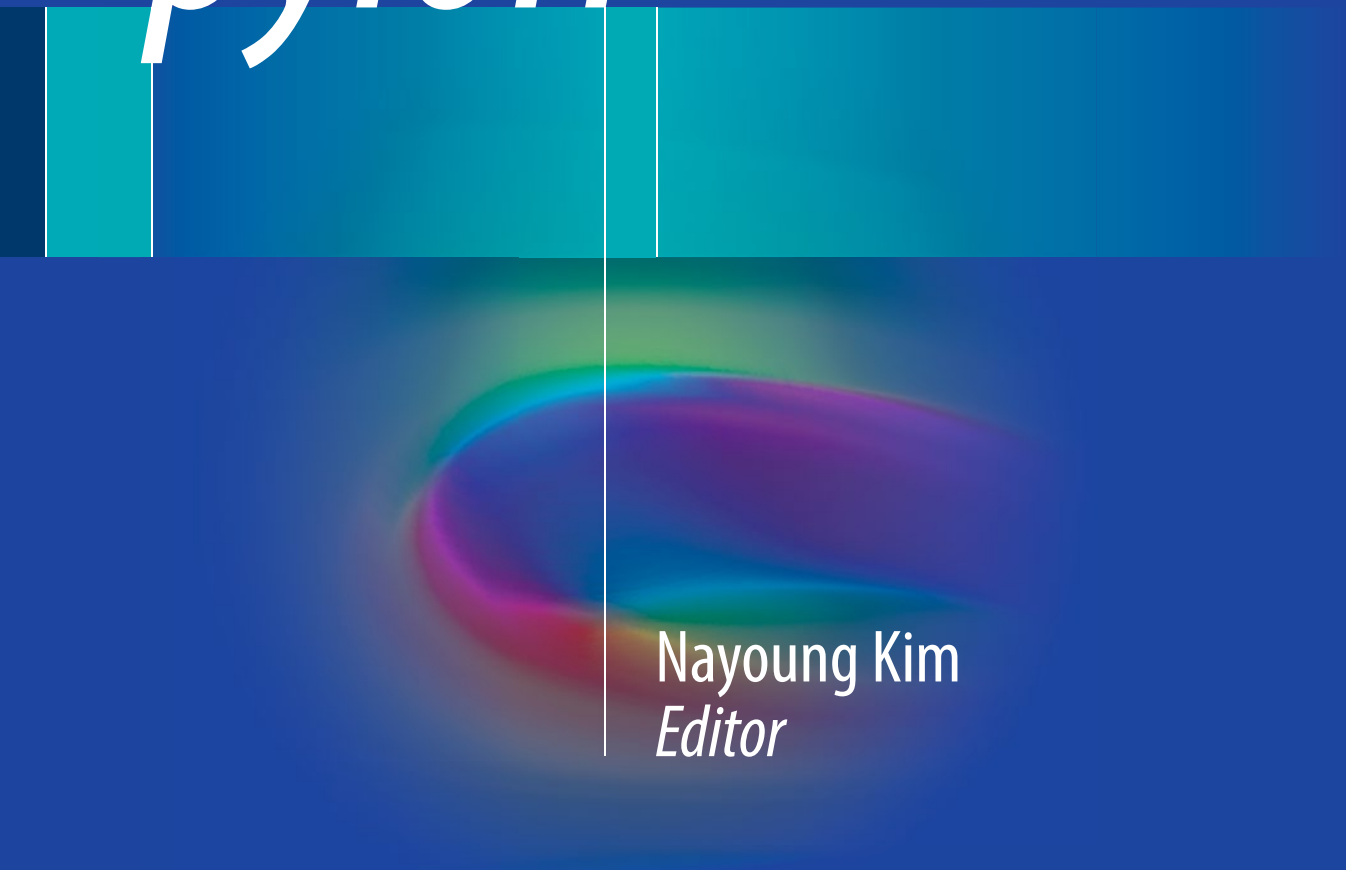
Helicobacter pylori



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Preface

After the discovery of *Helicobacter pylori* (*H. pylori*) by Prof. Barry Marshall and Robin Warren in 1982, the paradigm of gastroduodenal diseases fundamentally changed. Long-term colonization of *H. pylori* over the gastric mucosa caused a big change of microenvironment in the aspect of acid secretion by way of chronic inflammation, intriguing the balance of gastric hormones. In addition, active interaction of *H. pylori* and mucosal immunology causes intragastric diseases and gives impact on the extraintestinal diseases such as asthma and allergic disorders. Actually it is well known that disrupted balance between the host and intestinal microbiota produces changes in the mucosal immune system from microscopic to overt inflammation, and this also results in gut sensory-motor function and immune activity. Before this information of microbiota was reported, *H. pylori*, a gram-negative spiral bacterium, was found to be the main causal factor of gastritis, peptic ulcer, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer. Accordingly, *H. pylori* has been classified as a group 1 gastric cancer carcinogen by the International Agency of Research on Cancer in 1994. Thus, these diseases could be categorized into a kind of infectious disease, and consequently eradication of *H. pylori* could be the key to the prevention of these diseases. However, the antibiotic resistance became the most important barrier for this strategy, and rationale for the *H. pylori* eradication became a hot issue. For instance, Prof. Martin Blaser mentioned that "Perhaps one day physicians will be restoring *H. pylori* to young children and eradicating it when they reach the age of 30 or 40 years, to maximize early life benefit and to minimize late-in-life risk". In contrast, Prof. David Y. Graham strongly insisted that the only good *H. pylori* is dead bug. As the infection rate of *H. pylori* has an inverse relationship with the socioeconomic state, research regarding *H. pylori* eradication is actively under way in developing countries such as Asia and Latin America. However, the world has already become globalized, and developed countries also show the disease pattern of developing countries when they enroll immigrants from the developing countries. This suggests that this issue is still very important even in developed countries. As new knowledge such as the Kyoto Consensus Guideline is continuously released, an updated comprehensive book regarding *H. pylori* is very necessary. This book covers all of the important issues of *H. pylori* from the basic area such as how it survives in the acid gastric mucosa, immunology, and pathophysiology to the clinical aspect such as epidemiology, diagnosis, symptom, disease, antibiotic resistance, treatment, consequences of *H. pylori*

eradication, the effect of *H. pylori* infection on the gastric microbiota, and animal model. I hope this book will be appropriate to deal with this complicated issue for medical students; trainees such as interns, residents, and fellows; and even boards who have interests in this area in the world.

I would like to thank the 26 Korean doctors for their contribution to the *Helicobacter* book. Actually, most of them had performed research and published the paper with me, and initially we published a Korean book entitled *Helicobacter pylori* in April 2015 by Daehan Medical Book Publishing. With warm encouragement by Korean doctors who read this book, we requested Lauren Kim, Springer Korea, to publish most of the upgraded knowledge in English by Springer. In this step I invited Prof. David Y. Graham, Francis Mégraud, Ernst J. Kuipers, and Elizabeth A. Marcus, who are the leading experts in the world, and happily they submitted precious manuscripts. I am very grateful to them. Ms. Mijin Han and Ms. Eunju Shin from Daehan Medical Book Publishing, skillfully redraw the figures from the original papers. Finally Prof. Su Youn Nam, Ms. Eun Ju Ko, and Ms. Jihyun Park edited the contents and references of all of the papers. Without their help this book could not be completed. I would like to express my sincere gratitude to them. I am pleased to publish this book from Springer.

Seongnam, Gyeonggi-do, South Korea Nayoung Kim

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Part I

Epidemiology

Prevalence and Transmission Routes of *H. pylori*

1

Nayoung Kim

Abstract

To establish health policies for the prevention of *Helicobacter pylori* (*H. pylori*)-related diseases, observation of prevalence trends and confirmation of risk factors for *H. pylori* infection are important. Prevalence of *H. pylori* has been falling due to improved sanitation and better living conditions over the decades. However, its prevalence has been reported to be still higher than 50 % in 2013 in Africa, Central/South America, Asia, and South/East Europe and at least twofold higher in countries with high gastric cancer incidence. In contrast, in Northern Europe and North America, about one-third of adults are infected. However, even in these countries, *H. pylori* remains highly prevalent in the immigrants coming from countries with high prevalence of *H. pylori*. The lower prevalence of infection in the younger generations even in the presence of high prevalence in adults suggests a further decline of *H. pylori* prevalence in the coming decades. Low socioeconomic conditions in childhood are the most important risk factors for *H. pylori* infection. Although the way of transmission is still unclear, interpersonal transmission appears to be the main route, especially, in the developed countries.

Keywords

Helicobacter pylori • Prevalence • Transmission • Epidemiology

1.1 Introduction

Helicobacter pylori (*H. pylori*), a cause of peptic ulcer disease, gastric adenocarcinoma, and low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma [1], has been falling due to improved sanitation and better living conditions over the decades in most countries [2, 3]. The changing epidemiology of the bacterium has

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been associated with a decline in the peptic ulcer disease (PUD) and gastric cancer [4] but it could increase gastroesophageal reflux disease and asthma which are related to acid or immunity [5]. There are many studies regarding the prevalence and risk factors of *H. pylori* infection, and older age was commonly considered as the main risk factor [6]. Adults have a continuous risk of *H. pylori* infection, resulting in the increased seroprevalence during lifetime as a function of age [7]. However, this does not mean that young people have a higher seroprevalence when they get older, as cross-sectional presentation does not necessarily give an accurate picture of lifetime trends. To compensate this there have been precious studies on lifetime trends for *H. pylori* seroprevalence [6, 8, 9]. In this chapter epidemiology and risk factors of *H. pylori* will be summarized over the decades depending on adults and children. In addition, the transmission route will be presented according to developed and developing countries.

1.2 Prevalence of *H. pylori*

This chapter has received much help from two review articles, which have been published in 2014 [10, 11]. Eusebi et al. [10] have searched PubMed up to September 2013, and Peleteiro et al. [11] made a search from Medline and PubMed databases for the period of April 2013–March 2014, in which results have been well introduced in each article. I also searched PubMed until July 2015 and summarized gathered information together in this chapter. The characteristics of the populations and tests in the publications were rather variable. That is, the clinical setting such as socioeconomic status was different. The assessment of *H. pylori* status was done mainly through enzyme-linked immunosorbent assay (ELISA) tests to determine IgG antibody titers in serum, but few studies used urine, saliva, or urea breath test (UBT) and joint information of blood and biopsy specimens. Thus, the results of prevalence rate of *H. pylori* in one country in the similar period could be at a wide range sometimes. As the age is a very critical factor for the prevalence of *H. pylori*, the prevalence results are shown separately for adults and for children in this chapter.

1.2.1 Prevalence of *H. pylori* in the Adults

The prevalence of *H. pylori* increased overall with age, decreasing in the older age groups such as over 60 or 70 years old in some countries (Chile [12], Ecuador [13], Japan [14], Mexico [15]), Latvia [16], and South Korea [17]). In the late 1990s/early 2000s, the prevalence estimates were generally higher among countries in Central/South America and Asia [10], which decreased in the late 2000s in most of these countries.

1.2.1.1 Asia Pacific Area

As the gastric cancer incidence is highest in Korea, Japan, and China, the trend of prevalence of *H. pylori* in these countries was compared. All of these countries showed the decrease of prevalence of *H. pylori*, but the detail was slightly different. That is, the prevalence of *H. pylori* was 71.4 % in China (35–64 years, 1989) [18] which decreased in 2000 (Table 1.1). That is, a total of 5417 healthy individuals aged between 30 and 69 years from areas of high incidence of gastric cancer in China were tested with ^{13}C-UBT, and the prevalence of *H. pylori* infection was 63.4 % in 2014 report [19]. When two Chinese prevalence studies using UBT or serum IgG antibody were compared by age, the 2004–2005 Jiangsu study [26] showed a higher rate than the 2003 Beijing study [27] regardless of age (Fig. 1.1a). Similarly, Japan showed *H. pylori* prevalence rate as 70.0 % among 40–79 years old in 1988–1990 [46] and 75.0 % among 40–69 years old in 1990–1992 [47]. However, three subsequent Japanese prevalence studies of *H. pylori* (using urine antibody or serum IgG antibody) in 1992 [74], 2002–2006 [14], and 2007–2011 [75] showed definite decrease of prevalence rate as time goes regardless of age (Fig. 1.1c). In South Korea, the

Table 1.1 Prevalence of *H. pylori* infection reported in adults

Country	Year reference	Setting	Number	Diagnostic method	Prevalence of *H. pylori* (%)
Argentina	2000 [20]	B	754 (261 children and 493 adults)	Serum IgG	Crude 37.5/Adjusted 35.7
Australia	2008 [21]	B	2413	Serum IgG (ELISA)	15.4
	2011 [22]	A	1400	Serum IgG (ELISA)	15.5
Bhutan	2013 [23]	A	372	Histology and RUT	73.4
	2014 [24]	A	244	Serum IgG (ELISA)	86
Canada	2013 [25]	A	203	Gastric biopsy tested positive for the bacterium	37.9
Chile	2007 [12]	A	3619	Serum IgG (ELISA)	Crude 73.0/Adjusted 73.4
China	2008 [18]	A	8280	*H. pylori* antibody in urine (ELISA)	71.4
	2008 [26]	A	1371	^{13}C expired air (UBT) and Serum IgG (ELISA)	62
	2009 [27]	A	1232	^{13}C expired air (UBT)	41.3–54.7
	2014 [19]	A	5417	^{13}C expired air (UBT)	63.4
Cyprus	2013 [28]	A	103	Gastric biopsy, PCR (primers for urea)	39.8
Czech Republic	2006 [29]	B	2509 (1234 men and 1275 women)	^{13}C expired air (UBT)	41.7
	2012 [30]	B	1837 (857 men and 969 women)	^{13}C expired air (UBT)	23.5
Ethiopia	2013 [31]	A	1388	Anti-*H. pylori* antibodies of all isotypes (IgG, IgM, IgA)	65.7
Finland	1999 [32]	A	173	Serum IgG (EIA)	61.0
	2001 [33]	A	730 (1983)	Serum IgG (EIA)	30.1 (1983)
			618 (1995)		13.1 (1995)
	2006 [34]	A	336	Serum IgG (not further specified)	65.0 (1977–1980)
					59.0 (1997–1998)
	2009 [35]	A	958	Serum IgG (EIA)	31.0 (1983)
					21.0 (1989)
					24.0 (1995)
					19.0 (2001)
France	1999 [36]	A	1597	IgG in saliva (ELISA)	25.4
Germany	1999 [37]	A	1834	Serum IgG (ELISA)	39.3
	2005 [38]	A	6545	Serum IgG (ELISA)	40.7
Iceland	2005 [39]	A	96 (only women)	Serum IgG (ELISA)	33.0
India	2013 [40]	A	530	Histology and RUT	58
	2013 [41]	A	530	Histology and RUT	62
Iran	2014 [42]	B	8459 (3575 men and 4172 women)	Serum IgG (ELISA) and stool antigen	30.6–82
Israel	2000 [43]	A	144	Serum IgG (ELISA)	46.5
Italy (Sardinia)	2015 [44]	A	11,202 (4160 men and 7042 women)	Histology, RUT, or ^{13}C expired air (UBT)	43.8

(continued)

Table 1.1 (continued)

Country	Year reference	Setting	Number	Diagnostic method	Prevalence of *H. pylori* (%)
Japan	2005 [45]	A	350	Serology	19.7
	2005 [46]	A	633 (349 men and 284 women)	*H. pylori* antibody in serum (not further specified)	70.0
	2006 [47]	A	511 (342 men and 169 women)	Serum IgG (ELISA)	75.0
	2007 [48]	B	4136	Serum IgG (ELISA)	54.7–67.5
Kazakhstan	2013 [49]	A	835	Serum IgG (ELISA)	76.5
Latvia	2012 [16]	A	3564 (1218 men and 2346 women)	Serum IgG (ELISA)	79.2
Lebanon	2012 [50]	A	308 (144 men and 164 women)	Serum IgG (ELISA)	52.0
Mexico	1998 [15]	B	11,605	Serum IgG (ELISA)	66.0
	2013 [51]	A	343	Serum IgG (ELISA)	52.2
Morocco	2013 [52]	A	429	Histology, RUT, culture	75.5
New Zealand	2014 [21]	11–85 (A)	4463	Serology	30.2
Nigeria	2013 [53]	A	125	Serum IgG (ELISA) / histology	93.6/80.0
Oman	2013 [54]	A	133 (100 men and 33 women)	Serum IgM, IgG, and IgA (ELISA)	69.5
Portugal	2013 [55]	A	2067	Serum IgG (ELISA)	84.2
Republic of San Marino	1995 [56]	A	2237 (1048 men and 1189 women)	Serum IgG (ELISA)	51.0
Saudi Arabia	2013 [57]	A	456	Serum IgG (ELISA)	28.3
Singapore	2002 [58]	A	261 (130 men and 131 women)	Serum IgG (ELISA)	50.2
South Korea	2001 [59]	A	3394	Serum IgG (ELISA)	66.9
	2005 [60]	A	344 (228 men and 116 women)	Serum IgG (ELISA)	80.8
	2007 [17]	A	15,916 (8616 men and 7300 women)	Serum IgG (ELISA)	56.0
	2008 [61]	A	25,536	Serum IgG (not further specified)	59.2
				Urease enzyme in biopsies (RUT)	
				H. pylori presence in histological examination	
	2013 [6]	A	10,796 (6085 men and 4711 women)	Serum IgG (ELISA and EIA)	54.4
Sweden	2003 [62]	A	3502	Serum IgG (ELISA)	18.0
	2004 [63]	A	499 (414 men and 85 women)	Serum IgG (ELISA)	40.0
	2011 [64]	A	117	Serum IgG (ELISA)	35.0
The Netherlands	2013 [65]	A	1550	Serum, *H. pylori* antibody and CagA antigen	31.7
	2013 [66]	A	6837	Serum IgG and CagA antibodies	46

Table 1.1 (continued)

Country	Year reference	Setting	Number	Diagnostic method	Prevalence of *H. pylori* (%)
Taiwan	2003 [67]	B	924	Serum IgG (ELISA)	16.7
Turkey	2003 [68]	A	675/260/148[a]	Serum IgG (ELISA)	13.1/30.4/44.5
	2013 [69]	A	4622	^{13}C expired air (UBT)	82.5
The United Kingdom	2002 [70]	B	10,118	Serum IgG (ELISA)	13.4
The United States	2000 [71]	A	7465 (3717 men and 3748 women)	Serum IgG (ELISA)	32.5
	2005 [72]	B	7462	Serum IgG (ELISA)	27.1
	2015 [73]	A	1200	Culture or histopathology	28.9

A adults, *B* both children and adults

EIA enzyme immunoassay, *ELISA* enzyme-linked immunosorbent assay, *RUT* rapid urease test, *PCR* polymerase chain reaction, *UBT* urea breath test

[a]Three subgroups were recruited encompassing 675 Germans (402 men, 273 women), 260 Turkish people born and raised in Germany (145 men, 115 women), and 148 Turkish people living in Turkey (91 men, 57 women)

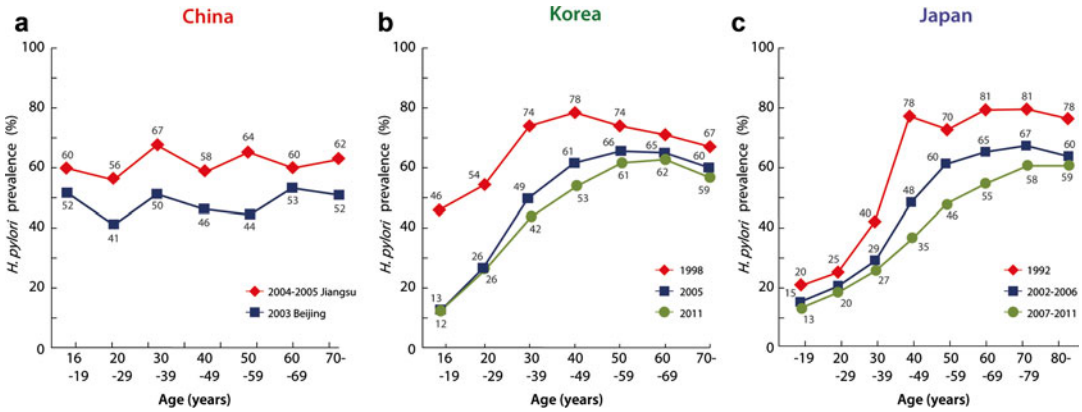

Fig. 1.1 Comparison of prevalence rate of *H. pylori* infection among China, Korea, and Japan. (**a**) Prevalence (using urea breath test or serum IgG antibody) by age in 2004–2005 Jiangsu [26] and 2003 Beijing in China [27]. (**b**) Seroprevalence in asymptomatic subjects without a history of *H. pylori* eradication in 1998 [19], 2005 [17], and 2011 [6] in Korea. (**c**) Prevalence (using urine antibody or serum IgG antibody) of *H. pylori* in 1992 [74], 2002–2006 [14], and 2007–2011 in Japan [75]

decrease of seroprevalence was significant across all age groups and in most areas of the country, especially in the age below 40 years old (Fig. 1.1b), which reflects the changes from developing to developed country. That is, in a large cross-sectional nationwide multicenter study, more than 10,796 asymptomatic subjects without a history of *H. pylori* eradication were enrolled in the adult age group greater than and equal to 16 years in 2011; its prevalence was 54.4 % [6]. This result was lower than that reported in the same country by similar surveys performed in 1993–1999 [60], 1998 [59], 2005 [17], and 2006 [61] where the prevalence of *H. pylori* was 80.8 % [60], 66.9 % [59], 59.6 % [17], and 59.2 % [61], respectively (Table 1.1), in the age of greater than and equal to 16 years [6]. This decreasing trend could be explained by cohort analysis [6] instead of continuous new infection over the age. All younger birth cohorts had a lower seroprevalence of *H. pylori* than older birth cohorts at the same age, and a decreased seroprevalence within the same birth cohorts was also accounted for in this phenomenon [6] (Fig. 1.2).

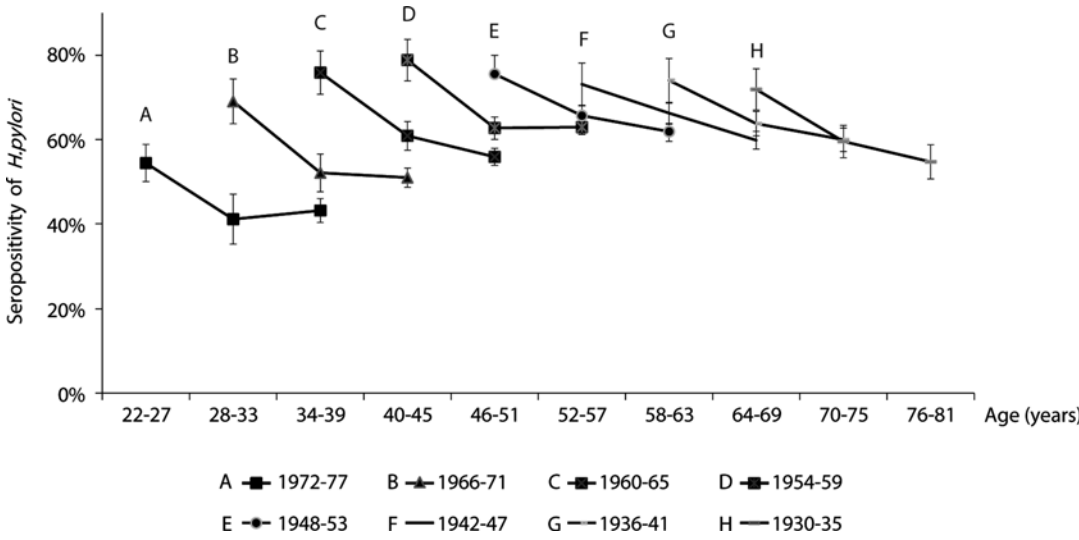

Fig. 1.2 Seroprevalence of *H. pylori* infection in asymptomatic subjects without a history of *H. pylori* eradication in birth cohort by age in Korea. Each line connects the values for the same cohort group in different age groups. For example, the first line shows the seroprevalence of *H. pylori* in a birth cohort in 1972–1977 for ages 22–39 years, and the second line shows the seroprevalence of *H. pylori* in a birth cohort in 1966–1971 for ages 28–45 years. All younger birth cohorts at the same age have a lower seroprevalence of *H. pylori* than older birth cohorts (Adapted from Lim et al. [6])

The prevalence in Singapore was already not so high in 1998, 50.2 % (55–69 years) [58] (Table 1.1). In India, the prevalence of infection ranged from 58 to 62 % in subjects with dyspeptic symptoms [40, 41], and in Kazakhstan, the prevalence of *H. pylori* infection was 76.5 % among symptomatic and asymptomatic cases [49]. Similarly, in Bhutan, the infection was present in 73.4 % of cases, although it was lower in the capital city, Thimphu, than in the rural areas, mainly related to sanitary conditions [23]. An even higher prevalence rate of 86 % was reported from another study in the same country [24] (Table 1.1).

In Oman, one of the Eastern Mediterranean regions, the *H. pylori* prevalence was reported to be 69.5 % among 15–50-year-olds by serology [54]. Similarly, in a population-based cross-sectional survey in Turkey, a weighted overall prevalence of infection was 82.5 % among more than 4600 subjects [69] (Table 1.1). Interestingly, the prevalence was lowest among individuals living in the southern part of Turkey who usually have a citrus fruit-rich diet, as this is the major citrus fruit-growing area [69]. In the Eshraghian review article regarding *H. pylori* prevalence in ten studies from Iran, the overall prevalence of *H. pylori* infection ranged from 30.6 to 82 % irrespective of time and age group [42]. In addition, the prevalence of *H. pylori* infection from Eastern Mediterranean region (five studies from the Kingdom of Saudi Arabia, four studies from Egypt, two studies from the United Arab Emirates, and one study from Libya, Tunisia, and Lebanon) ranged from 22 to 87.6 % [42] (Table 1.1). In the case of Israel, the prevalence rate was 46.5 % (mean age 18.7 years, 1986–1995) [43]. This wide variation in Middle Asia is probably related to the various age groups and methodology of *H. pylori* tests.

In Austria two seroprevalence rate studies performed in 2002 for 1–59 years old [21] and during 2002–2005 [22] showed 15.4 % and 15.5 %, respectively (Table 1.1). In the case of New Zealand where several ethnic groups live, *H. pylori* prevalence was highest among Pacific people (ranging from 39 to 83 %) followed by Maori (18–57 %) and then European (7–35 %) [76] (Table 1.1). The absolute ethnic differences in seroprevalence are decreased in subsequent

cohorts, but the relative ethnic differences increased [76].

1.2.1.2 Europe

In Europe, the prevalence of *H. pylori* seems to be lower in Northern countries than in Southern and Eastern countries [11]. In the Netherlands, a randomly selected sample of 1550 blood donors from four different regions was tested for the presence of antibodies against *H. pylori* and the CagA antigen [65]. This study where only native Dutch subjects were evaluated excluding non-European immigrants reported a 31.7 % prevalence of *H. pylori* infection (Table 1.1), with 28 % of *H. pylori*-positive subjects carrying a CagA-positive strain [65]. The seroprevalence of *H. pylori* declined from 48 % in subjects born between 1935 and 1946 to 16 % in those born between 1977 and 1987, as a likely consequence of a birth cohort effect. Also the proportion of CagA-positive subjects decreased from 38 % to 14 % in the same age cohorts. Additionally, from the Netherlands, a population-based prospective study of a cohort of more than 6500 pregnant women was published [66]. This study found that the prevalence of *H. pylori* in Dutch women was 24 %. In contrast, the prevalence of *H. pylori* in non-Dutch women was much higher, 64 % [66]. Moreover, in the latter group, infected subjects born abroad (first-generation immigrants) had a higher risk of *H. pylori* infection than second-generation immigrants [66].

In Sweden, the prevalence of *H. pylori* was 18.0 % (17–79 years, 1995) [62], 40.0 % (51–79 years, 1995–1997) [63], and 35.0 % (16–40 years, 1968–2001) [64] (Table 1.1). In Finland, the proportion of pregnant women infected declined to nearly half between 1983 (30.1 %) and 1995 (13.1 %) [33] and between 1983 (30.1 %) and 2001 (19.3 %) [35] (Table 1.1). *H. pylori* prevalence in adults who took part in a large population-based health survey was 65.0 % (1977–1980) and 59.0 % (1997–1980) [34]. In addition, *H. pylori* prevalence in the aged people who were older than 100 years was 61.0 % (mean age, 101 years and 1 month; 1991) [32]. In the Czech Republic, between 2001 and 2011, the prevalence decreased from 41.7 % [29] to 23.5 % [30] (Table 1.1). In the case of the United Kingdom, it was 13.4 % (1–84 years, 1986–1996), lower than the other countries because the children were included [77]. In France, *H. pylori* prevalence in adults was 25.4 % by ELISA method IgG in saliva between 1995 and 1997 [36] (Table 1.1). Similarly, the prevalence rate was 23 % in Hungary (19–23 years, 1999–2000) [78], 33 % in Iceland (mean age, 27 years; 1975–1997) [39], and 39.8 % in Cyprus (2013) [28] (Table 1.1). In Germany *H. pylori* prevalence in adults was 39.3 % (18–89 years, 1987–1988) [37] and 40.7 % (18–79 years, 1997–1999) [38] by ELISA tests to determine IgG antibody titers in serum (Table 1.1). In Italy a dramatic decrease in the prevalence of infection occurred over the 19-year observation period due to the improvement in socioeconomic conditions in the dyspeptic Sardinian patients from 1995 to 2013 [44]. The overall prevalence of *H. pylori* infection in Sardinians was 43.8 % (M: 46.6 % vs. F: 42.0 %; $p = 0.0001$) [44] and 51 % in San Marino (20–79 years, 1990–1991) [56] (Table 1.1). In contrast, a higher prevalence of *H. pylori* was reported in Portugal, where the prevalence of *H. pylori* infection was 84.2 %, with 61.7 % of strains also positive for CagA [55] (Table 1.1). In addition, based on a proportion of included subjects, an incidence rate of infection was 3.6/100 person-years, showing that Portuguese rates of *H. pylori* infection remain very high in Europe [55]. Similarly, Latvia also showed higher prevalence, 79.2 % in the age group of 17–99 ($n = 3564$) [16].

1.2.1.3 North America

In the United States, *H. pylori* prevalence in adults yielded small declines between 1988 and 1991 (32.7 %) [71] and 1999–2000 (27.1 %) [72]. In the cross-sectional study from a Veteran's Affairs Center in the United States among patients aged 40–80 years old, the overall prevalence was 28.9 %, but ethnicity was the most important factor [73] (Table 1.1). That is, *H. pylori* was highest among black males aged 50–59 (53.3 %), followed by Hispanic males aged 60–69 (48.1 %), and lowest in non-Hispanic white males aged 40–49 (8.2 %) [73]. In a

Canadian study where the presence of *H. pylori* infection was evaluated in 203 aboriginal patients with dyspepsia referred for gastroscopy, *H. pylori* infection was reported by histology in 37.9 % of patients [25] (Table 1.1).

1.2.1.4 Latin America

In the late 1990s/early 2000s, the prevalence estimates were generally higher among countries in Central/South America (around 20 years, ranging from 30 % in Argentina [20] to 70 % in Mexico [15]; around 60 years, ranging from 70 % in Chile [12] to 90 % in Mexico [15]) (Table 1.1). However, in 2013, a study from Mexico showed a seroprevalence rate of 52.2 % among 343 pregnant women living in rural areas in Mexico [51], which is a decreased rate than the previous high seroprevalence rate in this area [15] (Table 1.1). Similarly in Ecuador, a cross-sectional seroprevalence study during 2001–2002 showed a 63.1 % seroprevalence rate [13], which is rather a decreased rate than the previous reports from Latin America (Table 1.1).

1.2.1.5 Africa

New data from African countries has been summarized in the same review article [11] (Table 1.1). Studies from Morocco and Ethiopia reported a prevalence of *H. pylori* infection of 75.5 % [52] and 65.7 % [31], respectively. Both studies also found a significant increase with age [31, 52], probably due to birth cohort effect. A survey from patients with dyspepsia in Nigeria reported higher values: the prevalence was 80 % when tested with histology and was even higher, reaching 93.6 %, when serology was applied [53]. In the case of Lebanon, the seroprevalence study performed during 2008–2009 ($n = 308$, greater than and equal to 18 years old) showed a rate of 52.0 %, which is a quite decreased rate [50] (Table 1.1).

1.2.1.6 Summary

In most countries, recent surveys yielded lower prevalence estimates in the developing countries. However, *H. pylori* prevalence in adults in Africa, Central/South America, Asia, South/East Europe was still higher than 50 %, and it is related to birth cohort effect. In the developed countries, only small variations were observed, and *H. pylori* prevalence in adults still showed a low rate, 20–40 %, but the proportion of immigrants from the high-prevalence countries affected the *H. pylori* prevalence in adults, suggesting that ethnicity became a strong predictor for *H. pylori* in the developed countries [10, 11].

1.2.2 Prevalence of *H. pylori* in Children

H. pylori infection occurs mainly during childhood, especially under the age of 5 years [45, 79, 80], and *H. pylori* prevalence in the adulthood depends on infection in the childhood [8]. It is important to determine the status of current *H. pylori* infection in children including prevalence, incidence, and origin of infection because such evidence can be used to expect the incidences of *H. pylori*-related diseases in the future and can also be incorporated into a prevention strategy for gastric cancer [10].

1.2.2.1 Asia

The nationwide report performed in 1998 in South Korea shows the distribution of seroprevalence according to age as Figs. 1.3 and 1.4 [59]. The seropositivity of infants from birth to 6 months was 24.4 % and decreased to 6 % in the group aged 1–3 years, suggesting the transfer of mother's *H. pylori* IgG into the fetal blood [59]. The prevalence of *H. pylori* infection was increased progressively and steeply with advanced age ($p < 0.05$) from 6 % in the 1–3-year-old group to 78.5 % in the 40–49-year-old group. A characteristic feature of this study was that the seroprevalence increased abruptly in 10–12 years, resulting in the overall seroprevalence of *H. pylori* infection among children below 16 years old, 17.2 % [59] (Table 1.2). In China, a total of 1634 children and adolescents with upper gastrointestinal symptoms, who underwent gastroscopy with gastric biopsies, were evaluated for the presence of *H. pylori* infection [81]. The histological examination of gastric biopsies showed a 32.1 % prevalence of *H. pylori* infection [81]. In

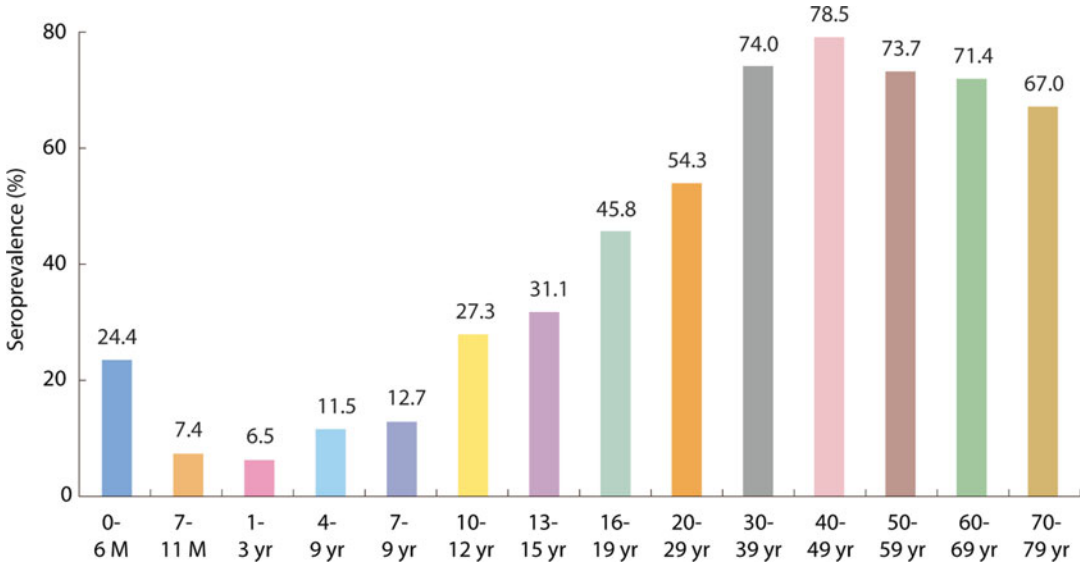

Fig. 1.3 Seroprevalence of *H. pylori* infection according to age. The seroprevalence was increased with age and was highest in people in their 40s (78.5 %). The characteristic feature of our study was that the infection rate was steeply increased in three age groups (10–12 years, 16–19 years, and people in their 20s). *M* months, *yr* years (Modified from Kim et al. [59])

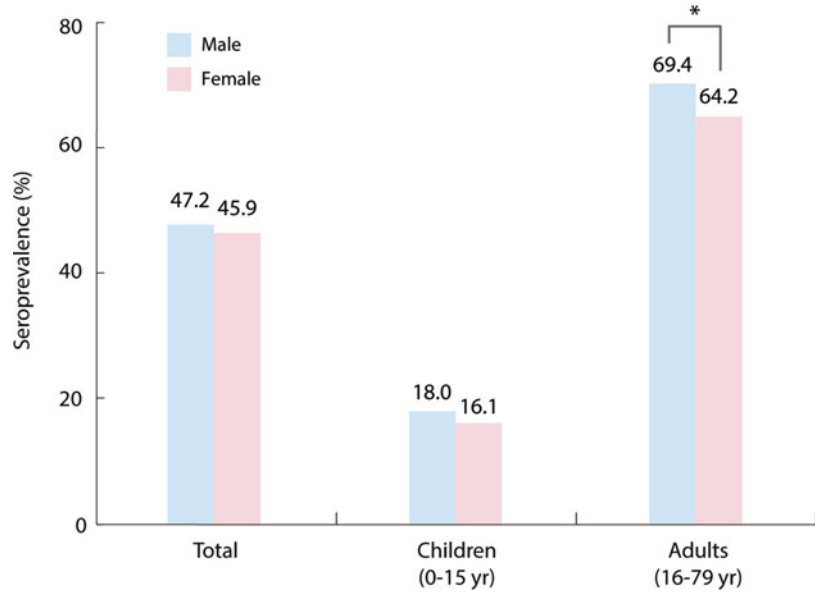

Fig. 1.4 Seroprevalence of *H. pylori* infection according to gender in children and adults. In adults, a significant difference was observed between genders. *$p<0.05$. *yr* years (Modified from Kim et al. [59])

contrast, *H. pylori* prevalence in Japanese children during 2010–2011 was approximately 1.8 % [82], which is much lower than that reported in Japanese adults. In detail *H. pylori* prevalence was 1.9 % among 689 children aged 0–8 years in 2010 and 1.8 % among 835 children aged 0–11 in 2011 [82]. No feco-conversion was observed in 430 children aged 0–8 years (170 were aged

Table 1.2 Prevalence of *H. pylori* infection in children

Country	Year reference	Age of included subjects (years)	Number	Diagnostic method	Prevalence of *H. pylori* (%)
Belgium	2013 [85]	12–25	516	^{13}C-UBT	11
Brazil	2014 [89]	2–19	129	Histology, RUT, culture	41.1
China	2014 [81]	1–18	1634	Histology, RUT	32.1
Ecuador	2004 [13]	6 months–16	257	Serum IgG (not further specified)	63.0
Germany	2003 [86]	7–20	540	^{13}C-UBT	9.4
Iran	2013 [84]	1–15	194	Serum IgG (ELISA)	50.5
Ireland	2005 [90]	24–48 months	327	^{13}C-UBT	8.6
Japan	2006 [79]	24–48 months	327	^{13}C-UBT	8.6
	2007 [80]	0–12 months	108	HpSA test	3.7
	2013 [83]	1–18	838 (389 boys and 449 girls)	Serum IgG (ELISA)	12.1
	2014 [20]	0–8/0–11	689/835	Stool antigen (PCR), UBT	1.9/1.8
Portugal	2013 [87]	13	1312	Serum IgG (ELISA)	66.2
South Korea	2001 [59]	0–15	2338	Serum IgG (ELISA)	17.2
Taiwan	2003 [91]	13–15	91/138	Serum IgG (ELISA)	82.4/71.0
Tunis	2003 [92]	1–15	191	Serum IgG (ELISA)	30.4
The United States	1996 [88]	6–19	2581 (1326 boys and 1255 girls)	Serum IgG (ELISA)	24.8

EIA enzyme immunoassay, *ELISA* enzyme-linked immunosorbent assay, *RUT* rapid urease test, *PCR* polymerase chain reaction, *HpSA H. pylori* stool antigen, *UBT* urea breath test

0–4 years) who provided follow-up stool samples after 1 year [82]. In contrast, another study in a small town in Japan showed that *H. pylori* prevalence in children was 12.1 % [83] (Table 1.2), suggesting again that different environments affect the seroprevalence in one country. In the case of Iran, a higher rate of seroprevalence in children was reported, 50.5 %, with 61.7 % of children positive for CagA [84] (Table 1.2).

1.2.2.2 Europe

In Belgium, a study carried out on children and young adults reported a prevalence of infection of 11 %, ranging from 3.2 % in Belgian-born children to 60 % in children born of foreign parents coming from countries with a high prevalence of *H. pylori* infection [85]. Similarly the seroprevalence of German children in Germany was 13.1 %, but that of Turkish children in Germany and Turkish children in Turkey was 30.4 % and 44.5 %, respectively [68], suggesting that seroprevalence could be different depending on race and socioeconomic status. Similarly, 7–9-year-old German children showed 9.4 % of *H. pylori* prevalence by UBT [86], but a subgroup analysis showed that German children prevalence was 7.1 % and that of the immigrant's children was 28.2 % [86] (Table 1.2). In contrast, Bastos et al. reported a very high prevalence of infection in Portuguese children [87], similar to the high prevalence in adults [55]. Among 13-year-old students from Porto, the prevalence was 66.2 % (Table 1.2). More than half of the

negative subjects were again tested after a median follow-up of 37 months, revealing an incidence rate of 4.1/100 person-years [87].

1.2.2.3 North America

In the United States, *H. pylori* prevalence in children and adolescents was 24.8 % (6–19 years, 1988–1991) [88] (Table 1.2).

1.2.2.4 Latin America

In Brazil, Pacheco et al. compared several diagnostic tests and reported a high prevalence of 41.1 % in subjects aged 2.1–19 years old [89] (Table 1.2).

1.2.2.5 Summary

H. pylori prevalence in children is very variable in the world, suggesting that still active *H. pylori* infection occurs in the childhood in some countries.

1.3 Risk Factors of *H. pylori* Infection

There have been many studies regarding risk factors of *H. pylori*. The most frequent independent risk factors for *H. pylori* infection were living in rural areas, poor sanitation, overcrowding, lower educational level, and low socioeconomic status [59]. In terms of gender, most studies reported no significant difference of *H. pylori* infection between men and women, both in adults [23, 24, 31, 41, 52, 65] and in children [59, 85, 87]. However, in South Korea, gender showed no difference in *H. pylori* prevalence among children, but the male gender became a risk factor in adults aged 13 years and over [6, 17, 59] (Table 1.3). In contrast, women showed higher *H. pylori* prevalence in Iran [43], suggesting that somehow intimate personal relationship could be related to *H. pylori* transmission. In terms of age, *H. pylori* seroprevalence became definitely higher in the adults than in the children recently [82]. However, no significant association was found between infection and age in the adult population [24, 40, 41, 66]. Instead, the age-specific gradient in *H. pylori* prevalence reported by some studies seems to be related to a birth cohort effect [6, 31, 52, 65, 81]. Several socioeconomic factors have been associated with *H. pylori* infection [11]. In particular, subjects with a low socioeconomic status which has been measured as a low family income had a higher likelihood of carrying *H. pylori* infection [19, 66], especially in the childhood [57] (Table 1.3). Furthermore, an inverse association between educational level and *H. pylori* infection was found in the majority of the studies [6, 17, 52, 55, 69]; except for two cases [19, 42], individuals with lower educational levels had a higher risk than those with a higher education [11]. The same association concerning the parents' education was also found in studies on children [85, 87], especially mother's education [57] (Table 1.3). Moreover, several factors related to residence have been found to be associated with the infection [11]. Indeed, living in a rural area [17, 23] and in crowded homes [24, 57, 87] and having contaminated sources of drinking water

Table 1.3 Multivariate analysis of risk factors for *H. pylori* infection in three nationwide epidemiologic studies in South Korea

	Children (1–15 years)	Adults (≥16 years)
2001 [57]	Age	Age
	Geographic areas	Geographic areas
	Drinking water (tap or well water)	Gender (male)
	Low mother's education	Crowding in childhood
	Family income per month	Low economic status in childhood
2007 [17]		Age
		Geographic areas
		Gender (male)
		Medium or low monthly income
		Medium education than high
2013 [6]		Age
		Geographic area
		Gender (male)
		Medium or low monthly income
		Medium education than high Cholesterol (240≥ mg/dL)

[57, 69] were risk factors for *H. pylori* infection (Table 1.3). Among the main lifestyle habits, smoking and alcohol consumption showed discordant results [11]. Although in most studies there was no significant association with *H. pylori* infection [6, 17, 19, 52, 57, 66], some authors reported that regular smokers in Turkey [69] and in Saudi Arabia [93] and drinkers in Saudi Arabia [93] were at higher risk [11]. In contrast, in one study, regular alcohol drinking was a protective factor for *H. pylori* infection in Turkey [69].

1.4 Transmission of *H. pylori*

Now it has been established that *H. pylori* is mainly transmitted through person-to-person, especially in the childhood and intrafamilial transmission [48, 94–97]. However, it is not clear how the bacteria are transmitted person-to-person and why the colonization does not occur in some persons, but it persists forever in others. The main route of transmission is regarded through oral-oral, fecal-oral, or gastric-oral. As the route of transmission could be different depending on developing or developed countries because the exposed age, race, and socioeconomic status are variable in these countries [92], the transmission of *H. pylori* was summarized in two conditions of the developing and developed countries in this chapter.

1.4.1 Transmission of *H. pylori* in the Developing Countries

In the past, fecal-oral transmission was regarded as the main route in the developing countries. That is, environmental transmission, such as drinking contaminated water, could play a major role. However, as more evidences came out, fecal-oral transmission became a minor route although it remains as a possible route. In addition, the transmission of *H. pylori* was found to be different from hepatitis A viral infection [67, 91, 98]. Instead, parental transmission has been frequently reported. Didelot et al. sequenced the genomes of 97 *H. pylori* isolates from 52 members of two families living in rural conditions in South Africa [70]. Transmission events were more frequent between close relatives and between individuals living in the same house. Turkish report has shown close relationship of colonized *H. pylori* between the dental plaque and gastric epithelium [68]. In addition, further evidence came from Brazil that an *H. pylori*-infected mother was a strong and independent risk factor (odds ratio 22.7, 95 % confidence interval, 2.31–223.21) [99]. Taken together, oral-oral transmission became a more important transmission route of *H. pylori* infection even in developing countries. This could be related to the characteristics of *H. pylori* which need good conditions such as humidity and anaerobic conditions with warmth.

1.4.2 Transmission of *H. pylori* in the Developed Countries

In the developed countries, parental transmission has been frequently reported [82], but the transmission route is not so simple because of the immigrants from countries with a high prevalence of *H. pylori*. For instance, the seroprevalence of German children in Germany was 13.1 %, but that of Turkish children in Germany was 30.4 % and Turkish in Turkey was 44.5 % [68], suggesting that seroprevalence could be different depending on race and socioeconomic status. Similarly, among 7–9-years-old Germans, *H. pylori* prevalence was 9.4 % by UBT, but a subgroup analysis showed that prevalence in German children was 7.1 % and that of the immigrants' children was 28.2 % [86]. In this study, the number of family members was found to be a risk factor, and the prevalence rate was high when a family member had gastrointestinal symptoms [86]. The intrafamilial transmission became strong from many evidences in the developed countries. One study which performed fingerprinting showed that among 35 families, the strain of 29 families (81 %) was the same [96]. In addition, the concordance between mother and children was 56 % (10 among 18 families), but

none between father and children, and 22 % between husband and wife, suggesting that a close relationship increases the possibility of transmission [96]. Osaki et al. performed a multilocus sequence typing DNA analysis using the stools of parents belonging to three families with a child positive for *H. pylori* infection [90]. The study showed an intrafamilial transmission in all selected families, with a mother-to-child transmission in at least two families. Similarly, Urita et al. investigated the intrafamilial transmission of *H. pylori* infection by testing 838 children and their family members from a small town in Japan [83]. The *H. pylori* prevalence in children was 12.1 %, and most risk factors were the siblings, mother, and grandmother, but the father and grandfather were not a risk factor [83]. Indeed, it seems that mothers transmit the infection through mouth secretions, using common spoons or tasting the child's food [11]. Grandmothers might take care of their grandchildren when mothers are at work increasing the risk of transmission. Similar report has came out from Ireland that children could be infected when the mother or siblings had *H. pylori* infection [79]. Taken together, *H. pylori* infection in the developed countries could have characteristics of developing countries due to the immigrants from countries with a high prevalence of *H. pylori*. However, the oral-oral especially mother-to-child transmission is the main route.

Conclusions

Data from recent studies show that the prevalence of *H. pylori* infection is still high in most countries worldwide. *H. pylori* seems to be less frequent in Northern European and North American populations; however, about one-third of the adults seem to still be infected. Even in these countries, *H. pylori* remains highly prevalent in immigrants coming from countries with a high prevalence of *H. pylori*. The most frequent independent risk factors for *H. pylori* infection were living in rural areas, poor sanitation, overcrowding, lower educational level, and low socioeconomic status. Parent-to-child infection is thought to be the main infection route of the infrequent infection for children. The lower prevalence of infection in the younger generations suggests a further decline in *H. pylori* prevalence in the community over the coming decades.

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Part II

Pathophysiology

Gastric Colonization by *H. pylori*

2

Elizabeth A. Marcus and David R. Scott

Abstract

Helicobacter pylori (*H. pylori*) is uniquely adapted to colonize the gastric mucosa. The key components for gastric colonization include motility, adhesion, and acid acclimation. The flagellar system allows the bacteria to move within the gastric mucus layer to the sites where conditions are optimal for survival. Adhesion to the gastric mucosa, via interaction between bacterial and host proteins, allows the bacteria to withstand bulk flow of gastric fluid. Acid acclimation is the system that allows for periplasmic and cytoplasmic pH regulation in the setting of an acidic environment. The bacteria are bioenergetically neutralophiles, meaning they are able to survive between pH 4 and 8 and grow between pH 6 and 8. The pH at the gastric surface in the presence of *H. pylori*, as shown by microelectrode, fluorescent dye, and in vivo transcriptome studies, is below the range for growth and near to below the limits for survival. The bacteria are able to sense acidic medium pH and stimulate trafficking of cytoplasmic urease and its accessory proteins to the proton-gated urea channel, UreI, in the inner membrane. The breakdown of urea into carbon dioxide and ammonia buffers the periplasm and cytoplasm to within the pH range optimal for a neutralophile. Understanding gastric colonization is clinically relevant because these systems that facilitate colonization can be targeted or interfered with to improve efficacy of eradication regimens.

Keywords

Helicobacter pylori • Colonization • Acid acclimation • Adhesion • Motility

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2.1 Introduction

Colonization of the host by a microbe refers to a state of infection resulting in a continuum of disease from none to significant. Colonization where

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no disease is present is synonymous with commensalism, as is seen with normal human gut flora. On the other side of the disease spectrum, bacteria that cause damage to the host are defined as pathogens. Therefore, colonization factors are distinct from pathogenic factors in that they are required for initial and persistent infection, but do not necessarily result in damage to the host. Pathogenic factors, on the other hand, are not required for initial and persistent infection but cause damage to the host. *Helicobacter pylori* (*H. pylori*) infection always results in chronic active gastritis, defining it as a pathogen, even though most infected individuals are asymptomatic. This chapter describes the gastric environment that *H. pylori* colonizes, followed by a discussion of the colonization factors used by *H. pylori* to overcome the impediments to infection that make it uniquely suited to colonize and persist in the gastric milieu.

2.2 Gastric Environment at the Site of Infection

The human stomach, in the setting of normal physiologic acid secretion, has a median luminal pH of about 1.4, with elevations up to about pH 4 at mealtimes due to buffering by food [1]. It was originally believed that *H. pylori* inhabited a more neutral niche at the gastric surface with protection from gastric acidity provided by bicarbonate secretion from epithelial cells and by the mucus layer. Studies done with glass-tipped microelectrodes suggested a pH gradient through the gastric mucus, with pH near neutral at the epithelium [2]. These studies may have been hindered by measurement technique as the open-tip microelectrodes may have prevented diffusion of protons. Later microelectrode studies using a similar measurement system in mice suggested that the presence of *H. pylori* removed any barriers to proton diffusion and suggested an acidic pH at the bacterial niche [3]. Fluorescent dye studies done in the externalized stomachs of anesthetized mice again suggested an acidic gastric surface pH, regardless of the presence of the mucus layer [4]. The pH at the gastric surface is a combination of regulation of acid and alkali secretion to a specific set point rather than a result of trapping of buffers or protons under the mucus layer [4].

Transcriptome analysis of *H. pylori* provides further evidence for acidic pH at the gastric surface. Several in vitro studies have outlined changes in gene expression at acidic pH, using a variety of incubation times and conditions [5–7]. The unifying conclusion from these studies, done using divergent methodology, is that there is a set of genes that change expression based on environmental pH, suggesting adaptation to allow for gastric colonization. The well-documented movement of *H. pylori* from its typical niche in the gastric antrum to the fundus in the setting of acid inhibitory therapy in both humans and gerbils again provides evidence for the need for the bacteria to inhabit a very specific environment with a specific pH range [8–11]. The *H. pylori* transcriptome has been studied in the gerbil stomach to correlate with in vitro pH changes [12]. The gerbil is a viable model system because the gastric pH profile and the advanced sequelae of *H. pylori* infection are similar to those seen in humans [13–15]. The pattern of *H. pylori* gene changes in the gerbil stomach were comparable to gene changes seen in vitro at acidic pH, providing additional evidence for an acidic environment at the site of infection [12].

2.3 Motility

H. pylori infection is acquired in early childhood through oral-oral or oral-fecal routes. In the first step toward colonization, the microbe must reach the mucosa of the gastric antrum, its preferred site of colonization. To reach its gastric niche, *H. pylori* has evolved a spiral shape and unipolar flagella to transit the mucus barrier overlying the gastric epithelial surface. Host factors orient the movement of *H. pylori* toward the gastric mucosa through the bacterial chemotactic response (Fig. 2.1).

The need for motility as a colonization factor was demonstrated using *H. pylori* strains with different degrees of motility. It was found that the gastric infection in gnotobiotic pigs was greater in the more motile strains of *H. pylori* [16]. Infection of gnotobiotic pigs by aflagellate mutants was

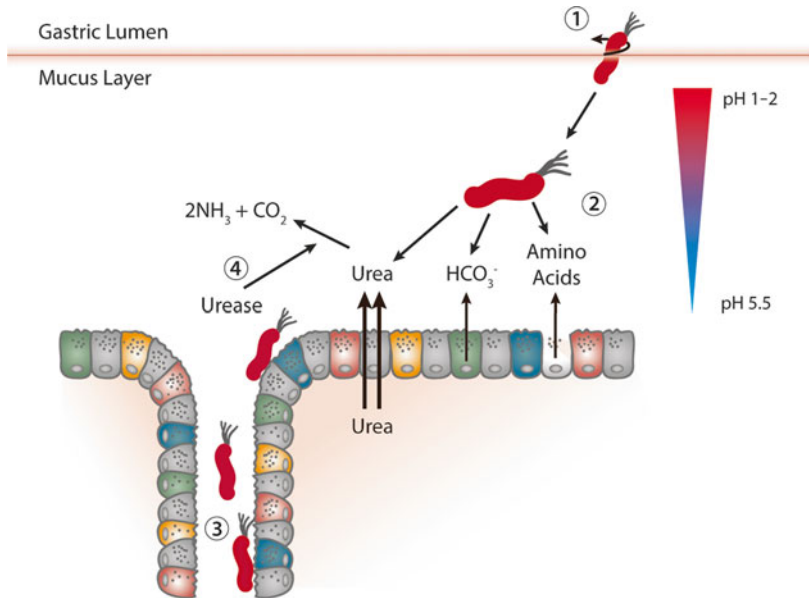

Fig. 2.1 Model of colonization of the gastric mucosa by *H. pylori*. (*1*) The spiral shape and unipolar flagella propel *H. pylori* in a counterclockwise rotation to auger through the gastric mucus and reach the gastric epithelial surface, the site of colonization. (*2*) *H. pylori* is lured to the gastric surface by the chemoattractant, urea, which enters the gastric lumen both trans- and paracellularly, bicarbonate released from surface cells, and various amino acids released from damaged surface epithelial cells. (*3*) Upon reaching the gastric surface, the bacteria adhere to adhesin receptors on the cell surface. (*4*) Through the mechanism of acid acclimation, *H. pylori* are able to survive and thrive in a highly acidic environment

cleared in 6 days, while the flagellate parent strain remained infective for at least 3 weeks [17]. The flagellar structure of *H. pylori* is similar to other bacterial flagella and is composed of two substructures, the hook-basal body (HBB), which drives flagellar movement, and the extracellular localized flagellum. The HBB has components located in the cytoplasm, the periplasm, and the inner and outer membranes. Flagellar gene expression is temporally regulated with transcription proceeding from intracellular localized components to the extracellular flagellar filaments.

In the normal acid-secreting stomach, *H. pylori* is found predominately within the first 15–30 μm of the mucus overlying the antrum, with about one-third of the bacteria in the mucus layer immediately adjacent to the epithelial cells (0–5 μm) [18]. About 2 % of the bacteria are found adhering to the gastric epithelium. To colonize this niche, the bacteria sense host attractants or repellents and move toward or away from them, respectively. Urea, bicarbonate, pH, zinc, nickel, arginine, glutamine, histidine, and other amino acids elicit chemotactic responses by *H. pylori* [19–23]. These chemotactic factors are sensed by methyl-accepting chemotaxis proteins (MCPs) that transduce the signal and alter flagellar rotation. *H. pylori* has at least four MCPs, the membrane proteins TlpA, TlpB, and TlpC and the cytoplasm-located TlpD. TlpA senses arginine, other amino acids, and bicarbonate [19]; TlpB is required for pH and urea taxis and also senses the quorum-sensing molecule autoinducer-2 (AI-2) [24]; TlpC regulates whether acid is sensed as an attractant or repellent [22]; and TlpD senses the internal energy state of the bacterium [25].

2.4 Adhesion

Adherence of *H. pylori* to the gastric epithelium is a necessary step in establishing successful infection because it provides protection from clearance mechanisms such as bulk liquid flow, gastric peristalsis, and the continuous shedding and replenishment of the mucus layer (Fig. 2.1).

H. pylori have evolved specific virulence determinates on their outer membrane which recognize distinct protein, proteolipids, or carbohydrates expressed on epithelial cells. These virulence factors are adhesins, which bind to receptors on the surface of the gastric mucosa. Many of the adhesins are outer membrane proteins (OMPs). The histo-blood group antigen Lewis b (Le^b) was identified as an adhesin receptor for *H. pylori* as shown by antibody competition assays and the lack of binding to gastric epithelia not expressing Le^b [26]. Le^b is the dominant antigen in the human gastric mucosa of individuals that secrete α1, 2-fucosyltransferase [27]. The Le^b phenotype is epidemiologically associated with the presence of the *cag* PAI [28, 29]. The adhesin recognizing the Le^b antigen was isolated by affinity purification. This adhesin is an *H. pylori* OMP and was named BabA (blood group antigen-binding adhesin) [29]. *H. pylori* infection of the gastric mucosa results in chronic active gastritis. This inflammation results in the replacement of the naturally produced Lewis antigens and the expression of sialylated glycans such as sialyl Le^a and sialyl Le^x [30, 31]. In the absence of BabA, an adhesin was identified that binds to sialyl Le^x antigen and named SabA (sialic acid-binding adhesin) [30]. *H. pylori* isogenic mutants lacking the OMP HopZ failed to adhere to the gastric carcinoma-derived AGS cells [32], indicating that it is an adhesin, but its receptor is unknown. Two other OMPs, the adherence-associated lipoproteins A and B (AlpA and AlpB), have been identified as putative adhesins due to loss of binding to the gastric epithelium of their respective isogenic mutants [33]. As with HopZ, the host receptors have not been identified.

2.5 Acid Acclimation

Gastric acid is an impediment to colonization. *H. pylori* is a neutralophile that grows between pH 6.0 and 8.0 and survives between pH 4.0 and 8.0. Since the median luminal pH of the stomach is less than 2.0 and *H. pylori* not only survives in this high acidity but flourishes, it has evolved the unique mechanism of acid acclimation. Acid acclimation is the ability of *H. pylori* to maintain periplasmic pH near neutral in an acidic environment [34]. This is distinct from the acid resistance mechanisms that allow bacteria to transit the stomach by maintaining a cytoplasmic pH near 5 [35]. Examples of proteins involved with acid resistance include the glutamate decarboxylase-glutamate aminobutyrate antiporter and the arginine decarboxylase-arginine-agmatine antiporter, which consume protons and produce carbon dioxide, and the proton transporters including the F_1F_0 ATPase and the $Na^+/2H^+$ antiporter [36, 37]. These systems are designed to buffer the cytoplasm but do not regulate periplasmic pH. Gastric colonization is not possible if cytoplasmic pH cannot be elevated to a level that allows critical metabolic processes such as protein synthesis, a level of buffering that requires periplasmic pH regulation [34]. While *H. pylori* expresses some of the known acid resistance or tolerance genes [38], these proteins complement rather than explain gastric colonization.

The principle component of acid acclimation is the neutral pH optimum, highly expressed cytoplasmic urease enzyme. The *H. pylori* urease gene cluster is made up of seven genes under the control of two promoters [39]. *ureA* and *ureB*, under the control of the first promoter, encode the structural subunits of the urease enzyme [39]. Urease is a hexameric heterodimer that requires nickel incorporation for activation. Downstream from the second promoter are *ureI*, *ureE*, *ureF*, *ureG*, and *ureH* [39]. *ureI* encodes the only integral membrane protein in the operon. UreE, UreF, UreG, and UreH are cytoplasmic accessory proteins that aid in nickel incorporation into apourease.

Urease is required for acid survival and gastric colonization [40, 41]. Production of urease by *H. pylori* is constitutive and accounts for about 10% of the total cellular protein [42, 43]. The cytoplasmic enzyme with a neutral pH optimum catalyzes the breakdown of urea into carbonic acid and then to carbon dioxide and ammonia. The activity curve of free urease shows the expected pH optimum near neutral, with minimal activity at acidic pH and inactivation as the pH

drops into the range found in the stomach. In contrast, in the intact bacteria, urease activity is minimal at neutral pH and rises to maximal below pH 6, down to about pH 2.5 [44]. This activity curve suggests a barrier of access of urea to the urease enzyme (Fig. 2.2). UreI, the only membrane protein in the urease gene cluster, was shown to be a proton-gated urea channel, allowing urea into the cytoplasm at acidic pH [45]. Deletion of *ureI* leads to loss of acid activation of urease [44]. At physiologic urea concentrations, *ureI* deletion mutants are unable to survive in acid. When medium pH drops, periplasmic pH drops as well. This leads to opening of UreI, movement of urea into the cytoplasm, and breakdown to the eventual end products of carbon dioxide and ammonia, catalyzed by the urease enzyme. These two gasses then buffer the periplasm into the pH range compatible with survival of a neutralophile, without the need for bulk pH change of the environment (Fig. 2.3).

2 Ni^{2+} per active site are required for activation of urease, and a large fraction of urease can be inactive, especially at neutral pH [46, 47]. Presumably this would prevent over-alkalization of this neutralophile in conditions where the pH rises and would also create a pool of urease that is ready and available to be activated in the setting of a drop in pH. The cytoplasmic accessory proteins are critical to urease activation and activity [48]. UreE forms a heterodimer with UreG and UreF with UreH, as evidenced by yeast two-hybrid and homology analysis, and these protein pairs bind urease most likely via UreB to aid with nickel incorporation and enzyme activation [49, 50]. Each accessory protein has a specific role in urease activation. UreE aids directly with incorporation of nickel into the active site [50]. UreF prevents premature nickel binding [51]. UreG provides energy for assembly of urease [52]. UreH provides stability for apourease [53]. The nickel regulatory protein NikR is able to directly and indirectly control a wide range of regulatory systems, many of which are involved in acid survival [54]. For example, NikR has been shown both in vitro and in vivo to positively regulate expression of *ureA* [55–58].

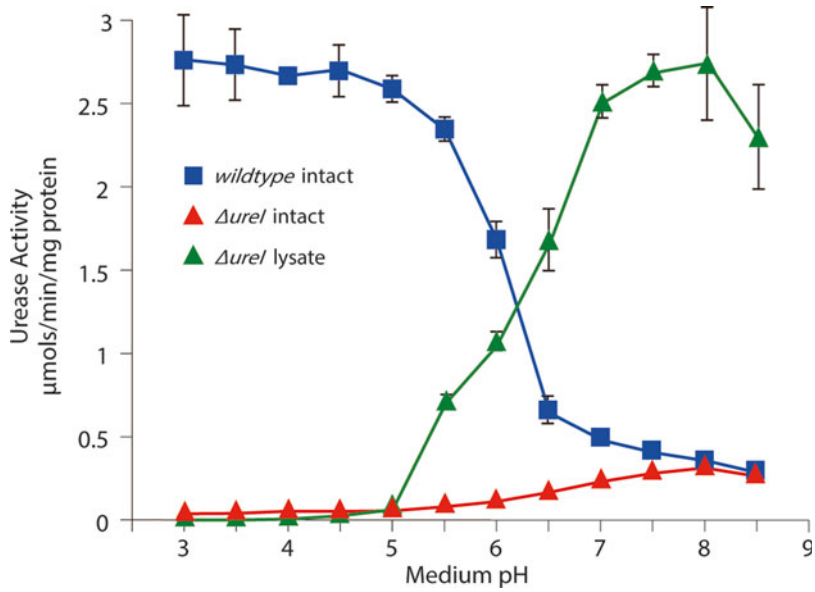

Fig. 2.2 *H. pylori* urease has a neutral pH optimum but is acid activated in intact bacteria. Activity of free urease enzyme, measured by release of ^{14}C-labeled CO_2 from ^{14}C-labeled urea, peaks near-neutral pH, and the enzyme is inactive below pH 4 (not shown). In intact bacteria, there is minimal activity at pH 7, when the proton-gated urea channel, UreI, is mainly closed, and activity increases as medium pH drops and urea gains access to cytoplasmic urease. In the absence of UreI, acid acclimation is not seen and activity is low at acidic medium pH. Activity of urease enzyme itself is unaffected by the absence of UreI, as evidenced by the normal-appearing activity curve in lysed bacteria

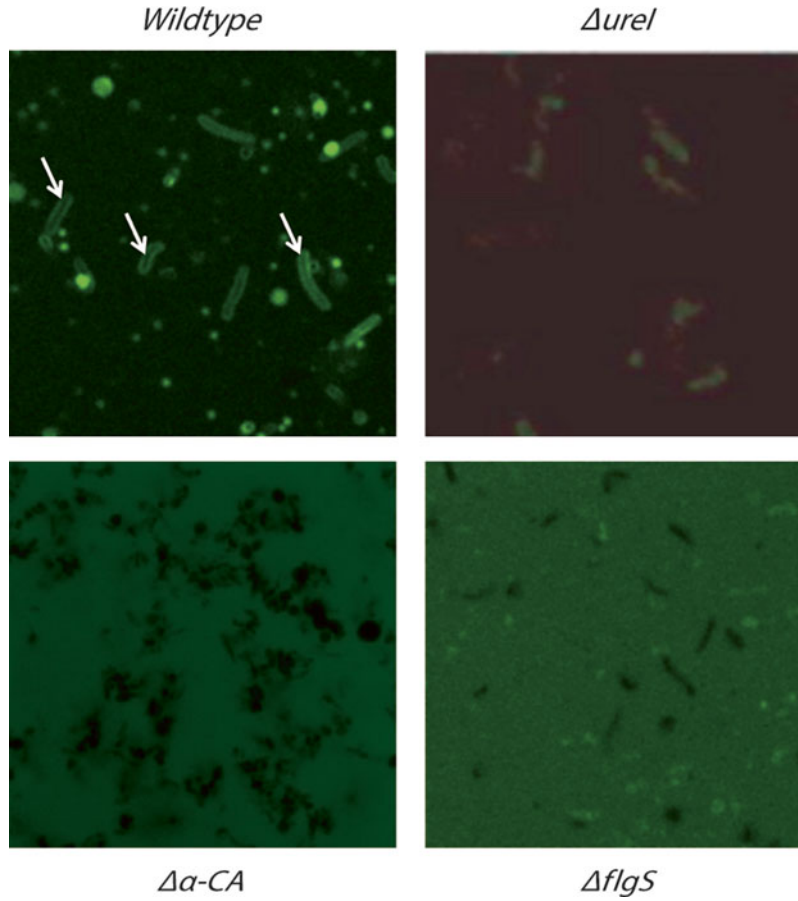

Fig. 2.3 Periplasmic alkalization is seen in *wild-type H. pylori* at acidic medium pH in the presence of urea. The fluorescent, impermeant, pH-sensitive dye BCECF free acid was used to look at periplasmic pH. At acidic pH, in *wild-type* bacteria, periplasmic buffering facilitated by urease activity and the urea channel, UreI, is shown by an increase in periplasmic fluorescence with the addition of urea (*arrows*). In the absence of expression of key acid acclimation proteins, such as UreI, α-carbonic anhydrase (CA), and FlgS, periplasmic pH change is significantly reduced

While the urease operon and regulation of nickel uptake are critical for gastric colonization, other proteins are also prominently involved in acid acclimation. *H. pylori* expresses a β-carbonic anhydrase enzyme in the cytoplasm that catalyzes the production of carbon dioxide from the intermediate products of the urease reaction. An α-carbonic anhydrase enzyme is localized to the inner membrane/periplasm and aids in generation of bicarbonate from carbon dioxide entering the periplasm. While ammonia can neutralize protons in both the cytoplasm and periplasm, the pK_a of ammonia/ammonium is about 9.2, so the buffering capacity of this product of urea hydrolysis is limited at the effective pH seen by the bacteria. In contrast, the pK_a of carbon dioxide/bicarbonate is around 6.1, which is closer to the range needed for *H. pylori* growth and for urea channel opening. Carbon dioxide is therefore a major contributor to the periplasmic buffering unique to *H. pylori* acid acclimation [34]. Inhibition or deletion of the α-carbonic anhydrase limits *H. pylori* acid survival, and periplasmic buffering is not seen using fluorescent dye and confocal microscopy [34] (Fig. 2.3).

Efficiency of response to medium acidification is also critical to *H. pylori* acid acclimation. If periplasmic buffering does not occur rapidly, the cytoplasmic pH will drop and critical metabolic processes will be impaired. The structural

subunits of the urease enzyme, UreA and UreB, interact with the urea channel, UreI, at the inner membrane, as demonstrated by native electrophoresis of purified membrane preparations, antibody isolation of the proteins, and electron microscopy [50, 59]. UreA and UreB are not found in purified membranes in the absence of UreI [50]. Urease is also activated, by incorporation of nickel, at the membrane. The products of urea hydrolysis, carbon dioxide, and ammonia, as gasses, are able to diffuse through the inner membrane to buffer the periplasm, and in addition, they are able to move back through UreI, which further enhances the efficiency of response [60].

Bacterial two-component systems (TCSs) allow bacteria to sense and respond to their environment. They are classically composed of a histidine kinase that senses changes in the environment and autophosphorylates and a response regulator that is activated by phosphotransfer to an aspartyl residue to regulate gene transcription [61]. *H. pylori* has relatively few TCSs compared with other bacteria, reflective of their environmental niche and the consistent environment in the stomach [38]. Two of these TCSs, ArsRS and FlgRS, are involved with sensing of environmental pH (Figs. 2.4 and 2.5). The histidine kinase ArsS is localized to the inner membrane and is able to sense periplasmic pH via protonation of at least histidine 94 [62]. Both acid-responsive TCSs are involved with trafficking of urease and the accessory proteins to the inner membrane in response to medium acidity [60, 63]. In the case of ArsRS, it has been demonstrated that this response is driven

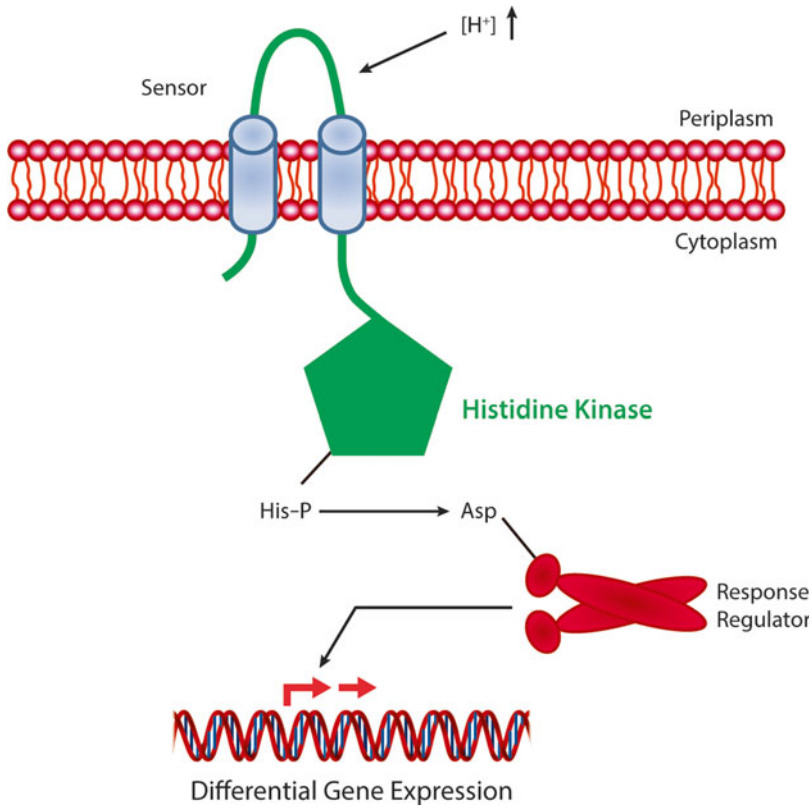

Fig. 2.4 Model of bacterial two-component systems (TCSs). *H. pylori* has 2 pH-sensitive TCSs. ArsRS responds to drop in periplasmic pH and FlgRS to drop in cytoplasmic pH. ArsRS is depicted in the model. The histidine kinase ArsS is in the membrane. When it senses a drop in pH, it is autophosphorylated, and then the phosphate is transferred to an asparagine residue on the cytoplasmic response regulator. The response regulator is then activated to regulate gene transcription, including expression of acid acclimation genes

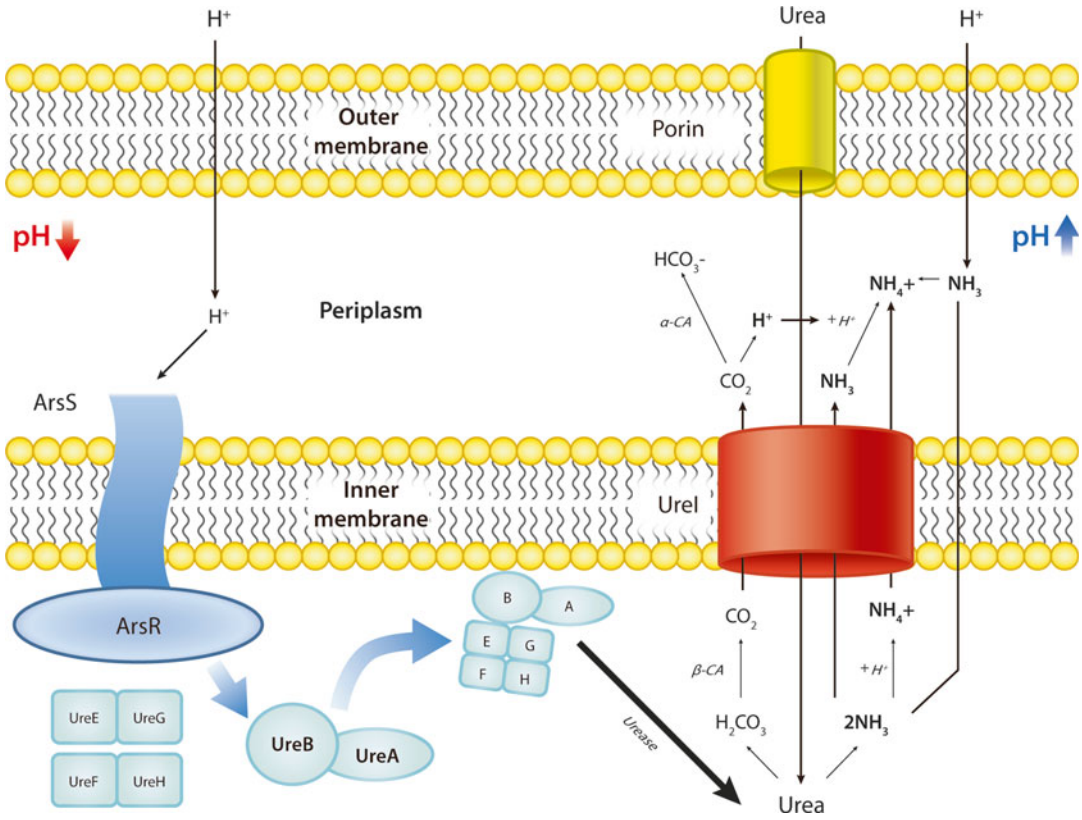

Fig. 2.5 Model of *H. pylori* acid acclimation. When medium pH drops, periplasmic pH drops as well. This leads to opening of UreI, the proton-gated urea channel in the inner membrane. Urea moves into the cytoplasm. At the same time, the fall in pH is sensed by ArsS in the inner membrane and, with further fall in pH, by FlgS in the cytoplasm (omitted for clarity). Activation of the TCSs leads to trafficking of urease and its accessory proteins to the urea channel at the inner membrane. Urease is then activated at the membrane via nickel incorporation, leading to breakdown of urea into carbonic acid and ammonia. Carbonic acid is converted to CO_2 with the help of the cytoplasmic β-carbonic anhydrase enzyme. The two gasses diffuse through the membrane and through the urea channel into the periplasm, where CO_2 is converted to bicarbonate with the help of the periplasmic α-carbonic anhydrase enzyme. The periplasm is then buffered to a pH within range for survival of a neutralophile

by the sensor kinase [64]. When medium pH drops, ArsS senses the drop in pH and stimulates movement of urease to UreI at the inner membrane [63]. Accessory proteins required for nickel incorporation and urease activation also move to the inner membrane [63]. In the absence of ArsS, the levels of urease and the cytoplasmic accessory proteins in the membrane fraction and total urease activity of this fraction are reduced [63]. *ΔarsS* deletion mutants are defective in colonization of the mouse stomach [65]. Proton gating of the urea channel, UreI, is unaffected by the absence of ArsS [63]. ArsR is essential for bacterial viability, but strains with a point mutation preventing phosphorylation are viable, and phosphorylation is not required for acid survival [64, 66]. ArsR phosphorylation is required for upregulation and transcriptional control of acid acclimation genes [67] and provides negative feedback on its own promoter to prevent over-alkalization [68].

The histidine kinase FlgS is cytoplasmic and therefore senses cytoplasmic pH. FlgS is required for *H. pylori* viability at medium pH 2.5 in the presence of urea, independent of the response regulator FlgR [69]. In addition to pH-dependent genes, FlgRS also controls intermediate flagellar genes [69, 70]. FlgS, like ArsS, is involved with trafficking of urease and its cytoplasmic accessory

proteins to the plasma membrane [60]. Since it is cytoplasmic, FlgS is positioned to respond to lower media pH and the resultant lower cytoplasmic pH [7, 60, 69]. Urease activity over time at acidic pH in a *ΔarsS* deletion mutant, initially decreased, is able to recover by 90 min, presumably because the cytoplasmic pH drops far enough by this point to activate FlgS [63]. The absence of *flgS* leads to an inability of the bacteria to buffer periplasmic pH in acid and to the loss of membrane potential at extreme acidity in the presence of urea, both requirements for gastric colonization [60].

2.6 pH Alteration and Treatment Efficacy

H. pylori is uniquely adapted to survive in the acidic gastric environment, but, as a neutralophile, the bacteria will divide and grow at neutral pH. There is an increase in transcription of growth-dependent genes at higher medium pH [71]. Many of the antibiotics used to treat *H. pylori* infection are dependent on bacterial growth for maximal efficacy. The action of ampicillin against *H. pylori* in vitro is significantly greater at near-neutral pH [71]. Addition of bismuth to treatment regimens also has, at least in part, a pH-based effect, as the compound impedes proton entry, leading to attenuation of the drop in cytoplasmic pH with medium acidification, allowing for increased bacterial metabolism and increased antibiotic efficacy [72]. With this in mind, the more bacteria that are dividing at the time of treatment, the more effective the treatment will be using standard, proton pump inhibitor, and antibiotic-based triple or quadruple therapy regimens. This concept is likely homologous to the concept of persisters seen across bacterial species. Persisters are members of a bacterial population that survive exposure to bactericidal antibiotics yet, when re-cultured, display the same antibiotic sensitivity as the population as a whole [73–75]. *H. pylori* that are not dividing at the time of antibiotic administration will not be killed by the antibiotics, leaving a small population of viable bacteria that can restore colonization of the stomach once the antibiotics are stopped. Medications currently available for acid blockade, at recommended doses, will not achieve the sustained pH change required to mimic the bactericidal effect seen in in vitro studies [71, 76]. The existing problems with treatment efficacy could be overcome through the development of non-antibiotic treatment regimens that take advantage of the colonization mechanisms described here, via prevention of or interference with motility, adhesion, or acid acclimation. The in vitro efficacy of the carbonic anhydrase inhibitor acetazolamide against *H. pylori* is one example of a potential treatment targeting acid acclimation and periplasmic pH regulation [34]. Continued study and understanding of the molecular mechanisms of gastric colonization of *H. pylori* are critical for the development of new and improved treatment regimens.

Conclusions

The pathogenic bacterium *H. pylori* has evolved a series of mechanisms to ensure persistent infection of the gastric environment. Among these are the ability to sense and transit to its preferred site of colonization at cell-cell junctions of the antral mucosa. The bacterium recognizes and binds to host adhesin receptors to prevent shedding into the stomach lumen. To combat gastric acidity, *H. pylori* is uniquely able to maintain periplasmic pH near neutrality through the urease system to maintain the bioenergetic profile of a neutralophile. Knowledge of the mechanisms that allow gastric colonization is critical for the development of novel treatment regimens.

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Immunological Reactions on *H. pylori* Infection

3

Nayoung Kim

Abstract

An immune network that responds to bacteria as a mucous membrane defense factor is called gut-associated lymphoid tissue (GALT), which is made up of distinctive tissues of B cell, T cell, and phagocytes. The purpose of GALT is to maintain stomach homeostasis via interaction between immune and nonimmune mechanisms. Actually stomach immune mechanism is mostly about *Helicobacter pylori* (*H. pylori*). Long-term *H. pylori* proliferation indicates failure of gastric immune mechanisms that causes chronic *H. pylori* inflammation, and various diseases emerge as a result. *H. pylori* delicately regulate innate and adaptive immunities to evade from host immunity. Especially, *H. pylori* disrupt T-cell activity such as Th1/Th2 immunological reaction balance. Th1 produces cytokines like interferon-γ and interleukin (IL)-2, and Th2 produces cytokines like IL-4, IL-5, IL-10, and IL-13 and engages with differentiation and activation of B cell. Finally it causes gastritis, peptic ulcer, and mucosa-associated lymphoid tissue (MALT) lymphoma and increases risk of gastric cancer.

Keywords

Immunity • Gut-associated lymphoid tissue • T helper cell • *Helicobacter pylori*

3.1 Introduction

The human stomach is constantly exposed to external antigens from food, gastric acid, and pepsin, so the stomach performs distinct and complex immune and non-immunological reaction mechanisms. Non-immunological reaction factors are mucus, epithelial cell phospholipid defense membrane, mucous membrane microcirculation, and nerve, immunity, and inflammatory

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mediators of mucous membrane recovery mechanism. These factors are directly related to ulcer or erosion recovery. An immune network that responds to bacteria as mucous membrane defense factor is called gut-associated lymphoid tissue (GALT), which is made up of distinctive tissues of B cell, T cell, and phagocytes. GALT utilizes a special epithelium called follicle-associated epithelia (FAE) to gather antigens from lumen and manages the functions of immune cells and nonimmune mucosal barrier compositions in a balanced condition. In other words, immune and nonimmune mechanisms have a close interaction to maintain stomach homeostasis.

While immune mechanisms in small and large intestines are well known due to their coexistence with bacteria, gastric immune mechanism is mostly about *Helicobacter pylori* (*H. pylori*), since gastric acid makes a difficult condition for a bacterial invasion, whereas *H. pylori* can survive. A disease occurrence due to long-term *H. pylori* proliferation indicates the failure of gastric immune mechanisms, and a chronic *H. pylori* inflammation means the failure of appropriate activity by host immunity. The results of these clinical features due to chronic *H. pylori* infection are asymptomatic gastritis, mucosa-associated lymphoid tissue (MALT), gastric cancer, and so on. These results are considered as the aftermaths of interactions between mucous membrane damage and defense mechanisms by immunological reactions on stomach epithelium, along with *H. pylori* toxin factors, host susceptibility, and other environmental factors. This chapter will discuss on general functioning of the gastric immune reaction and how immunological reactions for *H. pylori* invasion are different from normal immunological reactions especially in terms of immune evasion [1].

3.2 Microbiota and General Immune Mechanism in the Stomach

The human gut, populated by complex communities of microorganisms, plays central roles in the digestion and the absorption of nutrients [2], the stimulation of intestinal epithelial renewal [3], and immune responses [4]. Keeping these communities in balance with the host is crucial for health maintenance and disease prevention [5]. The human stomach as an ecological niche for bacteria received attention after the discovery of *H. pylori* in the 1980s. Before the discovery of *H. pylori*, human stomach environment was thought to be sterile due to pH values <4, peristalsis, and high bile concentration suppressing the microorganisms from the oral cavity. Recent development of molecular methods provided more detailed insights into the human microbiota in various organs including the skin, gut, and so on [6–9]. Analysis of the 16S ribosomal RNA (rRNA) gene contents of microbial samples after amplification by polymerase chain reaction (PCR) has revolutionized the characterization of microbial communities not only *H. pylori* but also other microbiota.

3.2.1 Microbiota in the Stomach and Their Possible Role

Although the stomach, along with the esophagus and the duodenum, is the least colonized region of the gastrointestinal (GI) tract, in contrast to the high bacterial counts (10^{10}–10^{12} CFU/g) in the colon, the stomach also supports a bacterial community with hundreds of phylotypes [10–12]. While it has been postulated that the indigenous stomach microbiota might be a reflection of transient bacteria from the mouth and esophagus, microbiota were distinguishable from microbiota found in the mouth, nose, and distal GI tract [13]. The most abundant phyla were *Proteobacteria*, *Firmicutes*, and *Actinobacteria* in *H. pylori*-positive subjects and were *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* in the absence of *H. pylori* [13]. In contrast, five major phyla were present in majority of the samples in Kim's study: *Proteobacteria,* 65.0%; *Firmicutes*, 20.0%; *Actinobacteria*, 7.8%; *Bacteroidetes*, 4.0%; and *Fusobacteria*, 0.8% [14]. In addition, *Proteobacteria,* the phylum to which *H. pylori* belongs, was the major phylum in the gastric mucosa of both *H. pylori*-positive and *H. pylori*-negative subjects: *H. pylori*-negative control,

60.3%; *H. pylori*-negative gastric cancer, 50.9%; *H. pylori*-positive control, 82.4%; and *H. pylori*-positive gastric cancer, 73.0% [14]. The mean proportion of *Proteobacteria* was lower in the gastric cancer group than that in the control group, and the mean proportion of *Actinobacteria* was higher in the *H. pylori*-negative gastric cancer group, but they did not reach statistical significance [14].

In the GI tract, the microbiota has a major impact on the functioning of the mucosal immune system and vice versa. That is, germ-free mice have small size of Peyer's patches (PPs), decreased number of lamina propria immunoglobulin (Ig) A-secreting plasmocytes, and low levels of serum Ig and demonstrate no Th17/Th1 in the intestine [15, 16]. The composition of the intestinal flora modulates the functioning of the immune system, for instance, the presence of *segmented filamentous bacteria* (*SFB*) in the microbiota is associated with the development of Th17 in the intestinal lamina propria [17]. The presence of some *Clostridia* strains within the human intestinal microbiota has been recently associated with the development of intestinal T regulatory (Treg) cell [18]. In addition, some commensal bacteria and microbiota-derived metabolites like short-chain fatty acids have been shown to inhibit inflammatory reactions at intestinal levels and promote pathogen clearance [19–21]. Inversely, defects in antibody response lead to a modification of the bacterial composition of the intestinal flora [22]. Although there was no report in detail, these data in the GI tract suggest that the colonization of the stomach mucosa by *H. pylori* and/or the associated microbiota might also impact the functioning of the immune system of the host and vice versa [16].

3.2.2 General Immune Mechanism of Stomach

The immunity of GI tract consists of innate and adaptive immunity. The innate immunity does not require acquired immunostimulation, and it is responsible for bacterial invasion as a first-line defense mechanism. A typical parameter of innate immunity is Toll-like receptor (TLR), which is a cluster of one of embryologically preserved eukaryotic cell receptors that are made of transmembrane proteins. This receptor engages with innate immunity by perceiving pathogenicity or molecular behavior of bacteria, and the receptor is related to the plasma membrane, ribosomal vesicle, or endosomal vesicle of stomach epithelium [23]. Eleven subtypes of TLR can be found among mammals, and the signal transduction via TLR ultimately activates pro-inflammatory genes via nuclear factor (NF)-κB activation [24]. Also, another important factor of innate immunity is nucleotide-binding oligomerization domain protein I (NodI) that exists in the cytoplasm. NodI detects peptidoglycans that are detached from cells and then induces epithelial cells to eliminate bacteria by themselves [23]. Adaptive immunity indicates a reaction on a previously known immunological stimulation that is specialized on a specific pathogen and depends on an immunological memory. However, adaptive immunity shares similar characteristics with innate immunity, since lymphocytes are activated and recruited by the stimulation of macrophages and dendritic cells (DCs), and derives specific reactions of T helper cells (Th cells). As *H. pylori* colonizes the mucosal surface of the stomach, mucosal defenses are very important which are physical, chemical, and immune-mediated [16]. The mucosal epithelium blocks invasion by pathogenic and commensal bacteria by forming multiple layers of physical (tight) junctions, chemical nitric oxide, and immune protection (local secretion of defensins, anti- and/or pro-inflammatory chemokines/cytokines and IgA/IgG/IgM transport) [16]. In addition, numerous bone marrow-derived cells belonging to the innate or adaptive immune systems colonized the intestinal mucosa to fight the invaders, but at steady state the same cells have to tolerate commensals [16]. Here, the epithelial defense mechanism of GI tract will be described experientially.

3.2.2.1 IgA and IgG Response of Stomach

A major defensive mechanism that excludes commensals and pathogens from the mucosal surface involves IgA [25]. Mucosal IgA

comprises antibodies that recognize antigens with high- and low-affinity binding modes. In general, high-affinity IgA neutralizes microbial toxins and invasive pathogens, whereas low-affinity IgA confines commensals in the intestinal lumen. High-affinity IgA is thought to emerge in PPs and mesenteric lymph nodes (MLNs) from follicular B cells stimulated via T cell-dependent pathways, whereas low-affinity IgA likely emerges in PPs, MLNs, and lamina propria from B cells stimulated via T cell-independent pathways [25]. IgA response is powerfully induced by the presence of commensal microbes in the intestine [26, 27] and has been shown to promote the maintenance of appropriate bacterial communities in specific intestinal segments [22]. In contrast to the lungs, vagina, and most of the GI tract, the healthy mammalian stomach produces very low levels of polymeric immunoglobulin receptor (pIgR) [28, 29], the receptor mediating IgA transport into the GI lumen. Studies in *H. pylori*-infected humans have shown that baseline pIgR expression by the gastric epithelium can be upregulated in response to gastric inflammation [30] due to increased local interferon (IFN)-γ production [31]. However, despite significantly increased pIgR expression and IgA plasma cell infiltration in response to *H. pylori* infection [32], there is no concomitant increase in IgA secretion into the stomach, and it is nonsecretory monomeric IgA which predominates in the stomach of *H. pylori*-infected individuals [33]. Hence, the IgA that is present in the gastric lumen would be unstable, susceptible to degradation by proteases. These observations suggest that, the stomach anti-*H. pylori* IgA responses do not play similar biological roles as compared with anti-commensal or anti-pathogen IgA response taking place in the intestine. In contrast to gastric IgA response, systemic IgG response to gastric commensal is rather weak. That is, in unmanipulated specific pathogen-free animals, it has been showed that there was no specific serum IgG response detectable directed against commensal bacteria [34]. In pathogen-free mice, the systemic immune system appeared to remain ignorant of the commensal microbes. However, in humans, a certain degree of systemic exposure to gut commensal bacteria and the associated priming of systemic immune response seem to be well tolerated, harmless, and common in healthy humans since systemic antibody responses against live gut commensal bacteria and fungi can be detected [35]. Most of the *H. pylori*-infected individuals develop systemic anti-*H. pylori* IgG responses [36]. However, in the case of children, these anti-*H. pylori* IgG responses could be weak that serum antibody test is not recommended for the test of *H. pylori* infection.

3.2.2.2 CD4⁺ T-Cell Responses

Since *H. pylori* is an extracellular bacteria, anti-*H. pylori*-specific CD8⁺ T-cell responses are inadequate to protect the host against such pathogen, but priming of CD4⁺ T-cell response is important [16]. CD4⁺ T-cell responses are initiated within the PPs and MLNs. DCs capture, process, and present antigens to naive T cells in PPs and MLNs [16]. In the stomach, DCs are penetrating the mucosa [37] to sample luminal antigens and migrate to the stomach lymph node [38]. At steady state, mucosal CD4⁺ T cells are tolerant to microbiota-derived antigens [39]. Remarkably, systemic CD4⁺ T cells are not tolerant to microbiota-derived antigens and conserved a naive state to these antigens [40]. Interestingly, antigen-specific intestinal IgA played a critical role in inhibiting the systemic CD4⁺ T-cell responses to commensal antigens by providing immune exclusion [39]. At mucosal surfaces, DCs maintain homeostasis by dampening inflammatory Th1 and Th17 cell responses [41] because they receive conditioning signals from intestinal epithelial cells (IECs) [42, 43]. One of these signals is provided by thymic stromal lymphopoietin (TSLP), which shifts the Th1/Th2 balance toward Th2 polarization by attenuating DC production of interleukin (IL)-12 but not of IL-10 [44]. In addition to TSLP, IECs release transforming growth factor (TGF)-β and retinoic acid, which stimulate the development of CD103⁺ DCs [41]. These DCs promote the formation of Treg cells via TGF-β and retinoic acid and suppress the development of inflammatory Th1 and Th17 cells [41]. In addition to initiating responses that create an overall tolerant state toward harmless intestinal antigens,

mucosal DCs are also implicated in the generation of protective immune responses aimed at the clearance of enteric pathogens [16]. A fundamental difference between the steady state and a state of infection may lie in the greater propensity of pathogens to invade and penetrate beneath the epithelial cell layer [16]. Invasion of IECs would allow for the activation of cytosolic pattern recognition receptors, TLRs, and both quantitative and qualitative changes in the secretion of pro-inflammatory cytokines and chemokines. Consistent with this, IECs produce CXC chemokine ligand 8 (CXCL8) when infected with strains of *Salmonella* spp. that are both invasive and flagellated [45]. CXCL8 may serve to attract neutrophils to the site of infection, furthering the inflammatory milieu [16]. As a result, the rate of blood-borne DC precursors migrating into the tissues and becoming DCs will increase. These cells will not have been subjected to IEC conditioning and can be directly activated by a combination of pathogens that have reached the epithelial cell barrier and the pro-inflammatory cytokine milieu [16]. In case of *H. pylori* infection, the initial tolerogenic response is progressively lost, showing that with time the mucosal immune system identified *H. pylori* as a pathogen [46, 47].

3.3 Immune Response to *H. pylori*

H. pylori successfully establishes a chronic infection by achieving a delicate balance between inducing immune responses and surviving in the inflammatory milieu by using an array of important virulence factors. In this part, the mechanisms by which *H. pylori* evades immune-mediated clearance will be discussed in detail which has been most adopted from Lina et al.'s review article [1]. In the next innate and adaptive immune responses to *H. pylori* will be discussed.

3.3.1 Immune Evasion

As discoveries about innate and adaptive immunities are continued, it can be inferred that innate immunity and adaptive immunity via antigen-specific route are activated, once various TLRs are stimulated by microorganism by-products as host-resistance mechanism to eliminate bacterial infection. The immunological reaction that *H. pylori* induces on human stomach mucous membrane includes both innate and adaptive immunities that normal bacteria also perform [48] (Fig. 3.1). However, *H. pylori* can survive on stomach epithelial cell for a long term, unlike other bacteria, because it has highly regulated techniques to avoid immunological reactions of its host. Host immunity evasion is the most important factor for the chronic survival of *H. pylori* in human stomach mucous membrane; *H. pylori* regulates innate and adaptive immunities for long-term survival [49] (Fig. 3.2). To maintain infection constantly, *H. pylori* performs six mechanisms to overcome innate immunity. It neutralizes gastric acid (Fig. 3.2a) and has flagella and lipopolysaccharide (LPS) that do not stimulate TLR, which is important for innate immunity [49] (Fig. 3.2b). TLR4 detects gram-negative LPS, such as *Escherichia coli* (*E. coli*), but it is immunologically anergic on the LPS of *H. pylori*. Also, flagellin of mucous bacteria, such as *Salmonella* or *E. coli*, activates TLR5, while *H. pylori* flagellin is not secreted and does not induce inflammation [49]. In terms of adaptive immunity transformation by *H. pylori* toxins, cytotoxin-associated gene A (CagA), which is a product of the *cag* pathogenicity island (PAI), restricts B-cell proliferation (Fig. 3.2c), and vacuolating cytotoxin A (VacA) limits T-cell proliferation (Fig. 3.2d), as host immunity mechanism alterations [49]. Moreover, *H. pylori* survives inside the cell by changing the host immunity mechanism (Fig. 3.2e) and performing genetic rearrangements (Fig. 3.2f) for its constant colonization [49]. In addition, *H. pylori* performs several mechanisms to restrict lymphocyte activity [24] (Fig. 3.3). VacA limits NFAT (nuclear factor of activated T cells) activity to decrease IL-2 production and to restrict T-cell proliferation [24] (Fig. 3.3). An arginase of *H. pylori* hinders T-cell receptor signal transduction, and low-molecular-weight protein, which is still unclear

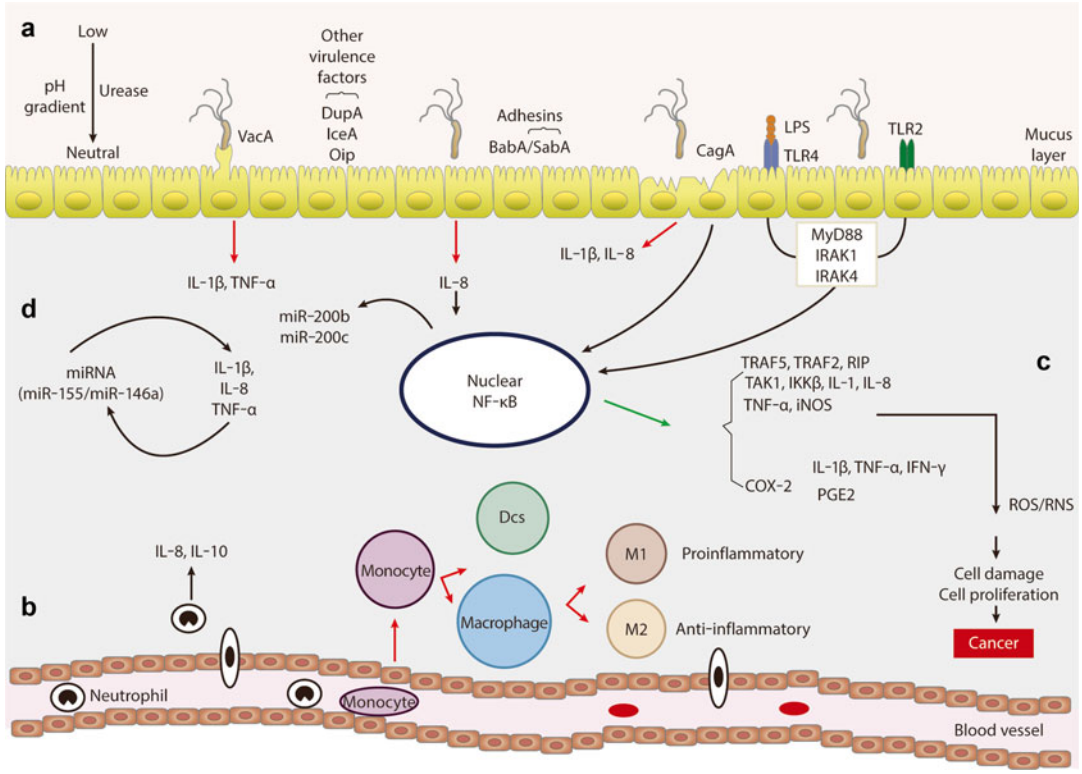

Fig. 3.1 Pathogenesis of *Helicobacter pylori* infection and host immune response. (**a**) Bacterial urease neutralizes the gastric pH, enabling the colonization of gastric epithelial cells by the bacteria and their motility in the mucus layer. Adhesion of the bacteria to the gastric epithelium is mediated by BabA and SabA adhesins, allowing the release of factors CagA and VacA into the host cells, which causes a strong systemic immune response and inflammation of the gastric mucosa. *H. pylori* LPS is recognized by Toll-like receptors, mainly TLR4 and TLR2, in cooperation with the adapter molecule MyD88 associated with IRAK1 and IRAK4 that leads to activation of transcription factor NF-κB, activating inflammatory signaling pathways. (**b**) The immune response is also activated, with the recruitment of inflammatory cells at the infection site, inducing the production of various pro- and anti-inflammatory mediators. (**c**) After NF-κB activation, rapid expression of multiple pro-inflammatory cytokines, chemokines such as the tumor necrosis factor (TNF)-alpha and interleukins, and consequently activation of oncogenic pathways may culminate in cancer. (**d**) The expression of some miRNAs is changed by *H. pylori* infection and the host immune response is regulated accordingly. *LPS* lipopolysaccharides, *IL* interleukin, *COX-2* cyclooxygenase, *RNS* reactive nitrogen species, *ROS* reactive oxygen species, *IFN* interferon (Adapted from Cadamuro et al. [48])

of its function, restricts T-cell cycle progression to limit T-cell proliferation [24] (Fig. 3.3). These T-cell restrictions lead to the constant *H. pylori* colonization, and MALT lymphoma can occur on some of *H. pylori*-infected patients due to T cell-dependent B-cell proliferation. One of the recent concepts on the evasion mechanism introduces bacterial homologous recombination that responds to host cell oxidative stress due to bacterial invasion. Theoretically, *H. pylori* is expert on genetic transformation, so the bacteria use homologous recombination to cause antigenic variation for structural transformation of outer membrane protein, such as BabA or BabB, so it evades host cell immunity and induces chronic gastritis [50]. In this review, we discuss how *H. pylori* avoids innate and acquired immune response elements, uses gastric epithelial cells as mediators to manipulate host T-cell responses, and uses virulence factors to avoid adaptive immune responses by T cells to establish a persistent infection as well as the genetic diversity of this pathogen for its survival [1].

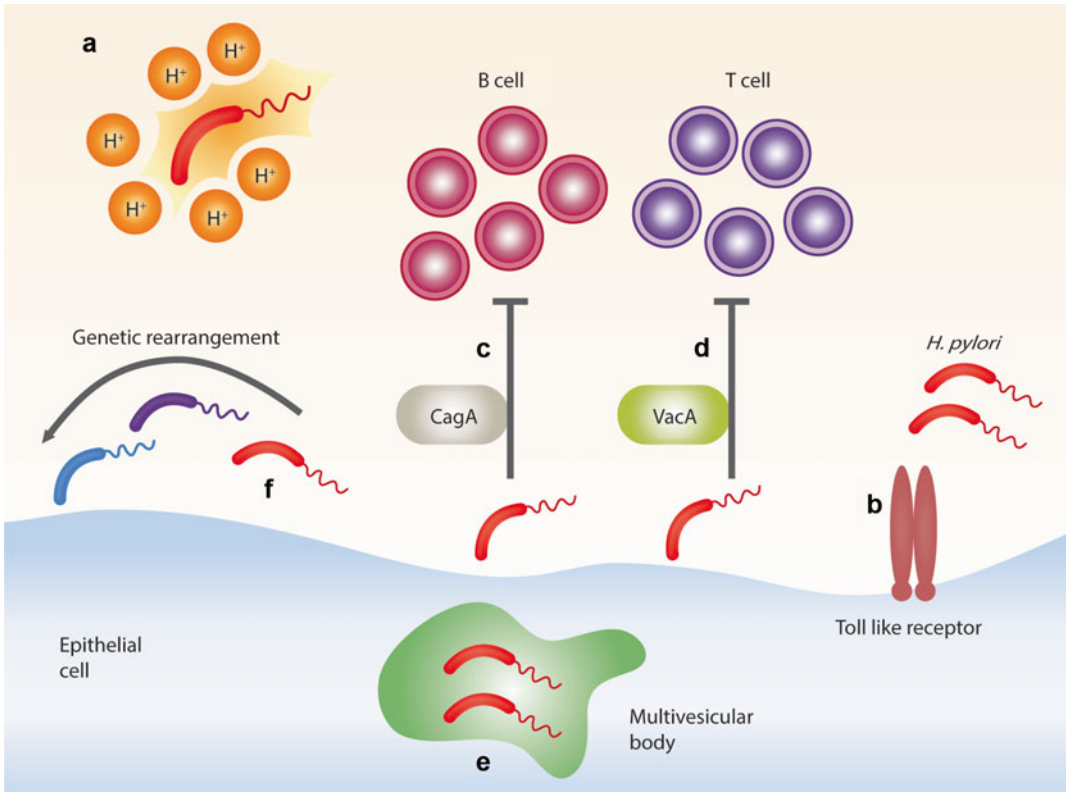

Fig. 3.2 Six mechanisms that *H. pylori* performs to maintain infection. *H. pylori* neutralizes gastric acid to overcome natural immunity (**a**). The bacteria have flagella and lipopolysaccharide that do not stimulate Toll-like receptors (**b**). For mutating adaptive immunity, CagA (a product of the cytotoxin-associated gene pathogenicity island (*cag* PAI)) is used to limit B-lymphocyte proliferation (**c**). Also, VacA (vacuolating cytotoxin A) toxin is utilized to limit T-lymphocyte proliferation in terms of adaptive immunity mutation (**d**). In addition, *H. pylori* survives inside cells by altering host immunity mechanism for sustained colonization (**e**), and the bacteria performs genetic rearrangement (**f**) (Adapted from Merrell and Falkow [49])

3.3.1.1 Inhibition of Innate Immune Recognition by *H. pylori*

Inhibition of innate immune recognition by *H. pylori* includes three aspects: (1) there is avoidance of detection by pattern recognition receptors (PRRs); (2) macrophages can engulf *H. pylori,* but the bacterium has developed mechanisms to avoid killing upon phagocytosis [51–53]; and (3) *H. pylori* produces catalase and superoxide dismutase to detoxify ROS [54, 55]. *H. pylori* can also downregulate CXCR1 and CXCR2 expression in human neutrophils, which act as receptors for the neutrophil-recruiting chemokine, IL-8, thus resulting in an inhibitory effect on neutrophil migration and reduced bacterial killing [56].

Evasion of Recognition by Pattern Recognition Receptors

H. pylori evades the innate immune system by a variety of mechanisms. One of these mechanisms is avoidance of detection by PRRs, which are proteins that recognize pathogen-associated molecular patterns (PAMPs) [48]. PAMPs include a large group of molecules that are part of microbes and can vary from microbial surface molecules to nucleic acids. When PRRs recognize PAMPs they induce several extracellular activation cascades such as the complement pathways and various intracellular signaling pathways, leading to inflammatory responses that are essential for clearance of pathogens [57]. *H. pylori* eludes identification by PRRs by multiple

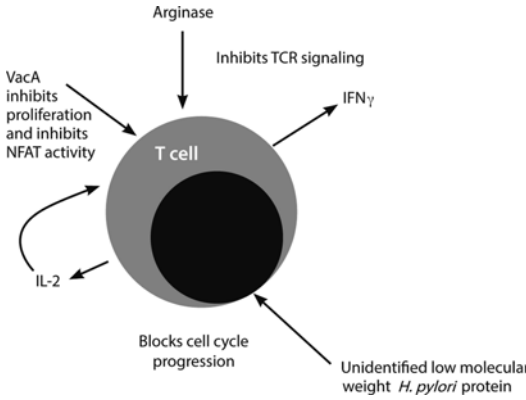

Fig. 3.3 The mechanism of hindering T-lymphocyte activity by characteristics of *H. pylori*. VacA (vacuolating cytotoxin A) limits NFAT (nuclear factor of activated T cells) activity to decrease IL-2 production and to limit T-cell proliferation. Arginase restricts T-cell receptor (*TCR*) signal transduction. Low-molecular-weight protein, which is still unclear of its characteristics, limits T-cell cycle to restrict T-cell proliferation. *IFN* interferon, *IL* interleukin (Adapted from Algood and Cover [24])

methods, including avoidance of recognition by TLRs and inhibition of c-type lectin (DC-SIGN)-mediated signaling [1]. To avoid recognition by TLRs, the bacterium modulates its surface molecules (including LPS and flagellin). LPS is a glycolipid found on the outer membrane of gram-negative bacteria [58]. It has three distinct units: lipid A, which is responsible for the toxic effects; a core polysaccharide of five sugars linked through ketodeoxyoctulonate to lipid A; and the O-antigen, an outer polysaccharide consisting of up to 25 repeating units of three to five sugars [59]. *H. pylori* expresses O-antigens with great diversity; the bacterium has Lewis antigens, which are made of carbohydrates that resemble human blood group antigens [60]. By exploiting this form of molecular mimicry, the bacterium is able to evade TLRs because the normally detectable O-antigen is recognized as a "self" molecule by this type of PRR. In addition, the bacterium also modifies the lipid A portion of the LPS molecule [1]. Reduced immunogenicity of *H. pylori* LPS could be due to uncommon phosphorylation and acylation of *H. pylori* lipid A [61]. *H. pylori* LPS binds poorly and at a slower rate to LPS-binding proteins, which are acute phase reactants that aid in LPS binding to CD14 and TLR4 on monocytes/macrophages. This reduced binding of LPS to its receptors results in decreased activation of monocyte-macrophages, preventing their contribution to innate immune response. Interestingly, *H. pylori* LPS has also been shown to possess anti-phagocytic properties in vitro [62]. Flagellin is the protein component of bacterial flagella needed for motility and colonization [63]. *H. pylori* relies on five or six polar flagella made of two separate subunits, FlaA and FlaB, to enable movement within the gastric mucus and to counteract peristalsis [64]. TLR5 is a PRR that recognizes flagellin. However, studies showed that *H. pylori* flagellin was not recognized by TLR5 and thus failed to induce NF-κB activation [65]. The study also reported that an eight amino acid stretch in the N-terminal D1 domain of flagellin differed from that of flagellin from bacteria that activated TLR5 [1]. Most flagellated bacteria are able to induce a pro-inflammatory state by promoting production of IL-8, but *H. pylori* flagellin seems unable to induce IL-8 production in gastric epithelial cells (GECs) [64].

Inhibition of Phagocytic Killing

H. pylori infection activates an inflammatory response in its host which leads to the recruitment of macrophages, neutrophils, and lymphocytes to the gastric tissue [66]. *H. pylori* can efficiently inhibit its own uptake by these professional phagocytes [1]. This anti-phagocytic phenotype depends on type IV secretion components encoded by the *cag* PAI [67, 68]. Even after macrophages engulf *H. pylori,* the bacterium has developed mechanisms to avoid killing upon phagocytosis [51–53]. Once inside the macrophage, *H. pylori* actively delayed actin polymerization and phagosome formation. *H. pylori*-containing phagosomes then underwent extensive clustering and fusion resulting in the formation of "megasomes" containing multiple bacteria, which caused resistance to intracellular killing [51, 69]. *H. pylori* type I strains were shown to reside in compartments with early endosome properties and did not fuse with lysosomes. VacA alone plays a significant role in the interruption of the phagosome maturation [53]. In fact, a study showed that, by interfering with endosomal traffic, VacA altered the presentation of

antigens by B cells [70]. A related recent study provided evidence that the effects of VacA on endosomal traffic may prevent the development of a strong Th1 response. The study showed that *H. pylori* VacA could redirect the endocytic pathway of the probiotic bacterium *Lactobacillus acidophilus*, which induces a polarized Th1 response, and does this by blocking the induction of key innate cytokines such as IFN-β and IL-12 [71]. Like other pathogenic bacteria, *H. pylori* also regulate host trafficking pathways by the selective modification of GTPases in macrophages during infection [1]. *H. pylori* has been shown to disrupt the actin cytoskeleton by suppressing *Rgs1/2*, *Fgd2*, and *Dock8* which are the key regulators of the Rho, Rac, and Cdc42 GTPases, respectively [71]. These are required for the organization and dynamics of actin cytoskeleton needed for proper cell function. This is another mechanism that disrupts phagocyte function and helps *H. pylori* survival in its host [71].

Inhibition of Killing by Reactive Oxygen Species and Nitric Oxide

A major pro-inflammatory factor produced by *H. pylori* is neutrophil-activating protein (NAP) [72]. *H. pylori* NAP (HP-NAP) is a 150 kDa oligomeric protein, which increases adhesion of polymorphonuclear cells (PMNs) to endothelial cells, stimulates phagocyte chemotaxis, and activates NADPH oxidase to produce reactive oxygen species (ROS) [73, 74]. However, *H. pylori* produces catalase and superoxide dismutase to detoxify ROS [54, 55]. *H. pylori* can also downregulate CXCR1 and CXCR2 expression in human neutrophils, which act as receptors for the neutrophil-recruiting chemokine, IL-8, thus resulting in an inhibitory effect on neutrophil migration and reduced bacterial killing [56]. *H. pylori* also disrupts NADPH oxidase targeting, which was shown to result in the release of superoxide anions in the cytoplasmic membrane instead of the accumulation inside *H. pylori* phagosomes [75]. One antimicrobial host defense mechanism is the generation of nitric oxide (NO) through inducible NO synthase (iNOS). *H. pylori* activates iNOS in macrophages [76]. A mechanism employed by *H. pylori* to activate iNOS involves urease, an important virulence factor of *H. pylori*. Despite the presence of iNOS, *H. pylori* infection persists, which suggests that iNOS production may be at suboptimal level. *H. pylori* arginase was shown to be an important factor that affords protection of the bacteria against NO-mediated killing since macrophages infected with *H. pylori* produce significantly less NO than arginase isogenic mutants [77]. A recent study showed that induction of macrophage arginase II (Arg2) restricts iNOS protein expression, elicits apoptosis of macrophages as well as pro-inflammatory cytokine production, and limits bacterial killing [78], suggesting another mechanism this bacteria uses to escape macrophage-mediated killing.

3.3.1.2 Modulation of Adaptive Immunity by *H. pylori*

H. pylori have evolved an array of mechanisms to actively dodge adaptive immunity by interfering with antigen presentation and modulation of T-cell responses. Antigen presenting cells (APCs), represented by macrophages, DCs, and B cells, internalize antigen by phagocytosis or endocytosis and process the antigens and present them to $CD4^+$ T cells via major histocompatibility complex (MHC) class II molecules. This leads to the initiation of antigen-specific T-cell response. The gastric mucosa of *H. pylori*-infected people has an increase in activated macrophages and DCs. Activated macrophages produce IL-6, IL-1β, IL-12, and tumor necrosis factor (TNF)-α which cause inflammation and help initiate Th1-type responses. In spite of the presence of these effector cells, *H. pylori* successfully establish a persistent infection, suggesting that these effector cells are unable to clear the pathogen. *H. pylori* has also been shown to cause the polarization of APCs. For instance, during atrophic gastritis macrophages are polarized to M1 subtype [79]. *H. pylori* can even control the functions of these APCs differently. A study showed that *H. pylori*-mediated activation of DCs and M1 macrophage leads to induction of T-cell proliferation and decreased phagocytosis. On the other hand, upon *H. pylori* infection, the M2 macrophages produced less

pro-inflammatory cytokines and increased anti-inflammatory cytokines compared to M1 macrophages [80].

3.3.1.3 Inhibition of Effective T-Cell Response

$CD4^+$ Th cells are major effector cells in the immune response to *H. pylori*. The response was initially characterized as a Th1-polarized response [46, 81], but more recently other $CD4^+$ T-cell subsets have been found in *H. pylori*-infected patients, and those include Treg and Th17 cells [82–85]. HP-NAP was shown to increase IL-12 and IL-23 production by neutrophils and monocytes, which promote Th1 responses. Addition of HP-NAP to antigen-induced T-cell lines caused a shift from a predominant Th2 to a Th1 phenotype of specific T cells. HP-NAP also elicited an antigen-specific Th1-polarized T-cell response in the gastric mucosa of *H. pylori*-infected patients [86]. Increased production of IFN-γ by Th1 cells was shown to cause chronic gastric inflammation [46, 87]. On the other hand, increased Treg cells produced during *H. pylori* infection suppress mucosal effector T-cell responses, which contribute to bacterial persistence, and are also a probable cause of gastric tumor progression [82]. Th17 cells, which produce IL-17A, appear to be crucial in the clearance of extracellular bacteria such as *H. pylori* [88]. IL-17 also acts on GEC to release IL-8, a chemokine that recruits neutrophils, and thus promote gastric inflammation. On the other hand, this IL-17-initiated recruitment of neutrophils is critical for the clearance of the bacteria [89]. A hallmark of *H. pylori* infection is that effector T-cell responses are generally impaired during *H. pylori* infection, and T cells from *H. pylori*-infected individuals are hyporesponsive [90]. As this is an important issue in vaccine design efforts, there has been a significant effort to address mechanisms that impair T-cell responsiveness. *H. pylori* virulence factors that have been reported to play a role in interfering with T-cell responses are VacA, γ-glutamyltranspeptidase (GGT), and arginase [91–96]. Recently *H. pylori* CagA also has been found to play an important role in modulating Th17 cell response indirectly by modulating expression of B7-H2 on GEC [97].

3.3.1.4 Evasion of Humoral Response

The majority of people infected with *H. pylori* develop a specific antibody response. This response is not normally enough to clear infection. Some studies suggest that infected children produce less antibodies, which may be concurrent with more Treg cells and less activated $CD4^+$ T cells to act as helper cells in the induction of B-cell responses [98]. Although most or all infected individuals are thought to mount an antibody response to *H. pylori*, differences in this response have been noted between those who develop gastritis or duodenal ulcers and those who develop gastric cancer [99]. By examining patient serum antibody levels, infected individuals who developed gastritis or duodenal ulcers were shown to have a greater IgG response than those who developed gastric cancers. In turn, gastric cancer patients mounted a more vigorous IgA response than those with gastritis and duodenal ulcers. In another study of serum antibody responses to *H. pylori* in Japan, the authors suggested that a weak antibody response was linked to a high risk of developing gastric cancer by infected individuals [100]. Another study suggested that development of antibodies specific to virulence factors of *H. pylori* may be linked to gastric cancer [101]. In this study, gastric cancer patients were more likely to develop antibodies to CagA and heat shock protein B, while no significant differences were found in the levels of VacA-specific antibodies between individuals with gastric cancer and other disease manifestations. These studies suggest that differences in humoral responses to infection may be linked to disease in infected individuals, but the mechanisms behind these differing responses remain elusive. Although most people respond to *H. pylori* with a high serum antibody titer, this response is not efficient in reducing bacterial burden as evidenced by studies in mice that lack B cells and by various vaccine studies. In a study of mice lacking B cells, mice were protected against *H. pylori* challenge suggesting that the humoral response is dispensable in protection against *H. pylori*. In addition to the viewpoint that protection against *H. pylori* challenge is independent of the B-cell response, there is also compelling evidence that antibodies

elicited against *H. pylori* may be harmful to the host. One group has shown in mice that specific antibody responses to *H. pylori* may actually aid in bacterial colonization and impair other immune responses against *H. pylori* [102]. This study showed that T cells, not B cells, were responsible for gastritis induced by infection and suggested the possible role for antibodies in inhibiting host resistance to infection in showing improved elimination of bacteria in the absence of antibodies in B cell-deficient mice. B cell-deficient mice were able to clear bacteria at 12–16 weeks postinfection, whereas wild-type mice still had a robust infection coupled with gastritis at this time point. Another compelling study showed that *H. pylori* evade antibody-mediated recognition because of a lack of surface binding of host elicited antibodies [103]. This study consisted of incubating bacteria with sera from patients who had detectable antibody responses to *H. pylori.* There was very little binding of antibodies to the surface of the bacteria, thus indicating another way the host immune response may be evaded. Another intriguing aspect of the humoral response to *H. pylori* are reports of autoantibodies that are induced during infection. These antibodies were against self-epitopes and potentially caused damage in the host. For instance, *H. pylori* induced antibodies against parietal cells in the stomach, which persisted after bacterial eradication and were linked to intestinal metaplasia [104]. In support of these results, another study examined autoantibodies in infected patient sera, revealing a prevalence of autoantibodies during gastritis associated with gland destruction and gastric atrophy [105]. Decreased acid secretion, but increased gastrin, was seen along with increased gastritis. This was shown in 20 % of duodenal ulcer patients coupled with a more severe disease manifestation. Likewise, detrimental effects of autoantibodies have been seen in gastric cancer as well. In a small panel of gastric cancer patients, spleen cells were isolated and immortalized with human hybridoma technologies, which allowed for characterization of 11 *H. pylori*-induced autoantibodies that reacted with gastric cancer cell-specific proteins [106]. Several of these antibodies stimulated gastric cancer cells to proliferate, interestingly enough, in contrast to normal epithelial cells.

3.3.1.5 Genetic Diversity in Immune Evasion

H. pylori is known as one of the most genetically diverse bacterial species since the strain of *H. pylori* 26695 was first sequenced [107]. When 26695 and J99 *H. pylori* strains were compared at the genome level, it was observed that 6 % of the genome represented strain-specific genes, which are mostly located in a region now referred to as the plasticity zone [108]. Since then, multiple other strains have supported the observation that such diversity occurs at the size of the genome, gene arrangement, and alleles [1]. This genetic diversity of *H. pylori* was found to be the result of high mutation rates and high recombination frequency [109]. The most well-known genetic diversity is found from two virulent factors, *cagA* and *vacA*. *cagA* is encoded in a 37 kb segment of DNA referred to as the *cag* PAI. An array of *H. pylori* isolates have been noted to differ in the rate with which they have the *cag* PAI in their genome [110], which was recently supported by a study that included 877 isolated from diverse populations and which highlighted the variability in the carriage of *cag* PAI by *H. pylori* strains. This *cag* PAI mostly encodes an array of structural constituents of a bacterial type IV secretion system (T4SS) in addition to a 128 kDa effector protein, CagA. When *H. pylori* adheres to GECs, CagA is translocated via the T4SS into the host cell cytoplasm where it becomes phosphorylated by host cell kinases and interacts with various signaling proteins [111]. As a result of the multiple interactions of CagA with host cell signaling proteins, multiple processes are affected leading to cell transformation [1]. This effector protein, CagA, also has a significant level of diversity, particularly in the C-terminal Glu-Pro-Ile-Tyr-Ala (EPIYA) repeat motifs where CagA is phosphorylated once it is inside the host cell. These EPIYA motifs differ between Asian and Western isolates. An interesting study of *H. pylori* isolates from experimentally infected mice and nonhuman primates showed that they have rearrangements in CagY of the T4SS [112], which in turn result in gain or loss of function in the *H. pylori* T4SS. These observations may be reflective of the overall variability in *H. pylori*

strains, which in turn contribute to immune escape and the establishment of chronic infection. Another famous toxin in *H. pylori* is VacA and it shows also frequent genetic polymorphisms. *vacA* encodes a preprotoxin of 139 kDa which includes an amino-terminal signal peptide and a 50 kDa carboxy-terminal domain that are both cleaved upon secretion to yield a mature toxin monomer of 87–95 kDa [113]. Sequence polymorphisms occur throughout *vacA*, but the two most diverse regions are the signal (s) region, encoding part of the signal peptide and the N-terminus of the mature protein (which may be type s1 or s2), and the mid (m) region, encoding part of the p58 domain (type m1 or m2) [113]. A third polymorphic determinant of vacuolating activity, the intermediate (i) region was found to be located between s-region and m-region within the p37 domain [113]. It has been suggested to be an important determinant of *H. pylori* toxicity and the best independent marker of VacA-associated pathogenicity [113]. As mentioned earlier in this chapter, VacA plays a significant role in the interruption of the phagosome maturation [53] and altered the presentation of antigens by B cells by interfering with endosomal traffic [70]. In addition, the effects of VacA on endosomal traffic may prevent the development of a strong Th1 response [1]. From this background, *vacA* genetic diversity could play a role in the immune evasion of *H. pylori* although there are not enough evidences so far.

3.3.2 Innate Immunity Activation Due to *H. pylori*

H. pylori enters the gastric mucosa and reaches the gastric epithelium surface that has a pH of 5 or 6. At this time, the human body activates innate immunity. Various factors like TLR and NodI derive innate immunity reactions to respond to LPS, flagellin, and peptidoglycan of bacteria [48] (Fig. 3.1), and antibacterial protein is secreted from gastric epithelial cells. In detail, peptidoglycan fragments that are delivered by *H. pylori cag* PAI reach the gastric epithelium and are detected by NodI inside the cytoplasm, and this detection acts as an important role when epithelial cells get rid of *H. pylori* directly [114]. Gastric epithelial cells produce various antibacterial proteins, such as α, β defensins and cathelicidin LL-37 [115, 116], that restrict bacterial growth and take important parts in host defense mechanism [117, 118]. At the same time, *H. pylori* derives inflammation-inducing gene expression of host cells via TLR; TLR4 detects LPS of bacteria and TLR2 detects peptidoglycan [119]. However, *H. pylori* flagellin is not detected by TLR5 of a host cell, unlike gram-negative *Salmonella enterica*, so the flagellin contributes to the immunity evasion of *H. pylori* [120].

As a result of innate immunity due to *H. pylori* invasion, gastric epithelial cells secrete cytokines that accelerate gastritis occurrence and various immune cell influxes, such as neutrophil [48] (Fig. 3.1). Neutrophil entrance can be observed at the beginning of bacterial influx on the stomach. However, the entrance can be also well observed among chronic *H. pylori* infection during adulthood because cytokines that are derived by *H. pylori*, such as IL-8 and growth-related oncogene (GRO)-α, activate neutrophils and regulate their movement [56]. VacA and HP-NAP of bacteria activate mast cells and increase inflammation [24]. HP-NAP works on TLR2 to derive IL-12 and IL-23 expression on neutrophils and monocytes to accelerate *H. pylori*-selective Th1 immunological reaction. Especially, IL-12 converts a naive Th cell into Th1 phase [121]. In addition, HP-NAP delays phagosome formation, which helps *H. pylori* from being eliminated by macrophages, so *H. pylori* proliferates inside epithelial and DCs [122]. Moreover, IL-10 that is produced from gastric epithelial cells has an ability to restrict Th1 immunological reaction, so it aids *H. pylori* to avoid the host immunity as well [24].

3.3.3 Adaptive Immunity Activation Due to *H. pylori*

H. pylori makes DC, T cell, and B cell interact to activate adaptive immunity. Both humoral and cellular immunological reactions are induced and

cause systemic or local immunological reaction to produce IgA, IgM, and IgG. Especially, serum IgM antibody usually appears during the first month of infection [36], serum IgA and IgG antibodies appear as they react with different antigenic determinants of *H. pylori*, and idiosyncratic secretive IgA antibody exists in gastric fluid as a local immunological reaction. Also, the immunity leads to inflammatory reactions by polymorphonuclear leukocyte and monocyte to secrete various cytokines, like IL-1β, TNF-α, IL-8, and IL-6. Remarkably, Th1 cell is activated once IL-12 is secreted from macrophage and DC, and then cytokines are produced, such as IFN-γ. A macrophage stimulated by *H. pylori* produces a pro-inflammatory substance called nitric oxide by iNOS and NOS2, but *H. pylori* also has *rocF* gene that neutralizes bactericidal action of NO. In other words, *rocF* gene encodes arginase to use arginine, so it relatively exhausts iNOS substrate (L-arg) of macrophage [77, 123] (Fig. 3.4). As a result, nitric oxide production by macrophages is relatively reduced, so *H. pylori* can protect itself from host immunity with pro-inflammatory substances.

T cell is the most important factor in immunological reaction on *H. pylori* infection [124]. It is well known that IFN-γ, IL-17, and TNF-α are produced when Th1 and Th7 cells are activated by *H. pylori* infection. However, a recent research has figured out that HP0175 is an important factor for Th17 reaction on mucous membrane [125]. HP0175 increases mRNA of IL-23 and IL-12 via TLR4; increases IL-6, IL-1β, and TGF-β production [126]; and accentuates polarized Th17 reaction by IL-17 and IL-21 production increment [127].

Meanwhile, the Th1/Th2 immunological reaction balance is important for etiologies of *H. pylori*-related diseases and the host defense mechanism. If Th1 immunological reaction is superior, tissue damage is also superior and *H. pylori* can be eradicated. However, Th2 immunological reaction induces a defense mechanism regarding stomach inflammation,

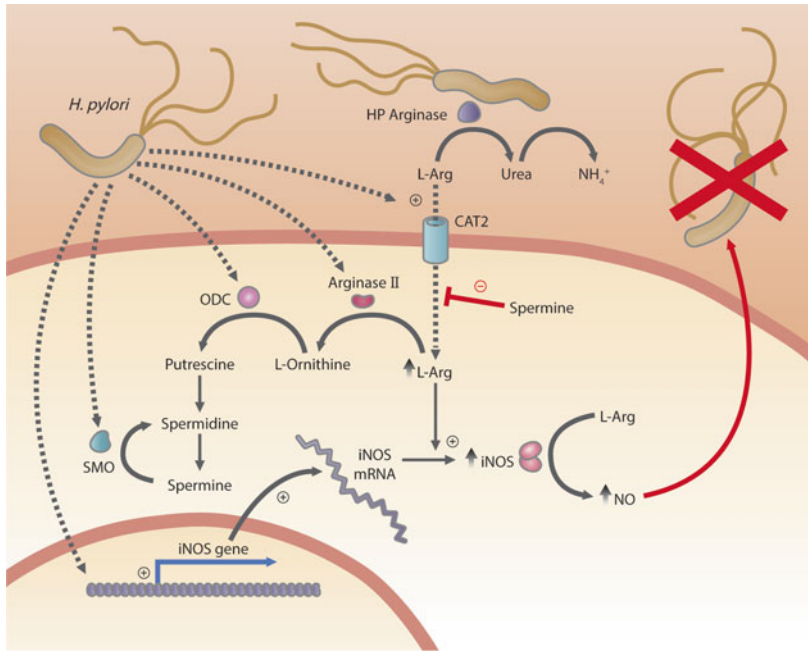

Fig. 3.4 Diagram that explains iNOS production and regulatory reaction on nitric oxide production by macrophage as a result of *H. pylori* stimulation. Once *H. pylori* stimulates a macrophage, arginase II is activated. However, *H. pylori rocF* gene encodes arginase to utilize arginine for the relative exhaustion of L-arg of iNOS on macrophage. Then, nitric oxide production by macrophage is relatively reduced. *CAT2* cationic amino acid transporter 2, *ODC* ornithine decarboxylase, *SMO* spermine oxidase (Adapted from Wilson and Crabtree [123])

less stomach epithelial cells are damaged, and *H. pylori* can survive, so it leads to chronic infection. Treg cell is important for adaptive immunity regulation [128]; the Treg cell not only regulates and protects pathological changes on stomach epithelium but also induces chronic infection [129].

Conclusions

H. pylori infection is typically acquired during childhood and usually becomes a lifelong infection, if left untreated [130]. Colonization by *H. pylori* induces various and complicated natural and adaptive immunity on gastric mucous membrane, creating a chronically inflamed environment with reduced gastric acidity that favors the growth of other bacteria in the gastric environment. However, this host immune response fails to clear the infection because the immune evasion manipulated by *H. pylori* is the most adroit. The reason of delayed *H. pylori* vaccine development would be the complexity of the immunological reactions on *H. pylori* infection and the skillful host immunity evasion mechanisms of the bacteria [131].

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Change of Acid Secretions, Ghrelin and Leptin, by *H. pylori*

4

Nayoung Kim and Yoon Jin Choi

Abstract

When *Helicobacter pylori* (*H. pylori*) begins to colonize on the surface of gastric epithelium, *H. pylori* induces strong inflammatory responses and causes a transitory hypochlorhydria. The main underlying mechanisms are, first, *H. pylori* represses the activity of promoter of the alpha-subunit (HKα) of H^+, K^+-ATPase, and second, cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α suppress gastric acid. Third, *H. pylori*-induced gastritis evokes decrease of parietal cell numbers. In the chronic *H. pylori* gastritis, the change of gastric acid is mainly determined by the pattern of corpus gastritis. That is, in the antral-predominant gastritis, acid secretion increases due to increase of gastrin secretion. In the situation of corpus-predominant gastritis, acid secretion is decreased similar to the acute gastritis. The acute response after *H. pylori* eradication includes the increase of gastric acid mainly due to the clearance of *H. pylori* itself and its secreted proteins, reversed activity of H^+, K^+-ATPase, and decrease of IL-1β and TNF-α. After this acute response phenomenon, the change of acid secretion depends on gastritis pattern. That is, in the situation of corpus-predominant gastritis, acid secretion increases slowly until 2 years depending on the severity of atrophic gastritis. In the

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antral-predominant gastritis, the increase of gastric acid secretion stops and it returns to normal as gastrin secretion decreases. In contrast to acid secretion, the effect of *H. pylori* and its eradication on ghrelin and leptin levels is under investigation.

Keywords
Helicobacter pylori • Acid secretion • Ghrelin • Leptin

4.1 Introduction

One of the important functions in gastric physiology is to regulate and sustain acid secretion to sterilize ingested nutrients in which system several hormones are involved. In regard to gastric acid secretion, gastrin and somatostatin have been well investigated. In the acute phase, *Helicobacter pylori* (*H. pylori*) is capable of inhibiting acid secretion directly as well as indirectly by activating intramural calcitonin gene-related peptide (CGRP) sensory neurons coupled to stimulation of somatostatin and inhibition of histamine secretion. Its infection of the human gastric mucosa inevitably alters the normal gastric physiology. Especially, activated cytokines deregulate secretion of gastric hormones including gastrin, somatostatin, ghrelin, and leptin. Chronic *H. pylori*-induced inflammation changes the gastric hormone and gastric acid depending on the pattern of gastritis such as antral- or corpus-dominant gastritis. *H. pylori* eradication causes the reverse process of gastric physiology which is determined by the severity of atrophy and pattern of gastritis. In contrast, ghrelin and leptin which are secreted from the stomach play a very important role in controlling appetite and satiety by an autocrine/paracrine manner in the central nervous system (CNS). They are also affected by *H. pylori*-induced inflammation. This chapter aims to review the data on gastric secretion and hormones which are produced in the stomach, focusing on the effect of *H. pylori* infection and its eradication.

4.2 Gastric Acid Secretion and H^+, K^+-ATPase with Regard to *H. pylori* Infection

There are three regulatory molecules (acetylcholine, histamine, and gastrin) that stimulate acid secretion and one regulatory molecule (somatostatin) that inhibits acid secretion [1] (Fig. 4.1). Acetylcholine is a neurotransmitter that is released by enteric neurons, while histamine is a paracrine that is released from enterochromaffin-like (ECL) cells. Gastrin is released from the G cells of the antral mucosa and travels through the bloodstream to the corpus where it stimulates ECL cells to secrete histamine which, in turn, stimulates the parietal cells to secrete acid (Fig. 4.1). In contrast, somatostatin, secretin, gastric inhibitory peptide (GIP), and vasoactive inhibitory peptide (VIP) inhibit acid secretion. Gastrin also has a direct effect on parietal cells, which stimulates parietal cells' proliferation. In oxyntic glands of the gastric body and fundus, somatostatin-releasing D cells are anatomically and functionally coupled to parietal and ECL cells [2] (Fig. 4.2). When the pH of the stomach gets too low, somatostatin secretion is stimulated, and it inhibits acid secretion by direct effects on parietal cells and also by inhibiting release of the positive regulators, histamine and gastrin. Inversely, endogenous gastrin release stops when the pH of the perfusate drops below 2.5. Gastrin release is suppressed primarily by direct contact of acid with the antrum [3]. Taken together, acid secretion and physiology of G and D cells are closely related to each other, and the effect of

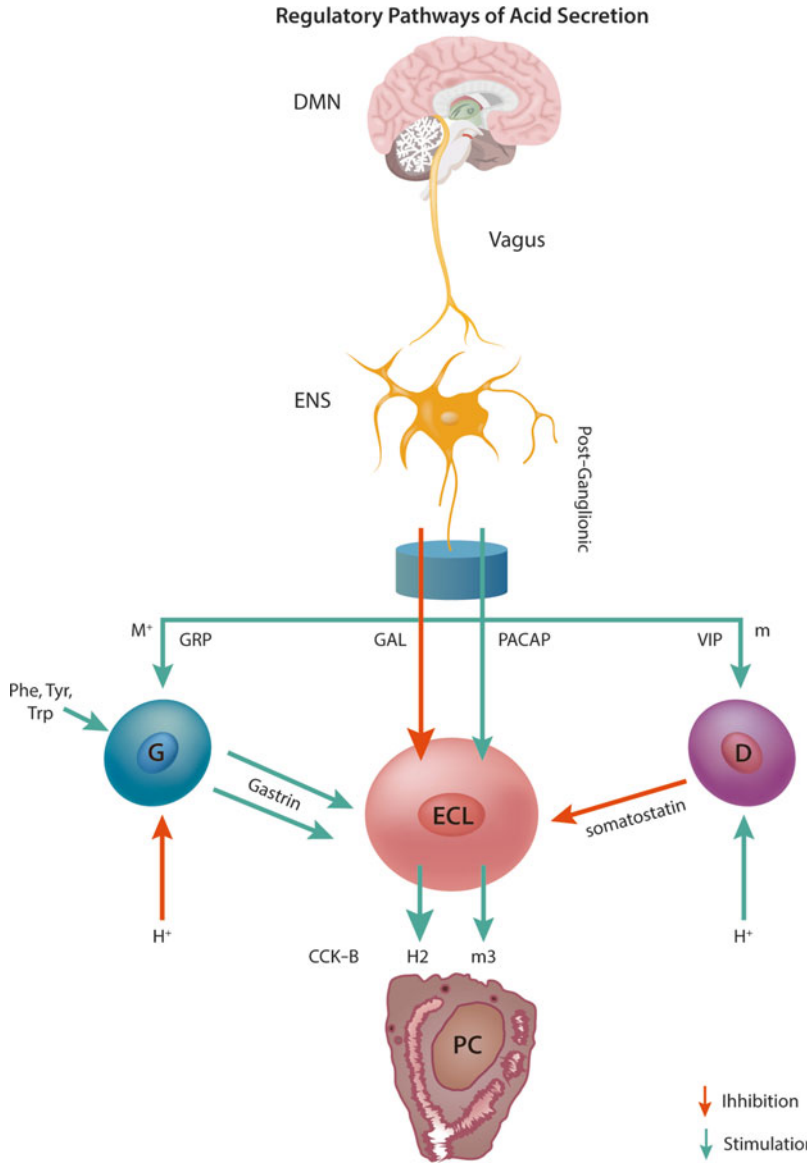

Fig. 4.1 Regulation of gastric acid secretion showing both activating and inhibitory pathways, neural, endocrine, and paracrine. *DMN* dorsal motor nuclei, *ENS* enteric nervous system, *GRP* gastrin-releasing protein, *GAL* gastrin ligand, *PACAP* pituitary adenylate cyclase-activating peptide, *VIP* vasoactive intestinal peptide, *D cell* somatostatin cell, *G cell* gastrin cell, *PC* parietal cell, *H2* histamine receptor type 2, *m3* muscarinic receptor subtype 3 (Adapted from Sachs et al. [1])

H. pylori on these cells is closely related with the acid secretory system. Finally H^+, K^+-ATPase is an acid pump located in the parietal cells and this pump is largely affected by *H. pylori*. This part focuses on the two major acid regulatory hormones, gastrin and somatostatin, and H^+, K^+-ATPase together with the effect of *H. pylori* infection or eradication on the acid secretion.

4.2.1 Gastric Acid Secretion and H^+, K^+-ATPase

Acid secretion depends on activation of the gastric H^+, K^+-ATPase, termed as the acid or proton pump. This enzyme was found uniquely in gastric parietal cells and in renal collecting ducts [1]. Parietal cells are located at oxyntic gastric gland of the corpus (Fig. 4.2). Mg^{2+}-dependent, K^+-stimulated, H_3O^+-transporting, P-type adenosine triphosphatase (H^+, K^+-ATPase, EC3.6.1.36) [4] is consisted of α-subunit and β-subunit [5] (Fig. 4.3). It is an electroneutral H^+ for K^+ exchange P_2-type (phosphorylating) ATPase with ten membrane-spanning segments (α-subunit, HKα, Mr. ~94,000) and with one transmembrane segment and six or seven glycosylation sites (β-subunit, HKβ, Mr. 34,000) [5] (Fig. 4.3). The cytoplasmic domain (HKα) with catalytic and transport functions has three loops, the N or nucleotide-binding domain, the A or activation domain, and the P or phosphorylation domain (Fig. 4.3). Human HKα gene resides on chromosome 19 (19q13.1), and HKα contains sequences responsible for apical membrane localization [6]. HKβ gene is located on the chromosome 13 (13q34), and HKβ protects the enzyme from degradation and is necessary for trafficking to and from the plasma membrane [7]. The morphology of the parietal cell becomes different depending on the activity of acid secretion [8] (Fig. 4.4). In the resting parietal cell, most of the ATPase is

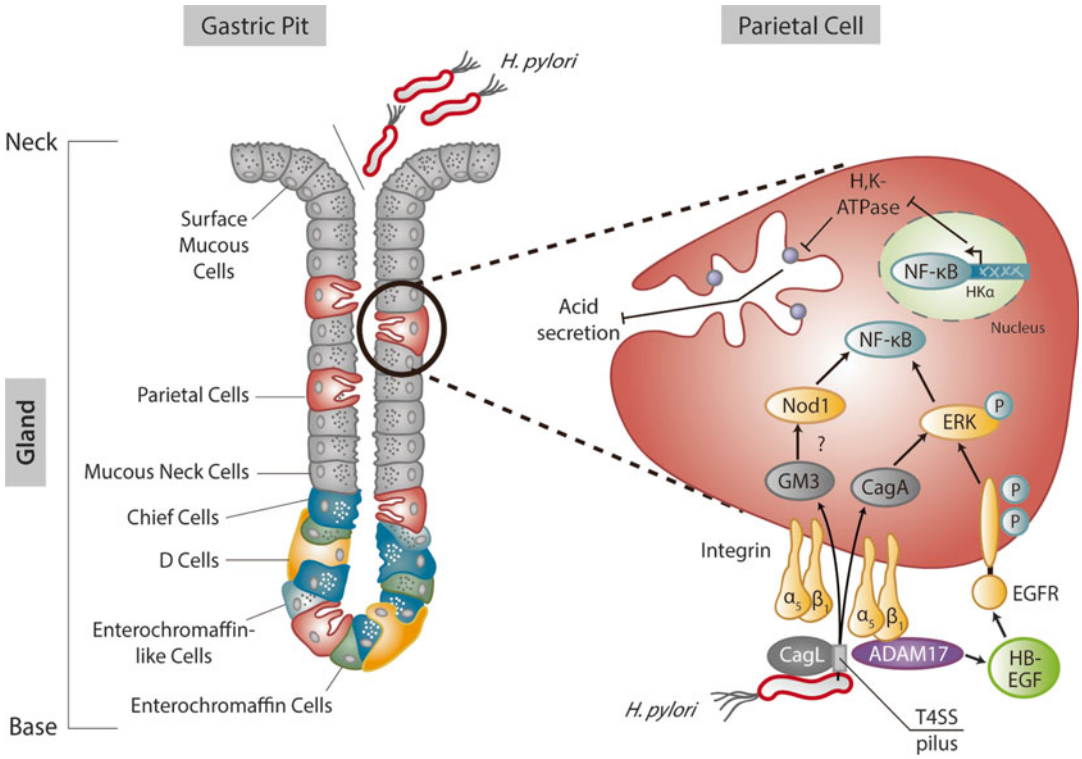

Fig. 4.2 Schematic illustration of a corporal gland in the human stomach focusing on the acid-secreting region. The location of various cell types in a gland of the human corpus was indicated in different colors. A gastric parietal cell is enlarged to the right, showing *Helicobacter pylori* interacting with integrins through CagL, injecting CagA and possibly the bacterial peptidoglycan-derived glycosylated tripeptide GM-3, leading to the activation of diverse host signaling pathways. The consequent mobilization of nuclear factor (NF)-κB p50 homodimers to the nucleus results in the repression of gastric H^+, K^+-adenosine triphosphatase (H^+, K^+-ATPase) α subunit transcription and the inhibition of acid secretion as indicated. *ADAM 17* a disintegrin and a metalloprotease 17, *EGFR* EGF receptor, *HB-EGF* heparin-binding epidermal growth factor, *T4SS* type IV secretion system (Adapted from Smolka and Backert [2])

sequestered in cytoplasmic tubulovesicles, as a form of $\alpha_2\beta_2$ complex, and upon stimulation, it is trafficked to the apical microvilli of the secretory canaliculus [8, 9] during which the surface area increases up to five times [10–12]. Actually parietal cells need huge amount of energy for the active acid secretion, and for this there are high densities of mitochondria, NAD^+/NADH, and tubulovesicles in the parietal cells [10–12].

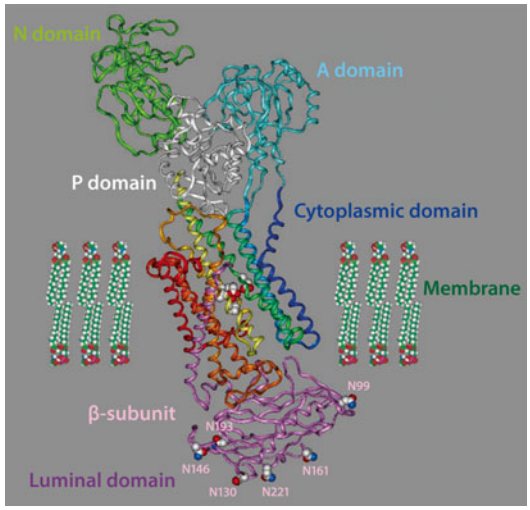

Fig. 4.3 A model structure of the gastric H^+, K^+-ATPase. The gastric H^+, K^+-ATPase α subunit has three lobes, N (ATP binding), P (phosphorylation), and A (activation) domains in the cytoplasmic domain and three transmembrane segments in the membrane domain. The gastric β subunit has short cytoplasmic region, one transmembrane segment, and a heavily glycosylated extracellular region. The number of Asn sites having carbohydrates is based on pig H^+, K^+-ATPase (Adapted from Shin and Kim [5], with permission from The Korean Society of Neurogastroenterology and Motility)

4.2.2 The Effect of *H. pylori* Infection on the Gastric Acid Secretion

Acute administration of *H. pylori* has been known to be capable of inhibiting acid secretion directly. However, recently there has been a report regarding an indirect pathway by activating intramural CGRP sensory neurons coupled to stimulation of somatostatin and inhibition of histamine secretion [13]. Since *H. pylori* is present in the upper regions of the gastric mucosa, whereas ECL cells are located from the base to the neck of glands (Fig. 4.2), it has been speculated that activation of CGRP sensory neurons may be one of the explanations to how initial patchy superficial colonization of the stomach can induce acute hypochlorhydria [13]. In addition, *H. pylori*-infected patients with acid hyposecretion tend to have corpus gastritis caused by a specific *H. pylori* product or by inflammatory cytokines, including IL-1β and TNF-α, which inhibit parietal cells [14]. IL-1β also inhibits ECL cells [15].

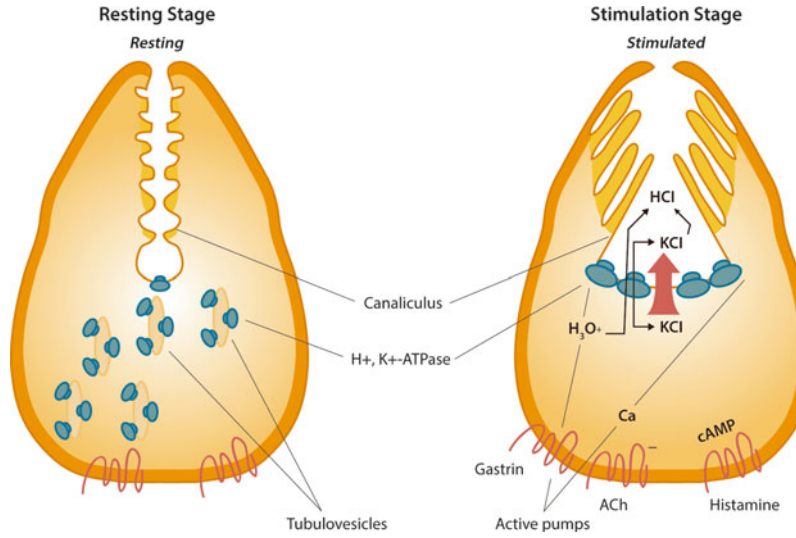

Fig. 4.4 Parietal cell structure of resting and stimulated stage. In the resting parietal cell, most of the H^+, K^+-ATPase is sequestered in cytoplasmic tubulovesicles and upon stimulation; it is trafficked to the apical microvilli of the secretory canaliculus (Adapted from Valle [8])

When *H. pylori* infection goes to chronic state, it accelerates the development of corpus atrophy, which further diminishes acid secretion through the loss of parietal cells [16]. A recent study clearly demonstrated that *H. pylori* (*cagA* positive) infection induced a decrease in acid secretion and an increase in serum gastrin, with these phenomena returning to control levels after treatment with an IL-1 receptor antagonist in Mongolian gerbils [17]. Because gastrin is a physiological stimulant of acid secretion, a decrease in intragastric acidity induced by *H. pylori* infection might precede the gastrin release.

4.2.2.1 Acute Phase of *H. pylori* Infection Causes Hypochlorhydria

In the acute phase of *H. pylori* infection, transient hypochlorhydria occurs [2], which status continues from several weeks to months [18–24]. Low acid secretion by acute *H. pylori* infection was demonstrated in the absence of loss of parietal cells [22], impaired permeability of gastric mucosa [22], and glandular atrophy [17]. Moreover, IL-1β which is produced by neutrophils and inhibits acid secretion was not involved in this stage. Instead, this low acid secretion in the acute stage is likely to result from direct contact parietal cells by *H. pylori* or its product [25, 26] and indirectly by activating intramural CGRP sensory neurons coupled to stimulation of somatostatin and inhibition of histamine secretion [13]. Studies about ultrastructure of the stomach reported that *H. pylori* was observed adjacent to parietal cells and even detected in secretory canaliculi of these cells [27, 28]. In human, *H. pylori* infection also suppressed acid secretion via histamine, acetylcholine, and cAMP [29, 30], and this inhibition was resolved soon after the eradication of *H. pylori* [31]. This transient inhibition of acid secretion in the acute phase facilitates the successful settlement of *H. pylori* in the stomach.

4.2.2.2 The Effect of *H. pylori* Infection on the H^+, K^+-ATPase

H. pylori-infected gastric mucosa or gastric epithelial cell lines showed the inhibition of HKα promoter activity in the endogenous or transfected H^+, K^+-ATPase [2]. Specifically, *H. pylori* inserts its protein to gastric cells through the type IV secretion system (T4SS), which look like cylindrical channel, and this inserted protein upregulated nuclear factor (NF)-κB (Fig. 4.2). Interestingly, the site which NF-κB combined in the promoter of H^+, K^+-ATPase was identified, and this fusion of NF-κB p50 homodimer resulted in repression of the transcription of HKα [2].

CagA protein encoded by *cag* pathogenicity island (*cag* PAI) and CagL, CagE and CagM, which consist of the T4SS, and lytic transglycosylase are mechanistically involved in NF-κB activation and repression of HKα transcription (Fig. 4.2). CagL, a T4SS pilus component, binds to the integrin α5β1 to mediate translocation of virulence factors into the host cell and initiate signaling. During acute *H. pylori* infection, CagL dissociates ADAM17 (a disintegrin and a metalloprotease 17) from the integrin α5β1 complex and stimulates ADAM17-dependent release of heparin-binding epidermal growth factor (HB-EGF), EGF receptor (EGFR) stimulation, ERK1/2 kinase activation, and NF-κB-mediated repression of HKα [2] (Fig. 4.2).

4.2.2.3 The Interaction Between *H. pylori* Infection and Gastric Acid Secretion Determining the Pattern of Gastritis

The interaction between *H. pylori* and gastric acid secretion also determines the pattern of *H. pylori*-induced gastritis (Fig. 4.5), which is a well known and also very important concept regarding the final outcome of *H. pylori* infection such as duodenal ulcer (DU) or gastric cancer. That is, progression to certain disease by *H. pylori* infection is known to be determined by the degree of acid secretion when the organism invades the stomach. In the case of subjects with high acid secretion, *H. pylori* escapes from the corpus and settles in the antrum leading to antral-predominant gastritis with excessive gastrin release (Fig. 4.5). By contrast, when the organism comes into the subjects with low acid secretion, it migrates into the corpus with adequate acidity. This subsequently causes atrophy

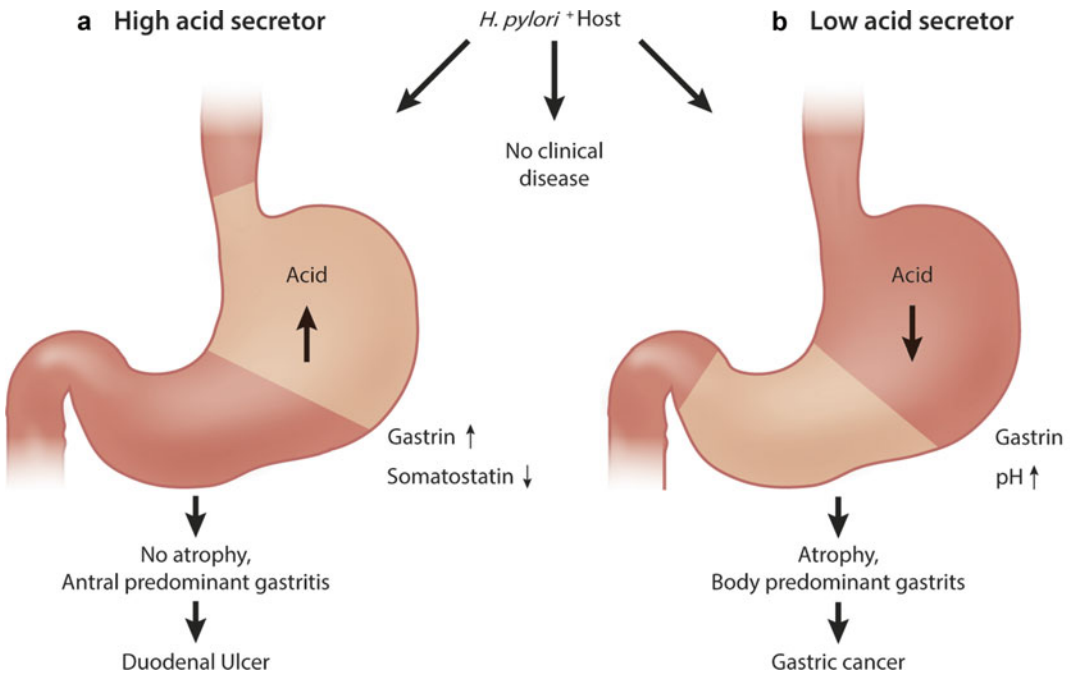

Fig. 4.5 Two patterns of *H. pylori*-induced gastritis. In the case of subjects with high acid secretion, *H. pylori* escapes from the corpus and settles in the antrum leading to antral-predominant gastritis with excessive gastrin release (**a**). By contrast, when the organism comes into the subjects with low acid secretion, it migrates into the corpus with adequate acidity (**b**). This subsequently causes atrophy of parietal cells and aggravates hypochlorhydria

of parietal cells and aggravates hypochlorhydria. When *H. pylori* succeed in the colonization, destroyed acid homeostasis influences the distribution of *H. pylori* in the stomach itself and the degree of gastritis. That is, *H. pylori* infection could diminish the number or function of D cells in the antrum, while it elevates the gastrin secretion of G cells via IL-8, IL-1β, or TNF-α [32, 33]. This disorganized endocrine system dumps excessive amount of acid into the duodenum leading to DU (Fig. 4.5). On the other hand, upregulation of IL-1β and TNF-α by *H. pylori* infection strongly suppresses acid secretion [14] and, at the same time, IL-1β reduces secretion of histamine from ECL cells [34]. This makes *H. pylori* thrive in the corpus and destroy parietal cells and finally aggravating impaired acid secretion. *H. pylori* infection, simultaneously, enhances the gastrin release. Consequently, the gastrin level in the blood was further accelerated due to reduced somatostatin secretion, since IL-1β and TNF-α inhibit somatostatin release during the Th1 immune response [35] (Fig. 4.6). This chronic low acidic milieu accompanying atrophy and elevated gastrin has been considered to be a good condition for developing gastric cancer (Fig. 4.5).

4.2.2.4 Chronic Phase of *H. pylori* Infection and Gastric Acid Secretion

As noted above the effect of *H. pylori* on acid secretion depends on the stage of infection or predominant location where the infection occurs. Chronic infection in the antrum causes upregulation of gastrin and subsequent elevated acid secretion, while chronic infection in the corpus leads to impaired acid secretion by direct suppression of H^+, K^+-ATPase or involvement of cytokines such as IL-1β or TNF-α. Therefore, the direct inhibition of H^+, K^+-ATPase, indirect inhibition through cytokines, and loss of parietal cells by ongoing inflammation were three mechanisms for the low acid secretion in the chronic corpus-predominant gastritis.

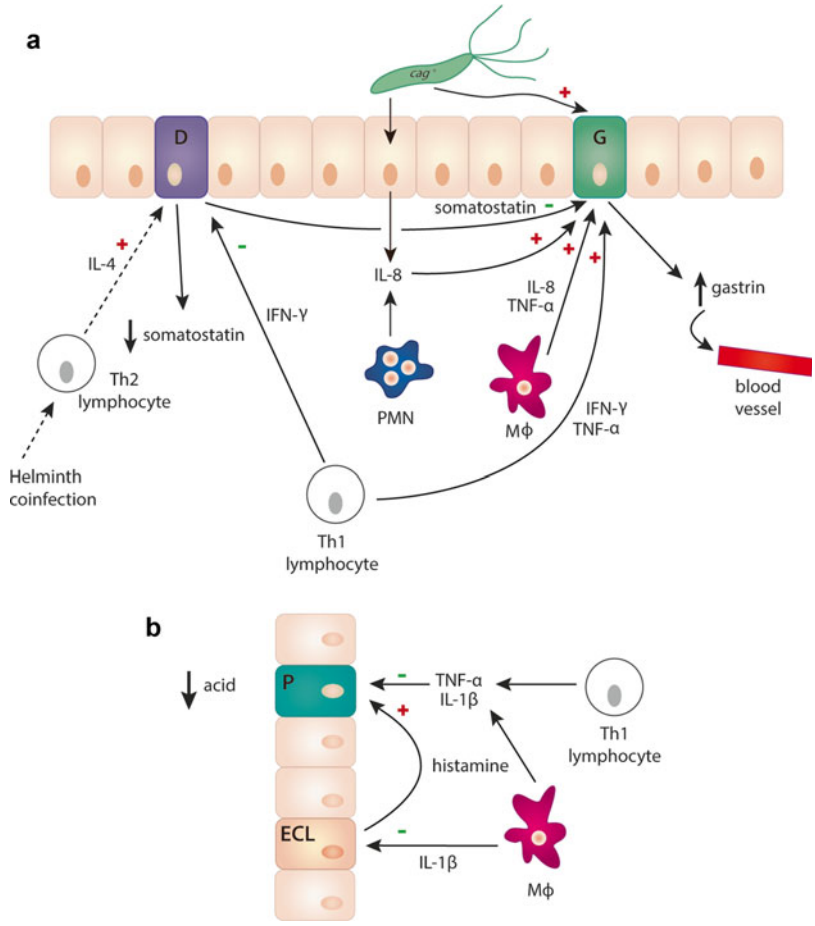

Fig. 4.6 Cytokine-mediated alterations in gastrin and somatostatin secretion (**a**) and enterochromaffin-like (*ECL*) and parietal (*P*) cell function (**b**) in *H. pylori* infection. (**a**) IFN-γ from Th1 helper cells, IL-8, and TNF-α stimulate gastrin secretion from G cells. In contrast, IFN-γ inhibits somatostatin release from D cells, decreasing its inhibitory effect on gastrin. IL-4 stimulates somatostatin secretion, which may be a potential mechanism by which helminth coinfection, and stimulation of Th2 responses, can protect against the development of corpus atrophy. (**b**) IL-1β suppresses ECL cell histamine release and IL-1β and TNF-α inhibit acid secretion from parietal cells. *PMN* polymorphonuclear cell (Adapted from Peek and Crabtree [35])

4.2.2.5 Gastrin and Somatostatin in Regard to *H. pylori* Infection

Gastrin release is stimulated by extramural cholinergic and intramural cholinergic and non-cholinergic factors and inhibited by somatostatin [36] (Fig. 4.7). Somatostatin release is stimulated by luminal (acid), paracrine, and hormonal factors (gastrin) and by intramural non-cholinergic factors and is inhibited by extramural cholinergic factors. Levi et al. [37] reported that both basal/stimulated acid secretion and basal/meal-stimulated plasma gastrin levels were significantly higher in *H. pylori*-positive DU patients ($n=25$) compared with *H. pylori*-negative DU patients ($n=6$), which phenomenon has been named as "the gastrin link." Their data clearly demonstrated that *H. pylori* infection induced hypergastrinemia that was followed by an increase in acid secretion in DU patients (Fig. 4.5). Consequently, eradication of *H. pylori* reduced the output of gastrin in the stomach [38].

In mammals, the gastrointestinal (GI) tract and pancreas contain the largest amounts of somatostatin. Most of the GI somatostatin immunoreactivity

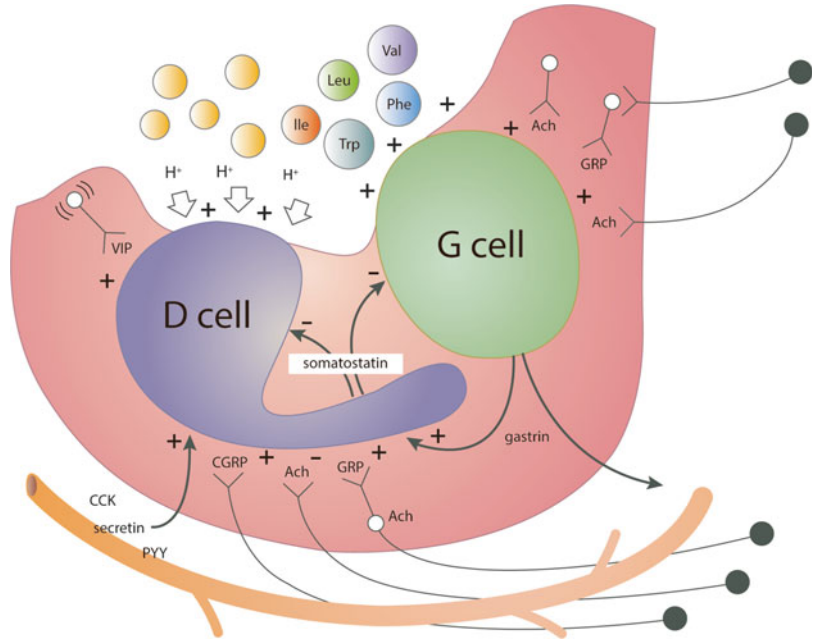

Fig. 4.7 Gastrin release is stimulated by extramural cholinergic and intramural cholinergic and non-cholinergic factors and inhibited by somatostatin. Somatostatin release is stimulated by luminal (acid), paracrine, and hormonal factors (gastrin) and by intramural non-cholinergic factors and is inhibited by extramural cholinergic factors. *CCK* cholecystokinin, *PYY* peptide YY, *CGRP* calcitonin gene-related peptide, *VIP* vasoactive intestinal peptide, *GRP* gastrin-releasing peptide, *Ach* acetylcholine (Adapted from Kaneko et al. [36])

is confined to the mucosal layer [39], where epithelial endocrine cells and D cells are localized. In the antrum, the D cells have apical membranes that are exposed to the lumen ("open cells"). In the corpus, the D cells are of the "closed" type; they are not exposed to the luminal surface of the mucosa [40]. Somatostatin plays an important role in the regulation of gastric acid secretion by inhibiting gastrin release. In the antral mucosa, the open D cell releases somatostatin in response to increased acidity in the gastric lumen. As the apical surface of D cells opens onto the gastric lumen, changes in pH may be sensed directly through chemoreceptors on the apical membranes.

The effect of *H. pylori* infection on gastric levels of somatostatin has been relatively well investigated. Antral somatostatin concentrations were decreased in *H. pylori*-infected patients, but not the corpus [41] which have been demonstrated by measuring the somatostatin concentration in the biopsy specimens from both sites [42, 43]. Moss et al. [44] demonstrated that eradication of *H. pylori* from patients with DU caused an approximately twofold increase in somatostatin mRNA in antral, but not in corpus biopsies. They also showed an increase in D cell numbers after *H. pylori* eradication in subjects with active DU [44].

4.2.3 The Effect of *H. pylori* Eradication on the Gastric Acid Secretion

The effect of *H. pylori* eradication on the gastric acid secretion also starts from the gastritis pattern such as antral- or corpus-predominant gastritis because the ongoing cascade of gastritis and gastric acid secretion caused by *H. pylori* will stop right away. It will also cause effect on the gastrin, somatostatin, and other gastric hormones. Basically, the changes of acid secretion after *H. pylori* eradication depend on the degree of inflammation in the corpus where parietal cells localize. More specifically, in the setting of antral-predominant gastritis with intact corporal glands, *H. pylori* eradication reduces gastrin and subsequently acid secretion, as well. On the contrary, in the setting of severe corporal inflammation, the inhibition of parietal cells by *H. pylori* disappears, leading to the elevated acid secretion [35].

Several studies reported that immediate increase in acid secretion after administration of anti-*H. pylori* agents [31, 35]. These results came from the termination of direct contact of parietal cells with *H. pylori* or its products [25, 26] and reduction of IL-1β or TNF-α which represses the

acid secretion. There was a question regarding the long-term change of acid secretion after *H. pylori* eradication, especially, for the relationship with the reversibility of atrophic gastritis in the corpus. Osawa et al. [45] performed a study in 111 subjects with chronic gastritis under the gastric cancer surveillance program and reported that mRNA of H^+, K^+-ATPase increased 12 weeks after *H. pylori* eradication even in severe atrophy without changes in the number of parietal cells. That is, median mRNA expression levels of H^+, K^+-ATPase in the gastric mucosa increased 250-fold after *H. pylori* eradication accompanied by attenuation of IL-1β. A large increase in H^+, K^+-ATPase expression was observed even in patients with severe atrophic gastritis. In contrast, fold increases in mRNA expression levels, including intrinsic factor, anion exchanger 2, and M3 muscarinic receptor which are involved in acid secretion export in the basal and apical sides of parietal cells, after eradication therapy, were limited to 1.4, 2.3, and 2.5 times, respectively [45]. These results that gastric H^+, K^+-ATPase mRNA expression was markedly restored 12 weeks after successful *H. pylori* eradication suggest a central role for the restoration of H^+, K^+-ATPase expression in gastric acid secretion recovery after *H. pylori* eradication in the absence of alteration of parietal cell number [45]. In addition, El-Omar et al. [46] reported that hypochlorhydria seen in patients with mild degrees of body atrophy improved readily after *H. pylori* eradication. As body gastritis preceded development of body atrophy by decades in *H. pylori* infection [16, 47], the duration of the infection, or age of the patients in many cases, could be considered to be a principal determinant for the reversibility of acid secretion by *H. pylori* eradication. Similarly, when Iijima et al. [48] assessed gastrin-stimulated acid output and histologic evaluation of biopsy specimens prior to and 1.7 months after eradication and gastric acid secretion over 5 years after *H. pylori* eradication in 12 patients with hypochlorhydria (<0.6 mmol/10 min), gastric acid secretion was reversed to normal range in nine of 23 patients (39 %) at 7 months after eradication [48]. In the long-term follow-up, gradual and significant recovery in gastric acid secretion was observed up to 2 years post-therapy [48]. However, there was no additional increase during the last 3 years of 5-year follow-up period, leaving the acid secretory levels subnormal in the majority of the patients [48]. This long-term follow-up study suggests that the pathologic process has already progressed to an irreversible stage in the majority of *H. pylori*-positive patients with marked body atrophy and profound hypochlorhydria [48]. However, further long-term studies are needed in the various clinical situations in the future as atrophy and intestinal metaplasia showed reversible changes with *H. pylori* eradication in part of persons even after 5-year follow-up period [49].

4.3 Ghrelin

Ghrelin is a 28-amino acid peptide hormone, primarily produced and secreted from the gastric mucosa [50]. The stomach is considered as the major source of circulating ghrelin, since severe reduction in blood ghrelin levels are observed in patients that undergone gastrectomy [51]. Ghrelin is produced in oxyntic cells that are prominent in the corpus of the stomach, and ghrelin-producing cells are located from the base to the neck of the glands. It is also secreted from the small intestine and the colon [52] and is also expressed in the hypothalamus [53], the pituitary [54], and several tissues in the periphery [55]. Although the major active product of the ghrelin gene is the 28-amino acid peptide acylated at Ser^3 with C8:0, recent studies have revealed that the ghrelin gene can generate various molecules besides ghrelin, which include des-acyl ghrelin [56]. The human ghrelin gene, located on the short arm of chromosome 3 (3p25-26), is composed of five exons and four introns [57] (Fig. 4.8). The main ghrelin mRNA transcript in human codes for a 117-amino acid long peptide, prepro-ghrelin (1–117). The signal peptide sequence, prepro-ghrelin (1–23) of prepro-ghrelin (1–117), is cleaved to form pro-ghrelin (1–94) (Fig. 4.8). A series of posttranslational steps, including the process of protease cleavage and acyl modification of the ghrelin precursor peptide, results in the production

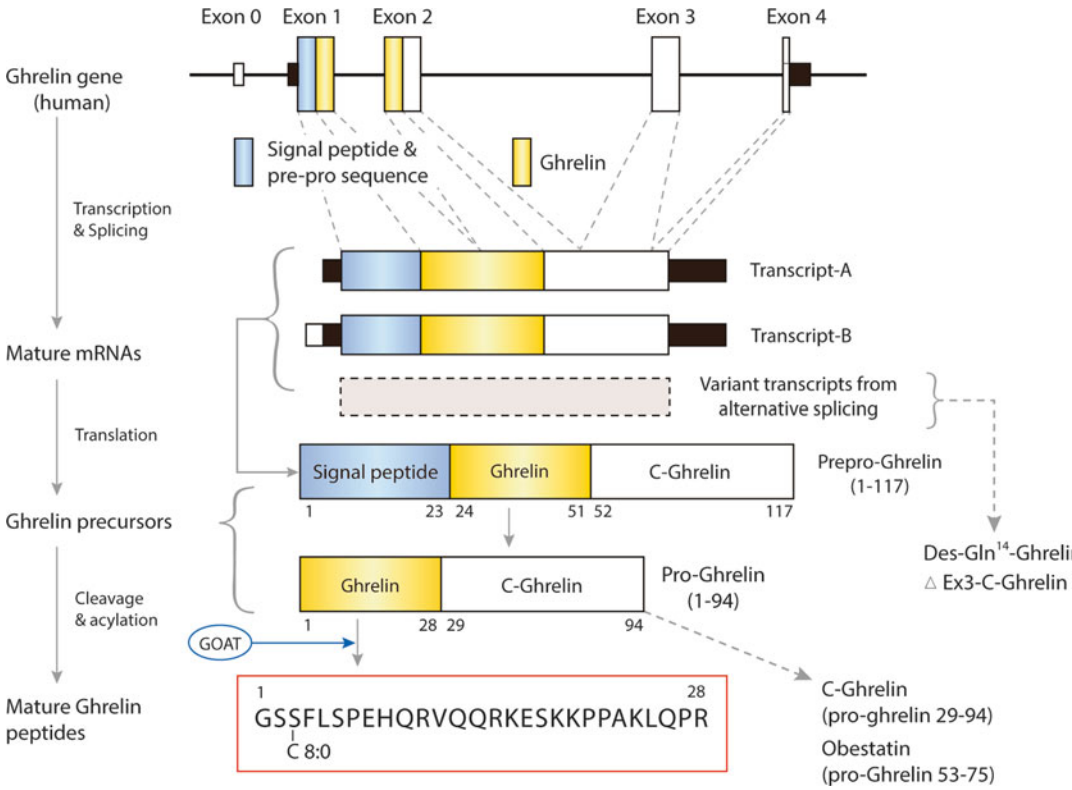

Fig. 4.8 Structure of the human ghrelin gene and processing steps from the ghrelin gene to acyl ghrelin, des-acyl ghrelin or other ghrelin-associated peptides. The human ghrelin gene is composed of five exons and four introns. The major mRNA transcript (transcript-A) of the ghrelin gene is translated into a 117-amino acid ghrelin precursor, prepro-ghrelin (1–117). The signal peptide sequence, prepro-ghrelin (1–23) of prepro-ghrelin (1–117), is cleaved to form pro-ghrelin (1–94). A series of posttranslational steps, including the process of protease cleavage and acyl modification of the ghrelin precursor peptide, results in the production of mature ghrelin peptides (acyl and des-acyl ghrelin) or other ghrelin gene-associated peptides (C-ghrelin and obestatin). Besides these peptides, several other ghrelin gene-derived peptides with or without acyl modification (des-Gln14-ghrelin, ΔEx3-C-ghrelin, etc.) are predicted to be produced from the splicing variants of the ghrelin gene transcripts. *GOAT* ghrelin-O-acyltransferase (Modified from Nishi et al. [57])

of mature ghrelin peptides (acyl and des-acyl ghrelin) or other ghrelin gene-associated peptides (C-ghrelin and obestatin) (Fig. 4.8). The ghrelin peptide is acylated by the enzyme ghrelin O-acyl transferase (GOAT) [58], which is expressed predominantly in the stomach, gut, and pancreas, but also at other sites [59]. This acyl modification of ghrelin is easily cleaved during sample extraction. Thus, acyl ghrelin should be isolated from blood specimens by adding ethylenediaminetetraacetic acid (EDTA) with aprotinin or *p*-hydroxymecuribenzoic acid. That is, the plasma should be separated by centrifugation and immediate acidification before freezing at −80 °C to ensure stability of acyl ghrelin during storage. The nonacylated form of ghrelin without octanoic acid modification at Ser^3 residue, des-acyl ghrelin, is also present at significant level in both the stomach and blood [56, 60]. Des-acyl ghrelin is the most abundant ghrelin-related molecule in the body, comprising 80–90 % of the total circulating ghrelin, and has a longer half-life.

4.3.1 Role of Ghrelin

In humans, ghrelin stimulates gastric motility [61] and acid secretion [62]. Acyl ghrelin has been

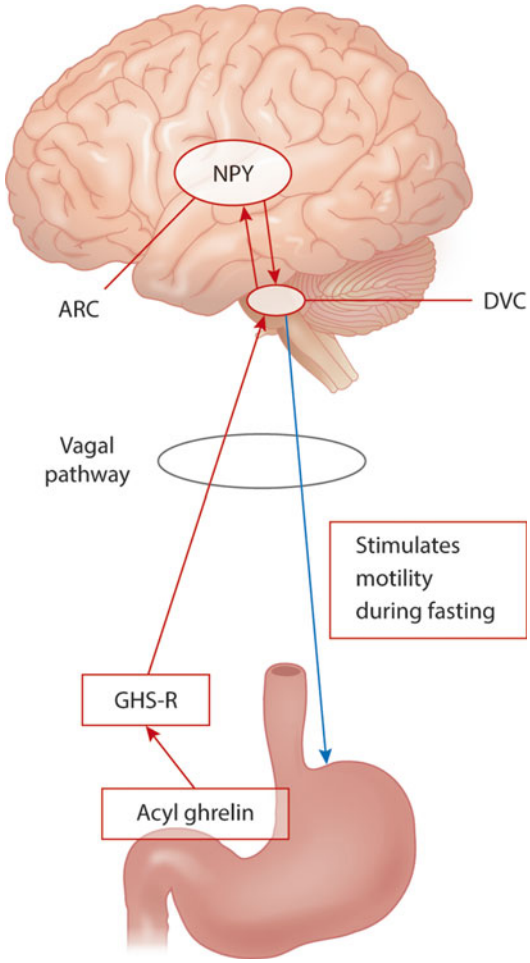

Fig. 4.9 The effects of acyl ghrelin via the brain-gut axis. *ARC* arcuate nucleus, *DVC* dorsal vagal complex, *GHS-R* growth hormone secretagogue receptor, *NPY* neuropeptide Y (Modified from Fujimiya et al. [64])

identified as the endogenous ligand of the growth hormone secretagogue receptor (GHS-R) [50]. Acyl ghrelin acts in GHS-R on vagal afferent nerve fibers in the stomach [63], which transmit this signal to the nucleus of the solitary tract (NTS) (Fig. 4.9). From the NTS, the information is projected to the arcuate nucleus (ARC) of the hypothalamus, where neuropeptide Y (NPY) neurons are activated (Fig. 4.9). The NPYY2 and/or Y4 receptor in the CNS may be involved in the upper GI motility because Y2 and Y4 receptor agonists can induce phase III-like contractions in the duodenum when given to animals in the fed state [64]. From the ARC, the signal is finally transmitted to the dorsal motor nucleus of the vagus nerve ((DVC) dorsal vagal complex) and via vagal efferent fibers, and fasted motor activity is induced in the gut [65] (Fig. 4.9). Acyl ghrelin is considered as a short regulator of food intake in both animals and humans [66, 67]. In rats, acute and chronic administration of ghrelin enhances food intake and weight gain [68, 69]. Peripheral administration of ghrelin produced a 28 % increase of food intake in normal-weight healthy volunteers [66]. The subjects who received exogenous ghrelin reported an increase in appetite and showed a higher caloric intake than after placebo [70]. This fasted motor activity of the GI tract has been considered to play a role of a mechanical cleansing of the stomach and the intestine in preparation for the next meal. In healthy volunteers, the peripheral administration of ghrelin induces the occurrence of phase III of the migrating motor complex about 20 min after administration of ghrelin [71]. Moreover, it induces a premature phase III originating in the stomach about 14 min after its injection [71]. A positive correlation was reported between preprandial ghrelin concentration and gastric emptying time.

4.3.2 Regulation of Ghrelin in Regard to *H. pylori* Infection

Since ghrelin is predominantly produced in the stomach, many researchers evaluated the effect of *H. pylori* infection on ghrelin expression in the gastric mucosa and blood. Theoretically, *H. pylori*-related gastritis may progress to atrophy with loss of oxyntic glands leading to a negative effect on ghrelin production in the stomach. Isomoto et al. [72] well demonstrated that the gastric ghrelin mRNA expression level in *H. pylori*-positive patients was significantly lower than that in *H. pylori*-negative subjects, and similar trend was noted for ghrelin peptide contents. In addition, plasma ghrelin concentrations in *H. pylori*-infected patients were significantly lower than in uninfected subjects and increased following cure of the infection [72]. Furthermore, plasma ghrelin levels correlated positively with the expression levels of ghrelin mRNA and peptide products. There was a significant stepwise decrease in

Table 4.1 Studies that compared circulating levels of ghrelin between *H. pylori*-positive and *H. pylori*-negative subjects

Author	Subjects	Sex	Age	No	Method	Specimen	Ghrl type	Results in HP (+)
Gokcel et al. (2003), Turkey [73]	Not stated	W	A	39	Commercial EIA	Plasma	Total	→
Isomoto et al. (2004), Japan [74]	Sick	B	A	68	Commercial RIA	Plasma	Total	↓
Isomoto et al. (2005), Japan [72]	Dyspepsia	B	A	81	In-house RIA	Plasma	Total	↓
Isomoto et al. (2005), Japan [75]	Sick	B	A	89	In-house RIA	Plasma	Total	↓
Osawa et al. (2005), Japan [76]	Healthy (health checkup)	M	A	160	In-house RIA	Plasma	Total	↓
Shiotani et al. (2005), Japan [77]	Healthy	B	A	132	Commercial ELISA	Serum	Total	↓
Konturek et al. (2006), Poland [78]	Healthy	B	B	180	Human RIA	Serum	Total	↓
Plonka et al. (2006), Poland [79]	Healthy	B	B	538	Commercial RIA	Serum	Total	↓
Plonka et al. (2006), Poland [80]	Healthy	B	C	287	Commercial RIA	Serum	Total	↓
Salles et al. (2006), France [81]	Hospitalized in Geriatric	B	A	62	Commercial RIA	Plasma	Total	↓
Alonso et al. (2007), Spain [82]	Type I DM	B	A	15	Commercial RIA	Plasma	Total	↓
An et al. (2007), Korea [83]	Gastric cancer	B	A	41	Commercial ELISA	Plasma	Total	→
Cindoruk et al. (2007), Turkey [84]	Sick (normal 24 pH without atrophy)	B	A	50	RIA	Plasma	Total	→
D'Onghia et al. (2007), Italy [85]	Colon cancer	B	A	29	RIA	Serum	Total	↓
de Martel (2007), USA [86]	Healthy + sick	B	A	110	Commercial ELISA	Serum	Total	→
Jun et al. (2007), Korea [87]	CG	B	A	63	Commercial RIA	Plasma	Total	→
Pacifico et al. (2008), Italy [88]	Healthy+ GI symptom	B	C	85	Commercial RIA	Serum	Total	→
Roper et al. (2008), USA [89]	Healthy	M	A	256	Commercial EIA	Serum	Total	→
Shak et al. (2008), USA [90]	BMI >35 kg/m^2	B	A	24	Commercial EIA	Plasma	Total and acyl	→
Chuang et al. (2009), Taiwan [91][a]	PU + FD	M	A	145	Commercial RIA	Plasma	Total	↓

(continued)

Table 4.1 (continued)

Author	Subjects	Sex	Age	No	Method	Specimen	Ghrl type	Results in HP (+)
Chuang et al. (2009), Taiwan [91][a]	PU + FD	W	A	196	Commercial RIA	Plasma	Total	→
Gao et al. (2009), China [92]	Healthy (health checkup)	B	A	100	Commercial RIA	Plasma	Total	↓
Kawashima et al. (2009), Japan [93]	Healthy + sick (PU + CG)	B	A	220	Commercial EIA	Plasma	Acyl	↓
Campana et al. (2009), Italy [94]	Healthy + CG (HP(−))	B	A	50	RIA	Plasma	Acyl	↑ in atrophy

A adults, *B* both, *C* child, *W* women, *M* men, *Ghrl* ghrelin, *HP H. pylori*, *No* sample size, *CG* chronic gastritis, *DM* diabetes mellitus, *FD* functional dyspepsia, *PU* peptic ulcer, *Sick* patients who visited clinics, but the specific condition is not stated, *EIA* enzyme immunoassay, *ELISA* enzyme-linked immunosorbent assay, *RIA* radioimmunoassay, *RT-PCR* real-time polymerase chain reaction

↑, higher; ↓, lower; → no significant difference

[a]Articles written in English and assay method were stated

gastric ghrelin mRNA expression, peptide contents, and density of ghrelin immunoreactive cells with progression of histologic severity of glandular atrophy in the corpus [72]. However, the effect of *H. pylori* on circulating level of ghrelin is not simple mainly due to complicated metabolism pathway of ghrelin. That is, the circulating level of ghrelin is determined by the balance among its rate of secretion, degradation, and clearance. While plasma esterases deacylate acyl ghrelin, plasma proteases degrade circulating ghrelin. Consequently, studies which have compared blood ghrelin levels between *H. pylori*-infected and noninfected subjects showed inconsistent results [73–95] (Table 4.1). More basically, there are several factors such as gastrin, insulin-like growth factor (IGF) 1, obesity, insulin resistance, hyperinsulinemia, and cholesterol and urine excretion in addition to *H. pylori* infection that could affect plasma ghrelin concentration. However, Nweneka and Prentice [95] concluded that circulating ghrelin was significantly lower in *H. pylori*-positive subjects in a meta-analysis.

4.3.3 The Effect of Eradication of *H. pylori* on Ghrelin

Three studies demonstrated that *H. pylori* eradication increases ghrelin mRNA [96–98]. Osawa et al. [98] demonstrated that median preproghrelin mRNA expression was increased nearly fourfold at the point of 12 weeks after *H. pylori* eradication in 134 subjects. Ghrelin-immunoreactive cell number/mm^2 also increased in 50 *H. pylori*-eradicated patients in the absence of significant change of atrophy and intestinal metaplasia [99]. However, Choe et al. [100] failed to demonstrate a change in ghrelin expression 4 weeks after *H. pylori* eradication. The effect of *H. pylori* eradication on circulating level of ghrelin was more evaluated than on the gastric levels but the results are still inconsistent (Table 4.2). At first, Nwokolo et al. [101] reported a rise in circulating plasma ghrelin levels following eradication of *H. pylori* in their study of 12 healthy subjects. Further studies reported elevated levels of blood ghrelin after the cure of *H. pylori* [93, 97, 102]. However, subsequent studies found no change [72, 74, 75, 84, 96, 100] or even decrease [88, 99] in circulating ghrelin levels. When Nweneka and Prentice [95] performed a meta-analysis, *H. pylori* eradication has no effect on circulating ghrelin levels. Nevertheless, pre-eradication elevation of ghrelin may be a predictor of a fall in plasma levels post-eradication [76, 95]. Regarding the relationship between ghrelin and functional dyspepsia (FD) in terms of *H. pylori* infection, two studies reported that fasting total ghrelin levels were significantly lower in patients with dysmotility-like FD than healthy volunteers [103, 104]. In addition, Shindo et al. [105] also showed significantly lower plasma acyl ghrelin levels in patients with postprandial distress syndrome (PDS) compared

Table 4.2 Studies that compared circulating levels of ghrelin before and after *H. pylori* eradication

Authors	Subjects	Sex	Age	No.	Method	Specimen	Ghrl type	FU (weeks)	Results after eradication
Nwokolo et al. (2003), UK [101]	Healthy	B	A	10	Commercial RIA	Plasma	Total	6	↑
Isomoto et al. (2004), Japan [74]	Sick	B	A	9	Commercial RIA	Plasma	Total	4	→
Isomoto et al. (2005), Japan [72]	Dyspepsia	B	A	43	In-house RIA	Plasma	Total	4	→
Isomoto et al. (2005), Japan [75]	Sick	B	A	10	In-house RIA	Plasma	Total	4	→
Osawa et al. (2006), Japan [98]	Healthy + PU/CG	M	A	134	In-house RIA	Plasma	Total	12	↓
Choe et al. (2007), Korea [100]	Sick	B	A	8	Commercial ELISA	Plasma	Total	4	→
Cindoruk et al. (2007), Turkey [84]	Sick (normal 24 pH without atrophy)	B	A	23	RIA	Plasma	Total	12	→
Czesnikiewicz-Guzik et al. (2007), Poland [102]	Sick	W	A	49	Commercial RIA	Plasma	Total	4	↑
Jang et al. (2008), Korea [97]	PU	B	A	16	Commercial RIA	Plasma	Total	NA	↑
Pacifico et al. (2008), Italy [88]	Healthy + sick (GI symptom)	B	C	22	RIA	Serum	Total	52	↓
Kawashima et al. (2009), Japan [93]	Both (PU + CG)	B	A	49	Commercial EIA	Plasma	Acyl	23	↑
Lee et al. (2010), Korea [96]	Healthy	B	A	9	ELISA	Plasma	Total	5	→

A adults, *B* both, *C* child, *W* women, *M* men, *Ghrl* ghrelin, *HP H. pylori*, *NA* not available, *No* sample size, *CG* chronic gastritis, *FU* follow-up, *GI* gastrointestinal, *PU* peptic ulcer, *Sick* patients who visited clinics, but the specific condition is not stated, *EIA* enzyme immunoassay, *ELISA* enzyme-linked immunosorbent assay, *RIA* radioimmunoassay, *RT-PCR* real-time polymerase chain reaction
↑, increased; ↓, decreased; → no significant change

with healthy volunteers. In our study, plasma acyl ghrelin level was lower in the PDS group than in the control and epigastric pain syndrome (EPS) group [106]. One year after the eradication of *H. pylori*, plasma acyl ghrelin level and gastric prepro-ghrelin mRNA expression were increased in the PDS type ($p=0.004$ and $p<0.001$, restrictively) [106]. In addition, patients with symptomatic improvement demonstrated an increase in plasma acyl ghrelin (11.51–21.00, $p=0.040$). From these results we concluded that the plasma acyl ghrelin plays a role in the development of PDS, and *H. pylori* eradication upregulates prepro-ghrelin mRNA and plasma acyl ghrelin, contributing to the improvement in PDS symptoms [106]. However, usually there is a difficulty of enrolling enough numbers of follow-up cases. Thus further studies are necessary to identify the true effect of *H. pylori* eradication on the gastric and circulating ghrelin levels as well as on the symptom changes in the patients with FD.

4.4 Leptin

Leptin was discovered in 1994 as a hormone produced by the adipose tissue with a modulatory effect on feeding behavior and weight control [107]. Leptin is a product of the obese (*ob*) gene, which is located on chromosome 7 in humans and acts through its receptor OB-R. Interestingly, the stomach has been

identified as an important source of leptin [107], and leptin-producing cells were found to be localized in the lower half of the fundic glands, a site similar to that of the pepsinogen-secreting chief cells. The soluble isoform of its receptor (Ob-R) is secreted by chief cells in the gastric mucosa [108]. Moreover, intestines do express membrane-bound leptin receptors on their brush border [107–109] (Fig. 4.10). Collectively, gastric exocrine and endocrine secretions of leptin

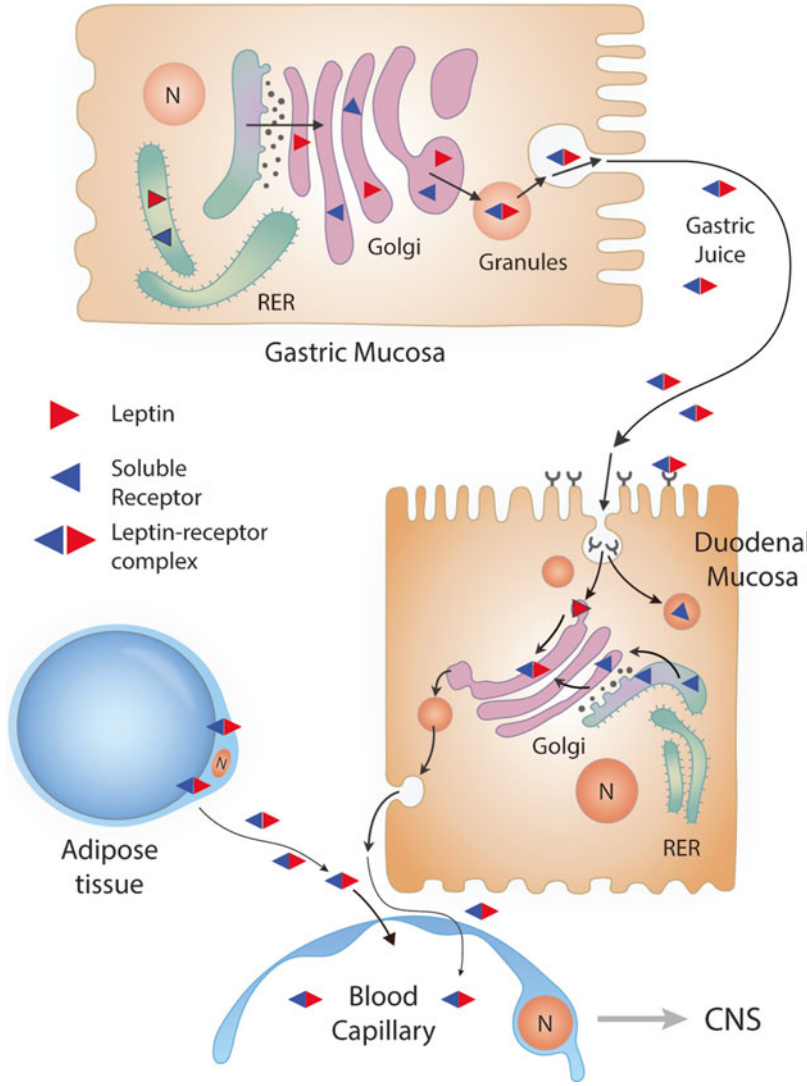

Fig. 4.10 Schematic drawing that illustrates the secretion of leptin by the adipocyte and the gastric chief cell. Both types of cell secrete the leptin receptor. Both the leptin and the leptin receptor are synthesized in the rough endoplasmic reticulum, transferred to the Golgi apparatus, and packaged into either small vesicles (adipocytes) or secretory granules (gastric cells). At the level of the trans-cisternae of the Golgi and in the secretory granules, leptin binds its soluble receptor to form the leptin-leptin receptor complex. This complex is discharged by both cell types through an exocytotic event. The adipose tissue secretes toward the blood circulation, while the gastric cells secrete in an exocrine fashion into the gastric juice. Leptin in the gastric juice is vehiculated to the duodenal lumen. In the duodenum leptin-leptin receptor complex is internalized and separated. The leptin is channeled toward the trans-Golgi cisternae, and it binds a newly synthesized soluble leptin receptor. The complex reaches the blood circulation. *RER* rough endoplasmic reticulum, *CNS* central nervous system, *N* nucleus (Adapted from Cammisotto and Bendayan [109])

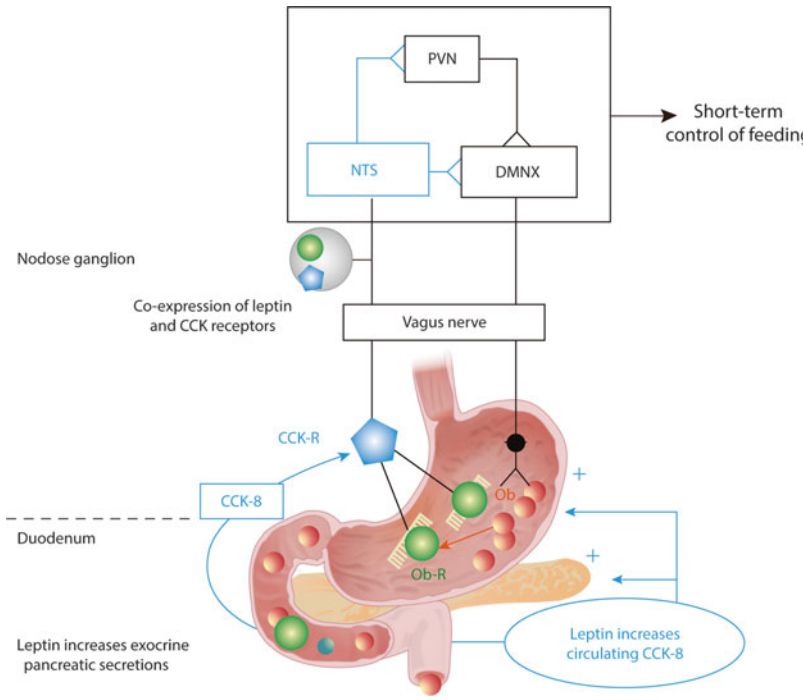

Fig. 4.11 Model of the action of gastric leptin. Leptin (secreted by the stomach) and CCK can be considered as short-term gastrointestinal signals in the control of feeding. These signals locally activate their receptors on vagal terminals to generate signals that are processed in the NTS and the paraventricular nucleus (PVN) of the hypothalamus. The stomach-derived leptin secreted in the lumen enters the intestine in an active form. *CCK-R* cholecystokinin receptor, *DMNX* dorsomotor nucleus of the vagus nerve, *Ob* leptin, *Ob-R* leptin receptors (Modified from Guilmeau et al. [111])

constitute a gastroenteric axis to coordinate its roles in the GI tract [109] (Fig. 4.11).

4.4.1 Regulation and Role of Gastric Leptin

Gastric leptin is sensitive to the nutritional status of the body. Fasting for 48 h induced a decrease in gastric leptin expression and content in rats [110]. Food intake quickly depletes gastric leptin while sustained feeding stimulates the leptin gene expression and leptin synthesis [110, 111]. Leptin derived from the stomach can be distinguished from adipocyte leptin through its rapid increased secretion following a meal and through its exocrine secretion (i.e., mainly in the gastric lumen). While the gastric mucosa secretes leptin within minutes after the beginning of food intake, it takes several hours for adipocytes to release significant amounts of leptin. Differences in time frame of secretion between the adipose tissue and gastric mucosa may reflect different roles. Gastric leptin remains stable in gastric juice even at pH 2 [112] which makes leptin to affect gastric functions. It has been considered that gastric leptin may exert paracrine effects within the gastric mucosa or stimulating vagal afferents to signal the central nervous system [108]. Gastric leptin controls food intake and satiety sensations by acting on the stomach itself. It potentiates the effect of cholecystokinin (CCK) by slowing gastric emptying and promoting gastric distension [113], without direct effect on CNS [114]. It also stimulates the production of glucagon-like peptides 1 and 2 (GLP1 and GLP2) which inhibit gastric emptying [115, 116].

Leptin receptor isoforms have been found in the rat nodose ganglion, which contains the cell bodies of vagal afferent neurons, and in the vagus

Table 4.3 Studies that compared gastric levels of leptin between *H. pylori*-positive and *H. pylori*-negative subjects (including *H. pylori* eradication)

Authors	Subjects	Sex	Age	No.	Specimen	Method	Results in HP (+) subjects
Breidert et al. (1999), USA [107]	Dyspepsia	B	A	39	Antrum and body	Commercial ELISA	↑Leptin mRNA in HP (+) in only the body
Azuma et al. (2001), Japan [121]	CG	B	A	201 HP (+)	Fundus	RT-PCR	↑Leptin mRNA in HP (+)/↓Leptin mRNA after cure (12 weeks)
Salles et al. (2006), France [81]	Hospitalized in geriatric	B	A (>75 years)	62	Body	RT-PCR	↓Leptin mRNA more so as atrophy increases
Jun et al. (2007), Korea [87]	CG	B	A	63	Body	RT-PCR	↑Leptin mRNA in HP (+)
Roper et al. (2008), USA [89]	Healthy	M	A	256	Antrum and fundus	Commercial ELISA	→ Leptin in the mucosa

Articles written in English and assay method were stated
A adults, *B* both, *C* child, *W* women, *M* men, *HP H. pylori*, *No* sample size, *CG* chronic gastritis, *Sick* patients who visited clinics, but the specific condition is not stated, *ELISA* enzyme-linked immunosorbent assay, *RT-PCR* real-time polymerase chain reaction
↑, increased (or higher); ↓, decreased (or lower); →, no significant change (or difference)

nerve proper [117, 118]. Signals arising from the upper GI tract are conveyed by the viscero-sensitive vagal afferent neurons to the nucleus of the solitary tract (NTS), then to the hypothalamus where they participate in the process of meal-induced termination of food intake [109, 119] (Fig. 4.11). CCK secreted from duodenal endocrine I cells typically functions as one of these short-term satiety signals via activation of the CCK-1 receptor [120]. In short, while adipose leptin acts on the long-term mainly through its interactions with the central nervous system, gastric leptin acts locally on the gastric mucosa for regulating food intake. Gastric leptin influences both the intestinal tract and the central nervous system [109] (Fig. 4.11).

4.4.2 Regulation of Leptin in Regard to *H. pylori* Infection

H. pylori infection significantly increased gastric leptin expression, and cure of the infection significantly reduced this expression with a concomitant increase in body mass index (BMI) [121]. *H. pylori*-infected gastric mucosa was found to be capable of releasing larger amounts of leptin mRNA than that without *H. pylori* infection [87, 107]. Although majority of studies have shown an increase of gastric leptin mRNA in *H. pylori*-infected subjects (Table 4.3), still there are discrepancies [81, 89]. Leptin-secreting endocrine cells are present in the gastric mucosa, but they are few in number and scattered in the gastric mucosa close to blood capillaries [111]. These cells do not reach the gastric lumen but secrete leptin to blood circulation. Since adipocytes are predominant source of leptin, whether *H. pylori* infection could affect circulating levels of leptin could be more complex (Table 4.4). It has been reported that rapid increase in the concentration of plasma leptin in response to CCK was involved in the mobilization of a gastric leptin store [122]. Similar to gastric leptin mRNA, higher serum leptin levels were reported in the subjects with *H. pylori* infection compared with *H. pylori*-negative subjects [78, 89, 123]. However, many studies reported no significant difference in plasma leptin according to *H. pylori* infection status [77, 81, 87, 91, 107]. Similarly, serum leptin level did not change significantly after

Table 4.4 Studies that compared circulating levels of leptin between *H. pylori*-positive and *H. pylori*-negative subjects (including *H. pylori* eradication)

Authors	Subjects	Sex	Age	No.	Specimen	Method	Results after eradication
Breidert et al. (1999), USA [107]	Dyspepsia	B	A	39	Plasma	Commercial ELISA	→
Azuma et al. (2001), Japan [121]	CG	B	A	201 HP (+)	Serum	Commercial ELISA	→ after cure (12 weeks)
Nwokolo et al. (2003), UK [101]	Healthy	B	A	10	Plasma	Commercial RIA	→ after cure (6 weeks)
LanKarani et al. (2004), Iran [123]	Dyspepsia + healthy	B	A	66	Serum	Commercial ELISA	↑
Shiotani et al. (2005), Japan [77]	Healthy	B	A	132	Serum	Commercial ELISA	→
Konturek et al. (2006), Poland [78]	Healthy	B	B	180	Serum	Human RIA	↑
Plonka et al. (2006), Poland [80]	Healthy	B	C	287	Serum	Commercial RIA	↓
Salles et al. (2006), France [81]	Hospitalized in geriatric	B	A (>75 years)	62	Plasma	Commercial RIA	→
Jun et al. (2007), Korea [87]	CG	B	A	63	Plasma	Commercial RIA	→
Roper et al. (2008), USA [89]	Healthy	M	A	256	Serum	Commercial ELISA	↑
Pacifico et al. (2008), Italy [88]	Healthy + sick (GI symptom)	B	C	85	Serum	Commercial RIA	↓ in HP (+) /↑ after cure
Chuang et al. (2009), Taiwan [91][a]	PU + FD	M	A	145	Plasma	Commercial RIA	→
Chuang et al. (2009), Taiwan [91][a]	PU + FD	W	A	196	Plasma	Commercial RIA	→

A adults, *B* both, *C* child, *W* women, *M* men, *HP H. pylori*, *No* sample size, *CG* chronic gastritis, *FD* functional dyspepsia, *GI* gastrointestinal, *PU* peptic ulcer, *Sick* patients who visited clinics, but the specific condition is not stated, *EIA* enzyme immunoassay, *ELISA* enzyme-linked immunosorbent assay, *RIA* radioimmunoassay
↑, increased (or higher); ↓, decreased (or lower); →, no significant change (or difference)

[a]Articles written in English and assay method were stated

curing *H. pylori* infection [101, 121]. In summary *H. pylori* infection seems to raise gastric leptin production, but its effect on circulating level is not much. Furthermore the effect of *H. pylori* eradication on leptin is not conclusive, yet.

Conclusions

Understanding the changes of acid secretion related with *H. pylori* infection gave basic insights regarding why the same *H. pylori* causes different outcomes among gastric ulcer,

duodenal ulcer, gastric cancer, or asymptomatic histologic gastritis. Gut hormones such as ghrelin and leptin, which are regulated by neuronal, hormonal, and immune process under *H. pylori* infection, are involved not only in gastrointestinal physiology but in systemic energy regulation including adiposity, appetite, or circulation. The change of physiology of G and D cells during *H. pylori* infection and after its eradication has been relatively well evaluated, while those of ghrelin and leptin are still under debate. Investigating these hormonal dynamics altered by *H. pylori* infection could provide an important clinical implication in the prevention and treatment of illnesses including obesity, FD, and GI cancer and give proper evidences of eradicating this microorganism.

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H. pylori Virulence Factors: Toxins (CagA, VacA, DupA, OipA, IceA)

5

Jung Mogg Kim

Abstract

Helicobacter pylori (*H. pylori*) specifically colonizes the human stomach. The majority of bacteria live in the mucus layer overlying gastric epithelial cells and only a small proportion of bacteria are found interacting with the epithelial cells. To succeed in the long-term colonization, *H. pylori* has developed a unique set of virulence factors, which allow survival in a harsh ecological niche. Clinical outcomes associated with *H. pylori* infections are largely mediated by a complex interaction between bacterial, host, and environmental factors. Over the past year, a variety of studies focusing on both host and bacterial factors have proceeded. Among the bacterial factors that contribute to the pathophysiology associated with *H. pylori* infections, it is remarkable that the cytotoxin-associated gene A (CagA) protein is delivered into gastric epithelial cells via bacterial type IV secretion system, leading to induction of diverse immune responses and gastroduodenal diseases. The vacuolating cytotoxin (VacA) is also one of the most important virulence factors and functions as an intracellular-acting protein exotoxin, in which one of the most important target sites for VacA is the mitochondrial inner membrane in the host. In addition, the outer membrane inflammatory protein (OipA), the induced by contact with epithelium (IceA), and duodenal ulcer promoting (*dupA*) gene have emerged as virulence factors, although the specific genes involved in virulence are still being determined. This chapter summarizes the results of the most relevant studies regarding *H. pylori* virulence factors such as CagA, VacA, OipA, IceA, and *dupA* gene and discusses their molecular mechanism for generating gastrointestinal diseases.

Keywords

CagA • *Helicobacter pylori* • VacA • Virulence factor

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5.1 Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative spiral-shaped bacillus, known to reside in the human stomach. It is presumed that the pathogen has undergone evolution along with human beings, ever since it was introduced in Africa at least 50,000 years ago. Accordingly, the primitive properties of *H. pylori* (e.g., the virulence factor) have changed over evolutionary time, whereby each *H. pylori* strain in different locales may have taken different evolution pathways. These bacteria have pathogenetically been identified as a cause of acute and chronic gastritis, peptic ulcers, and gastric cancer. By definition, the potential for pathogens to cause disease is referred to as the virulence, and a factor associated with this virulence is called the virulence factor. In the case of *H. pylori*, the virulence factors for gastrointestinal diseases consist of its structural components and secretory materials. Specifically, its structural components include lipopolysaccharide, outer membrane proteins (OMPs), flagella, and the type IV secretion system (T4SS), while its secretory materials comprise ammonia by urease, cytotoxin-associated gene A (CagA), vacuolating cytotoxin (VacA), and other secretory enzymes [1] (Fig. 5.1).

According to geographical differences with person-to-person variation, *H. pylori* infection rate and disease occurrence are known to be different, supposedly due to the various host factors, types of food intake, environmental factors, etc. In addition, the genetic polymorphism that controls the host inflammatory responses can either increase or decrease the degree of the inflammation and thereby contributes these different clinical aspects of diseases upon *H. pylori* infection [2]. More on the topic regarding genetic polymorphism will be described in the next chapter, while this chapter focuses on *H. pylori* virulence factors, among which are CagA, VacA, outer inflammatory protein (OipA), induced by contact with the epithelium (IceA), and duodenal ulcer promoting (*dupA*) gene.

5.2 Cytotoxin-Associated Gene A (CagA)

CagA is by far one of the most actively investigated virulence factors of *H. pylori. H. pylori* strains isolated from patients can be classified according to the presence or absence of the *cagA* gene, either into the "*cagA*-positive" strain or the "*cagA*-negative" strain. The following experimental results concordantly suggest the possibility of the *cagA* gene being an oncogenic gene of *H. pylori*. Infection of a Mongolian gerbil with a *cagA*-positive strain results in gastric cancer, whereas the exact same strain with *cagA* mutated (the isogenic *cagA* mutant strain) has not been observed to cause gastric cancer [3]. In addition, transgenic mice into which the *cagA* gene was artificially transformed was reported to develop gastric cancer [4]. However, in Western countries, *cagA*-positive strains are reportedly more commonly isolated from gastric ulcer or gastric cancer patients than *cagA*-negative strains [5, 6], whereas in East Asian countries including South Korea, noteworthily, most of the isolated strains have the *cagA* gene regardless of the gastrointestinal diseases [7]. Therefore, rather than association of specific gastrointestinal diseases solely with the presence of the *cagA* gene alone, it would be more appropriate to comprehensively take other virulence factors and host genetic susceptibility into account.

5.2.1 cag Pathogenicity Island (cag *PAI*)

H. pylori strains are considered to be a highly heterogeneous pathogenic entity associated with genetic recombination and gene mutability. As a result of lengthy evolutionary periods of time, therefore, genetic variants originating from even a single strain are found in the stomach of one individual [8]. As a result, although this leads to difficulty in identifying the correlation of virulence factors with diseases, a specific genetic region, called the *cag* pathogenicity island (*cag* PAI), has been presumed to be associated with

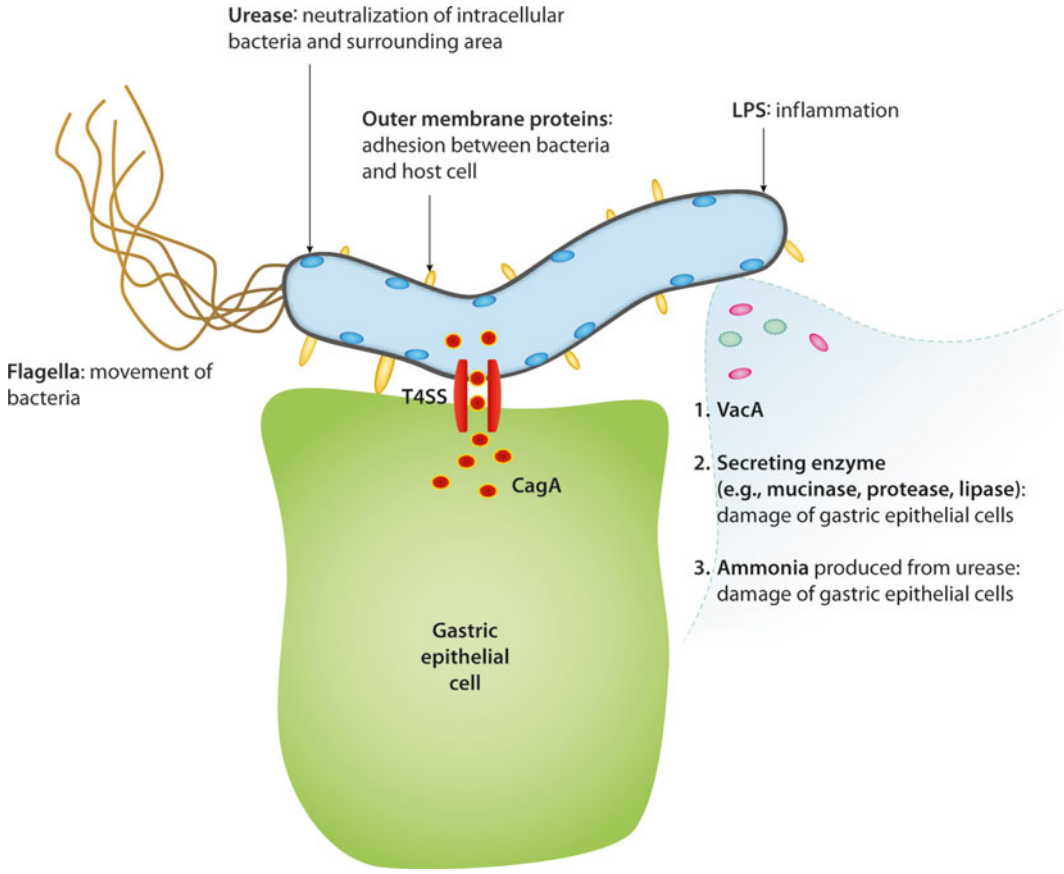

Fig. 5.1 Virulence factors of *H. pylori* and their functions. *CagA* cytotoxin-associated gene A, *T4SS* type IV secretion system, *VacA* vacuolating cytotoxin, *LPS* lipopolysaccharide

the tumor formation. The *cag* PAI is the 40-kb DNA insertion element in the bacterial genes, where about 32 genes – which are used in encoding the genetic information for *cagA*, the type IV secretion system (T4SS), etc. – are located [9]. Therefore, the CagA protein can be considered to be a marker for the presence of the *cag* PAI gene. Specifically, *cag* PAI can be found in 60–70 % of the strains isolated in Western countries, whereas it is found in almost every strain isolated in East Asia. The *cagA*-positive strains are observed adhering to gastric epithelial cells or being located nearby, whereas most of the *cagA*-negative strains are identified in mucus layers [10]. Therefore, the *cag* PAI genotype is a probable factor attributable to influencing the colonization location inside the stomach.

T4SS, which has a needle-shaped protrusion, is involved in transporting the materials from the bacterial cytoplasm into host cells. This structure is found not only in *H. pylori* but also in other bacteria such as genera *Bordetella*, *Bartonella*, *Legionella*, and *Anaplasma*. The bacterial cytoplasmic materials that are injected by *H. pylori* T4SS consist only of the peptidoglycan of the cellular wall component and the CagA protein [11]. When inactivating a gene encoding CagE, which is a protein component of *H. pylori* T4SS, the *H. pylori* proteins no longer move into the gastric epithelial cells. CagL, an adhesion factor, binds to the α5β1 integrin of the gastric epithelial cells in order to activate the CagA protein, which in turn stimulates the transport of toxic materials into the host cells [12]. In addition, CagL, known

to bind to integrin and fibronectin, participates in bridging the bacterial T4SS and the host's α5β1 integrin, which leads to the activation of the focal adhesion kinase (FAK) and Src signaling pathway of the host cells. Meanwhile, CagA, CagI, and CagY bind to the β1 integrin, thus inducing the structural transformation of integrin heterodimer in order to facilitate moving the toxic material [13].

Effects following intracellular delivery of CagA have been proposed as follows [9] (Fig. 5.2): translocation of CagA by the bacterial T4SS may lead to activation of host signaling pathways such as promoting epithelial responses with carcinogenic potential. CagA is phosphorylated by Src and Abl kinases. Consequently, phosphorylated CagA activates the Src homology-2 domain-containing phosphatase 2 (SHP2) and the Erk. Activated Erk signal is associated with morphological changes of gastric epithelial cells, including cellular elongation. In addition, the interaction between phosphorylated CagA and SHP2 lead to inactivation of FAK, which can activate Src. In this model, excessive phosphorylation of CagA can be inhibited by c-Src kinase (Csk). Therefore, unphosphorylated CagA (unmodified CagA) leads to changes in the epithelial cell motility and the proliferation through binding Grb/Sos/Ras and to activation of the Raf/MEK/Erk pathway. On the other hand, unphosphorylated CagA can associate with the tight junction proteins ZO-1 and JAM-A as well as the adherens junction protein E-cadherin, leading to dysregulated junctional complexes [9]. Many studies have been conducted to elucidate molecular mechanisms regarding the CagA-induced clinical outcomes, and it expects to reveal new signaling pathway.

5.2.2 Diversity of the *cagA* Gene

As mentioned, the *cagA* gene is highly heterogeneous among each isolated strains from patients.

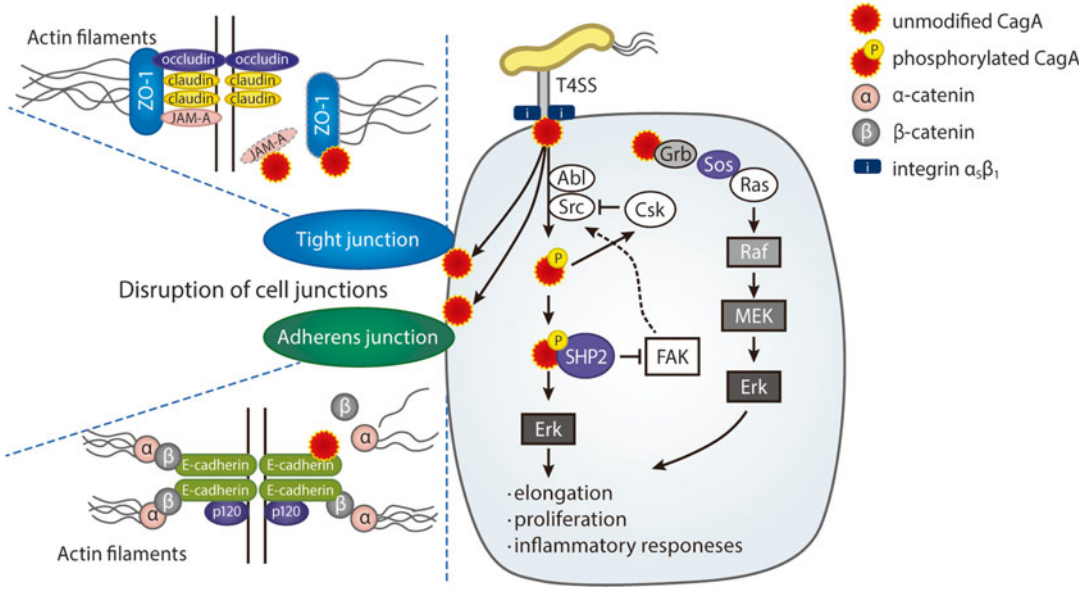

Fig. 5.2 Effects of intracellular delivery of CagA on gastric epithelial cells. Translocation of CagA by the *H. pylori cag* type IV secretion system (T4SS) leads to activation of host signaling pathways that promote morphologic changes and proliferation of gastric epithelial cell, in which their responses may be associated with carcinogenic potential. Unmodified CagA can also associate with the tight junction proteins ZO-1 and JAM-A as well as the adherens junction protein such as E-cadherin, leading to disrupting the cell junctional complexes. In addition, these signaling are associated with the induction of inflammatory responses. *SHP2* the Src homology-2 domain-containing phosphatase 2, *Csk* Src tyrosine kinase (Modified from Noto and Peek [9])

In particular, the numbers for the repeat sequence located at the 3′ region of the *cagA* gene are different across *H. pylori* strains. The repeat sequence is primarily classified into two types: a first repeat and a second repeat. Specifically, the sequences for the second repeat region in the strains isolated from Eastern and Western show substantial differences [6]. The repeat region of the CagA protein has the Glu-Pro-Ile-Tyr-Ala (EPIYA) motif, whose tyrosine (Tyr) residue, being phosphorylated, will be described next. The first repeat region is named sequentially, the EPIYA-A and EPIYA-B segments, and similarly, the second repeat region contains the EPIYA-C and EPIYA-D segments [14].

5.2.3 The Relevance Between the EPIYA Segment and Pathogenicity of CagA

Theories have been recently established for gastric cancers in relation to the number of EPIYA segments. Compared to strains containing only one EPIYA-C segment, published studies have demonstrated that gastric cancer is twice as likely to occur in a patient infected by strains with more than one EPIYA-C segment (e.g., ABC vs. ABCCC) [15, 16]. However, it may be rather questionable to conclude pathogenicity based upon only the number of repeat sequences due to the following considerations: first of all, *H. pylori* strains isolated in Eastern Asia contain mostly only one EPIYA-C segment [17], and, secondly, *H. pylori* strains with multiple EPIYA segments in the repeat region have lower resistance against gastric acids [18], which entails a possible hypothesis that the *H. pylori* strain with multiple EPIYA segments survive upon atrophic gastritis or gastric cancers only in the case of their lower secretion of gastric acids.

5.2.4 Tyrosine Phosphorylation of CagA

Tyrosine residues of the EPIYA motif in CagA protein can be phosphorylated, which then plays a significant role in the pathogenicity of *H. pylori*. Upon injecting CagA into gastric epithelial cells via T4SS, the tyrosine residues of the EPIYA motif undergo phosphorylation by Src or Abl-family tyrosine phosphorylating enzymes [19–21]. Meanwhile, approximately 20 substances in the host cells are estimated to bind to CagA [22], among which, about 10 host cell proteins are phosphorylation-dependent binding partners to CagA [22, 23]. The most representative example for this type of interaction with phosphorylated CagA is the SHP2. SHP2 binds with EPIYA-B, EPIYA-C, and EPIYA-D segments [14]. In particular, the fragment sequence of phosphorylated tyrosine in the EPIYA-D segment (EPIYATIDF) perfectly matches with a high-affinity-binding sequence of the SH2 domain of SHP2. In addition, the EPIYA-A and EPIYA-B segments show a tendency to bind with C-terminal Src tyrosine kinase (Csk), whereas the EPIYA-C segment preferably binds with Ras-GAP and Grb7 [23]. In conclusion, phosphorylated CagA, upon interaction with proteins in host cells, may induce morphological changes of gastric epithelial cells and chromosomal instability [11].

5.2.5 Phosphorylation-Independent Signaling of CagA

Unphosphorylated (unmodified) CagA proteins are also able to induce various responses in host cells. More than ten host cell proteins are known to interact with unphosphorylated CagA [22]. Additionally, in the host cell, the CagA proteins can form phosphorylation-independent dimerization, whose binding contact site is estimated to be the CagA multimerization sequence (FPLxRxxxVxDLSKVG) [24]. In other words, the CagA multimerization sequence binds to partitioning-defective 1 (PAR1)/microtubule affinity-regulating kinase (MARK) in order to form a complex, where the unphosphorylated CagA component of this complex inhibits PAR1 activity in order to induce a variety of cellular transformation including the loss of cell polarity [24–26]. The CagA multimerization sequence is also called the “MARK2/PAR1b kinase inhibitor

(MKI)" [27]. The same region of CagA, which can be involved in the interaction with the c-Met of host cells, is called the "conserved repeat responsible for phosphorylation-independent activity (CRPIA)" [28]. The interaction between CRPIA and c-Met can induce the activation of transcription factors, β-catenin, and nuclear factor (NF)-κB, which, respectively, can stimulate cell growth and the inflammation response. For reference, nowadays, CagA multimerization, MKI, and CRPIA terminologies are used interchangeably, but there has been an increasing need to unify the terminologies from the perspective of all referring to the same genetic sequence.

The host responses incurred by unphosphorylated CagA are exemplified in the destruction of the cell-cell junction between gastric epithelial cells, loss of cell polarity, induction of inflammatory responses, and stimulation of mitogenic responses. Moreover, the unphosphorylated CagA is involved in the interaction with the runt-related transcription factor 3 (RUNX3), where RUNX3, a tumor suppressor, is observed to be mostly in the inactivated status in gastric cancer cells. Therefore, it is postulated that CagA induces ubiquitination of RUNX3 and its decomposition which, in turn, causes gastric cancer [29].

5.3 Vacuolating Cytotoxin (VacA)

VacA can induce the cellular vacuolation and cause direct damage to human cells. Since there is a correlation between this virulence factor and the severity of gastric disease in persons infected with *H. pylori*, VacA, in addition to CagA, is possibly another important factor in the pathophysiology of *H. pylori*.

5.3.1 VacA Structure

VacA, a secretory protein of *H. pylori*, is initially composed in a precursor form with a molecular weight of 140-kDa in the bacteria. The VacA precursor first passes through the bacterial inner membrane with the help of Sec structures and then goes through the β-barrel-type pore of the outer membrane created by the autotransporter of the 40-kDa C-terminal structure protein and is finally secreted as an 88-kDa monomer. In a laboratory condition, VacA, after being purified from the *H. pylori* culture medium, is a water-soluble oligomeric complex or more specifically takes the form of either a hexameric ring or heptameric ring [30]. Therefore, in order to convert the purified VacA proteins to demonstrate virulence when binding to a host cell, either an acidic or alkalic condition should be required to decompose the VacA oligomeric complex into monomers.

5.3.2 *vacA* Gene Diversity

Although almost every *H. pylori* strain possesses the *vacA* gene, the degree of forming capacity of vacuoles in the bacterial culture medium is different for each strain [7, 31]. Various factors have been suggested to address this difference; it may result from the level of *vacA* transcription or the effectiveness of secretion of VacA proteins [32], but the fundamental difference should be attributed to genetic mutation such as nonsense mutation, internal duplication, deletion, or insertion of the *vacA* gene [33]. Consequently, the *vacA* genotype may reflect the difference in the amino acid sequence of VacA proteins, which in turn determines the degree of cell activity in response to the different VacA toxins.

VacA-coding genes are known for its characteristic of high mutability. Thus, the *vacA* gene is shown to have several allelic variants with a different base sequence. Especially, most diversity in the *vacA* gene is concentrated in the signal sequence and mid-region. For the signal sequence, "s1" and "s2" alleles are in the decoding area of the "s-region," where s1 is further divided into its three subtypes of "s1a," "s1b," and "s1c." The "m-region," corresponding to the mid-region, has the two alleles of "m1" and "m2." According to laboratory experiments, the s1-/m1-type strains show high toxicity, whereas the s1-/m2-type strains correspond to low toxicity. In addition, the s2-/m2-type strain does not

have toxicity, and the s2-/m1-type strain is highly rare in its presence [6, 34, 35]. In contrast to East Asia, in Latin America, Middle East, Europe, and Africa, persons infected with *H. pylori* strains with s1 or m1 type are shown to have a higher incidence rate for gastritis and gastric cancer compared to *H. pylori* strains with s2 or m2 type [6].

The "intermediate (i)-region" in the *vacA* gene is located between the "s-region" and the "m-region" [36]. Recently, three primary i-region types (i1, i2, and i3) were identified [37]. Although the role of "i-region" regarding the activity of VacA in host cells has not been elucidated on much, the i1 type, among the three, is reported to generate the most powerful vacuole [38]. In addition, the "i-region" has been suggested to be a more promising prognostic factor for gastrointestinal diseases than the "s-region" or the "m-region" of *vacA* genes, and the i1 type shows a high correlation for gastric adenocarcinoma and gastritis [36, 39]. However, it is acknowledged that there are other research studies with opposing points of view [6]. Currently, the majority of the *vacA* genes isolated in Korea take s1/i1/m1 types [40].

Recently, the "deletion (d)-region" has been found somewhere between the "i-region" and the "m-region" [41]. The "d-region" contains alleles of d1 (no deletion) and d2 (69–81 bp deletion). For *H. pylori* strains isolated in Western countries, d1 is proposed to be a risk factor for gastric mucosal atrophy. Referentially, for the strains isolated in East Asia, s1/i1/d1 types are the most frequently isolated [6, 41].

5.3.3 *vacA* Genotype in Relation to Gastroduodenal Diseases

Similar to the *cagA* genotype, researchers have investigated the relevance between particular *vacA* alleles (i-region, m-region, and s-region) and gastroduodenal diseases. For example, individuals infected with *H. pylori* with *vacA* s1 allele compared to those infected with the *vacA* s2 allele have been reported to be highly correlated with gastroduodenal diseases [34, 42–44]. In addition, *H. pylori* strains with *vacA* s1 allele compared to with *vacA* s2 allele are known to correspond with greater risk for gastric adenocarcinoma [45–47]. Similarly, the *vacA* m1 allele, compared to the *vacA* m2 allele, reveals a greater correlation with gastric adenocarcinoma [45–47], gastric epithelial damage as a pre-stage before gastric cancer, atrophic gastritis, and intestinal metaplasia [34, 48]. Meanwhile, *H. pylori* strains with both *vacA* s1 and *vacA* m1 alleles are not only attributed to greater numbers of bacteria colonized in gastric mucosa and a greater degree of neutrophils invasion into the mucosa [49] but also lead to a greater correlation with gastric and duodenal ulcers [34, 43] and gastric cancers [42, 47, 50]. However, care should be taken with the aforementioned findings due to controversies associated with the possible confounding factors such as the region and age of the people from which those pathogens have been isolated.

5.3.4 Biological Functions of VacA

VacA can form an ion-conducting channel in human cell membrane, in which the channel is selectively permeable to negatively charged ions. Thus, VacA monomers bind to the lipid membrane of the cell surface membrane to create a channel. Although biological effects of VacA seem to depend on the location of the channel being formed, ions and small organic molecules from host cells may be leaked through the channel accordingly. For example, VacA on the cell membrane becomes incorporated into the endosome by endocytosis, in which various ion channels operate to induce water influx in order to form massive vacuoles. In addition, VacA-induced channel formation in the inner membrane of the mitochondria can result in the inner membrane depolarization and eventually apoptosis. This kind of apoptosis is observed not only in gastric epithelial cells but also in eosinophil and dendritic cells [51, 52].

VacA proteins also modify the cytoskeleton organization of gastric epithelial cells [53, 54] and subsequently induce autophagy [55, 56]. This autophagy is presumed to take the role of

restricting the cellular toxicity. In addition, VacA proteins can inhibit T cell activation and proliferation [57] and prevent antigen presentation by B cells [58, 59]. VacA can increase the production of proinflammatory cytokine in mast cells and the infiltration of mast cells [60]. Moreover, VacA can upregulate the calcium influx, the production of reactive oxygen intermediate, and the chemokine expression via NF-κB activation [61]. Furthermore, VacA is capable of affecting neutrophil functions [41, 62]. Based on these reports, immune cells exposed to VacA may result in decreasing the antigen recognition and increasing the cellular toxicity that give rise to gastrointestinal diseases.

Nevertheless, the underlying mechanisms of VacA and CagA in gastric epithelial cells are different. Since CagA proteins require T4SS, which is to be directly injected into the cytoplasm of gastric epithelial cells, it can only operate on cells colonized with *H. pylori*. On the contrary, VacA, a secretory protein, can function not only on gastric epithelial cells with attached pathogens but also extensively spread to the cells that have not been infected yet. The interaction of VacA proteins and CagA proteins may also exert antagonism to each other. For example, CagA can stimulate the expression of the apoptotic suppressor, Mc11, which inhibits the apoptosis induced by VacA [63–65].

5.4 Outer Membrane Inflammatory Protein (OipA)

The outer membrane proteins refer to proteins that present on the outer membrane of a cell, external to the cytoplasm of *H. pylori*. The space between the inner membrane (cytoplasmic membrane) and outer membrane is called the periplasmic space. This space is to store a variety of materials to be secreted to the outside, as well as those from the outside. Recently, more than 32 outer membrane proteins in *H. pylori* have been identified. Most of them are involved in bacterial adhesion [58]. The representative examples include OipA, blood group antigen-binding adhesin (BabA), sialic acid-binding adhesin (SabA), and adherence-associated lipoprotein, of which OipA will be further described.

Among the various OipA functions, the most well-known is the function to induce the expression of interleukin (IL)-8, which stimulates the infiltration of neutrophils. Specifically, OipA and *cag* PAI regulate the activation of the transcription factors such as NF-κB, activator protein-1 (AP-1), and interferon-stimulated responsive element (ISRE)-like element within the IL-8 promoter region in gastric epithelial cells infected with *H. pylori*. Consequently, the activation of these transcription factors results in IL-8 production, leading to the infiltration of neutrophils into gastric mucosa.

In addition to CagA, OipA is involved in β-catenin signal transduction. Thus, the inactivation of the *oipA* gene is reported to decrease the transfer of β-catenin into the nucleus, lowering tumor incidences [3]. Therefore, it is presumed that there may be some type of interaction between OipA and *cag* PAI or between OipA and CagA, which is still to be elucidated. Referentially, the *oipA* gene and *cagA* gene are shown to be highly correlated (correlation coefficient = 0.82) [66]. In addition, *cagA* genes are related to the s-region type of the *vacA* gene and also closely to the *babA* gene [6]. These relationships among virulence factors may raise the possibility of their conveying biological implications and some level of interaction among them. From this viewpoint, rather than assessing the virulence factors individually, it is more appropriate to consider pathogenesis in association with the interaction of these virulence factors integratedly. For the record, the correlation between *cagA*(+), *vacA* s1/i1/m1, *oipA*(+), and *iceA1*(+) strains and the incidences of gastric ulcers are illustrated in research on the strains isolated from South Korea [40].

5.5 Induced by Contact with Epithelium (IceA)

IceA was named to reflect the fact that it is expressed only in the case of *H. pylori* attached to gastric epithelial cells. The *iceA* genes consist of two alleles, *iceA1* and *iceA2* [5]. The sequence of

the *iceA1* allele is similar to that of the *nla*IIIR of *Neisseria lactamica*, another pathogen [67], whereas any homology with the *iceA2* allele has not been found with other pathogens. In addition, a correlation of the *iceA* allele with either the *cagA* or *vacA* genes has not been identified.

The *iceA1* allele is highly correlated with peptic ulcers [5]. The expression of *iceA1* genes requires its direct contact with mucosal epithelial cells, after which activation of the genes results in an increase of IL-8 production, leading to acute inflammatory response [67, 68]. Recently, a research on strains isolated from South Korea has elucidated the high correlation between the presence of the *iceA1* allele and peptic ulcers [40]. The *iceA2* allele is known to be more prevalent in strains isolated from Western countries than in Japan, which suggests a regional difference [69]. Despite these findings, a correlation between the *iceA2* allele and diseases has not been established.

5.6 Duodenal Ulcer Promoting Gene (*dupA*)

Recently, duodenal ulcer promoting (*dupA*) gene is reported to be associated with *H. pylori*-induced diseases such as duodenal ulcer [69]. The length of the *dupA* open reading frame (ORF) depends on the isolated *H. pylori* strains [70, 71]. Based on the sequence of the putative 5′ regions, it has been proposed that *dupA* has two genotypes such as "long type" and "short type" [69]. However, Hussein et al. classified a *dupA* allele with 1884 bp (short-type) as *dupA1* and a truncated version by the mutations as *dupA2* [72]. Some strains have a presence of mutations in *dupA*, which created a premature stop codon [72–74]. Based on these results, it is possible that *H. pylori* strains with these mutated sequences may not produce intact DupA protein.

The *dupA* gene encodes homologues of VirB4 ATPase, which is thought to be involved in DNA uptake/DNA transfer and protein transfer. The absence of the *dupA* gene was associated with increased susceptibility to low pH [76]. The presence of the *dupA* gene was associated with increased IL-8 production from antral gastric mucosa as well as from gastric epithelial cells. In addition, the *dupA* gene is involved in the activation of transcription factors that bind to the IL-8 promoter, such as NF-κB and AP-1 [76]. These results suggest that the *dupA* gene can contribute the inflammatory responses to *H. pylori* infection.

Many *vir* genes exist before and after the region of the *dupA* locus [75]. Thus, the genes such as *virB2*, *virB3*, *virB4* (*dupA*), *virB8*, *virB9*, *virB10*, *virB11*, *virD4*, and *virD2* are found in a strain Shi470 [71]. Since these are structurally similar to the T4SS called *cag* PAI and ComB, it is proposed that their gene cluster may be the "third T4SS" [69]. Considering that complete *dupA* cluster (possessing *dupA* and all adjacent *vir* genes) was significantly associated with duodenal ulcer in the United States [77], complete *dupA* cluster with intact long-type *dupA* might be true virulence factors. Therefore, only strains that are intact *dupA* positive and form a novel T4SS might be involved in gastroduodenal diseases [69]. However, an actual function of *dupA* is still not fully understood, and further studies will help to elucidate of the clinical importance of *dupA*.

Conclusions

Many studies have been conducted to elucidate pathological mechanisms regarding the components originated from and materials secreted from *H. pylori* associated with human gastrointestinal diseases. So far, these researches suggest that the virulence factors, including CagA, VacA, OipA, IceA, and *dupA*, play a key role. Nevertheless, it should be acknowledged that the aforementioned pathological mechanisms of these virulence factors in *H. pylori* infection still do not seem to fit all the pieces together. Above all, the fact that *H. pylori* strains contain more than 1600 genes opens the possibility of finding additional new pathogenic genes in the future. Moreover, host factors (e.g., IL-1 genetic polymorphism) are expected to play a substantial role, more of which will be discussed in the next chapter. Whole genome analyses may be useful for the investigation of genetic

factors that are related to differences in the virulence among strains. Therefore, larger amounts of data will become available in the near future, and other important novel virulence factors can be discovered.

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H. pylori Virulence Factors: Genetic Polymorphism and Disease

6

Young Sun Kim

Abstract

Helicobacter pylori (*H. pylori*) virulence factors are important for clarifying the role of *H. pylori* in the regional differences in the gastric cancer distribution and the pathogenesis of clinically significant diseases such as gastric cancer or peptic ulcer. Genetic polymorphism of *H. pylori* virulence factors differs by geographic region, in which East-Asian-type *cagA* is known to be more virulent than Western type. As there are more repetitions of Glu-Pro-Ile-Tyr-Ala (EPIYA)-C segment in *cagA*, *H. pylori* becomes more virulent, which is associated with gastric cancers in the West. Between genotype m1 and m2 of *vacA* middle region, m1 is more virulent, which is thought to be the cause of the increased prevalence rate of gastric cancers in many East-Asian regions. If all the studies to date are put together, *cagA*, *vacA*, and *oipA* are the factors associated with gastric cancer and *dupA* can be considered to be an important virulent factor for duodenal ulcer, but because different studies have showed different results and particularly results were different by geographic region, more research is needed.

Keywords

Helicobacter pylori • Virulence factor • Genetic polymorphism

6.1 Introduction

Helicobacter pylori (*H. pylori*) has a genome of 1,600 genes, which are well conserved, but its variants are very diverse and complex in terms of combination and structure of the genes by region depending on the physiological and ecological changes in the strains and hosts, which makes an interesting subject as a model for adaptation and evolution of microorganisms [1, 2].

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H. pylori strains have evolved continuously within the human body when mankind left Africa and moved to America and Oceania approximately 58,000 years ago, and it is predicted that the virulence of *H. pylori* has been changed along [1, 3] (Fig. 6.1). For example, a genetic analysis of a strain isolated from Peruvians in South America demonstrated that while *cag* pathogenicity island (PAI) gene of Peruvian natives did not contain cytotoxin-associated gene A (*cagA*) gene, *cagA* gene of Europeans was newly inserted after the arrival of Europeans. And by a comparison of *cagA* DNA sequences, it was also confirmed that genes of Indo-Aryans and Neolithic man in the Crescent region coexist in the Indian strain. These genetic diversities of virulence factors including *cagA* and vacuolating cytotoxin (*vacA*) depending on the region suggest a possibility that pathogenicity of *H. pylori* is an acquired characteristic for survival and adaptation through the process of evolution and transfer [3–5].

H. pylori live in the human stomach for a long time, but most of those infected do not have symptoms and only some of those infected are taken with diseases, which are not necessarily same diseases. Thus, the reason why those infected show different results is because bacterial factors,

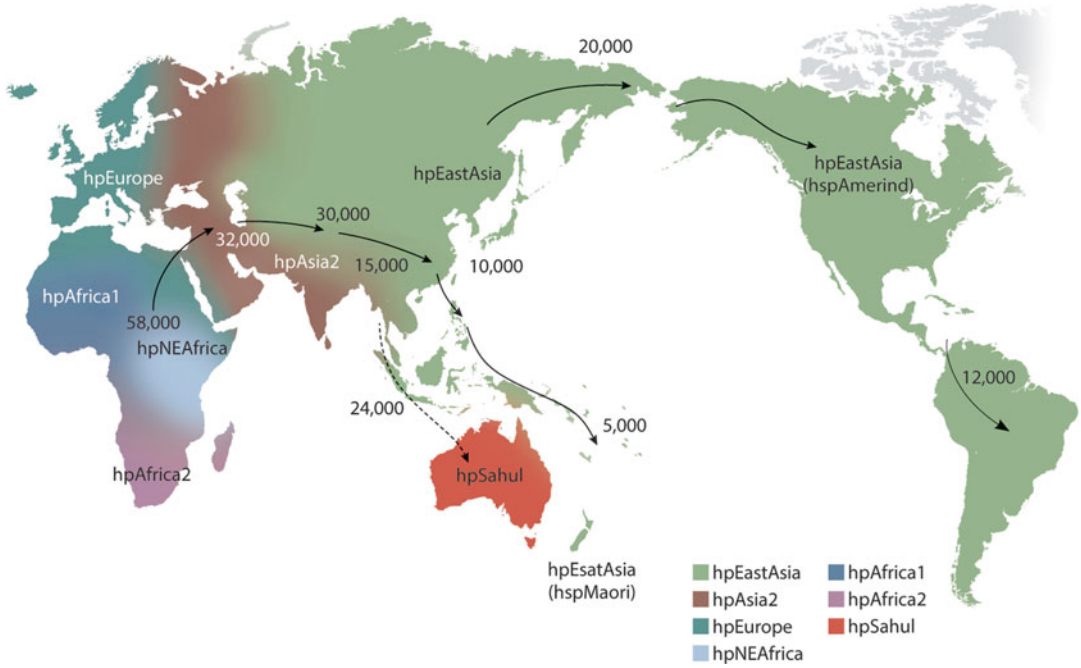

Fig. 6.1 Distribution of *H. pylori* genotypes before Columbus found the New World and human migration to America and Oceania began. There are seven modern *H. pylori* population types: hpEurope, hpEastAsia, hpAfrica1, hpAfrica2, hpAsia2, hpNEAfrica, and hpSahul. hpEurope includes almost all *H. pylori* strains isolated from ethnic Europeans, including people from countries colonized by Europeans. Most *H. pylori* isolates from East Asia belong to hpEastAsia, which includes hspMaori (Polynesians, Melanesians, and native Taiwanese), hspAmerind (American Indians), and hspEAsia (East Asia) subpopulations. hpAsia2 strains are isolated in South, Southeast, and Central Asia; hpAfrica1 in West Africa, South Africa, and African Americans. hpNEAfrica is predominantly made up of isolates from Northeast Africa. hpAfrica2 is very distinct from any other type and has currently only been isolated in South Africa. hpSahul is a novel group specific to *H. pylori* strains isolated from Australian Aborigines and Highlanders of New Guinea. *H. pylori* is predicted to have spread from East Africa over the same time period as anatomically modern humans (~58,000 years ago) and has remained intimately associated with their human hosts ever since. Estimated global patterns of *H. pylori* migration are indicated by *arrows*, and the *numbers* show the estimated time since they migrated (years ago). The *broken arrow* indicates an unconfirmed migration pattern (Adapted from Yamaoka et al. [9])

in particular virulence factors, as well as host and environmental factors play an important role. Although genetic studies of virulence factors have been actively conducted based on the already known DNA sequence of *H. pylori*, crucial virulence factor genes responsible for inducing diseases have not been fully established yet, which can be explained by high degree of variations in the DNA sequences of each *H. pylori* (genetic polymorphism). Such diverse variations are caused by point mutation, substitution, insertion, or deletion, and occasionally several strains of *H. pylori* with different genetic backgrounds are observed in a single individual. Through these observations, it is thought that not only endogenous mutations but also chromosomal rearrangements or recombination occur between each strain [1]. So far, there have been many studies to examine the relationship between diseases and known virulence factors, such as CagA, VacA, induced by contact with epithelium (IceA), outer inflammatory protein (OipA), duodenal ulcer promoting gene (*dupA*) and blood group A antigen-binding adhesion (BabA) [6–14] (Table 6.1). In this chapter, we will discuss the relationship of the genetic polymorphism of these virulence factors and gastroduodenal diseases with geographic differences.

6.2 Cytotoxin-Associated Gene A (*cagA*)

CagA is the most studied virulence factor of *H. pylori*, which is located at one end of the *cag* PAI. The *cag* PAI encodes a type IV secretion system, responsible for the injection of the CagA protein into the host cells [15–17].

CagA-producing strains are reported to be associated with severe clinical outcomes, especially in Western countries. Approximately 60–70 % of isolated *H. pylori* strains from Western countries are known to be positive for *cagA*, which cause more severe inflammatory reactions with increased interleukin (IL)-8 production. It has been reported that individuals infected with *cagA*-positive strains of *H. pylori* are at a higher risk of peptic ulcer or gastric cancer than those infected with *cagA*-negative strains [6, 18]. The studies conducted in Western

Table 6.1 Association of *Helicobacter pylori* virulence factors with host responses and disease outcome

Virulence factor	Colonization/cell damage	Inflammation/immunity	Disease outcome
cag PAI	Increased bacterial density	Increased production of cytokines and antimicrobials Infiltration of immune cells	Gastritis Peptic ulcer Gastric cancer
CagA	Disruption of epithelial junctions Epithelial motility and scattering	Cytokine production Up regulation of oncogenic proteins	Atrophic gastritis Gastric cancer
VacA	Colonization Epithelial permeability Epithelial erosion and necrosis Vacuolation Apoptosis	Disruption of antigen processing Inhibition of T-cell proliferation	Ulceration
OipA	Adherence to epithelial cells Colonization	Cytokine production	Dysplasia Gastric cancer Duodenal ulcer
DupA		Cytokine production	Duodenal ulcer Decreased gastric cancer

Modified from Allison and Ferrero [14]
cag PAI cag pathogenicity island, *CagA* cytotoxin-associated gene A, *VacA* vacuolating cytotoxin, *OipA* outer inflammatory protein, *DupA* duodenal ulcer promoting gene

countries showed that the prevalence of CagA antibodies was significantly higher in peptic ulcer patients or duodenal ulcer patients compared to control, that is, 100 % for peptic ulcer disease, 85–100 % for duodenal ulcer, and 30–60 % for control [7, 8].

A study using Western blotting method for serological detection of antibodies against *cagA* reported that serum antibodies to *cagA* were detected more frequently in gastric carcinoma patients (91 %) than control (72 %) [19].

A cohort study (mean follow-up period of 11.5 years) for 58 subjects infected with *H. pylori* showed that infection with *cagA*-positive *H. pylori* strains is associated with an increased risk for the eventual development of atrophic gastritis and intestinal metaplasia [20]. However, it is difficult to determine the importance of *cagA* in clinical outcomes in East-Asian countries including Korea because nearly all *H. pylori* strains possess *cagA* [8, 10, 12, 13].

6.2.1 *cagA* Type: Western Versus East Asian

There has been an increasing tendency in the last decade to explain the higher incidence of gastric cancer in East Asia using the concept of East-Asian-type *cagA* and Western-type *cagA* [9]. There are different numbers of repeat sequences located in the 3′ region of the *cagA* gene of different *H. pylori* strains. Each repeat region of the *cagA* protein contains Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs, including a tyrosine phosphorylation site. It has now become more common to name the first repeat region as EPIYA-A and EPIYA-B segments and to name the second repeat region as EPIYA-C or EPIYA-D segments [21]. Western-type *cagA* contain EPIYA-A, EPIYA-B, and EPIYA-C segments. By contrast, East-Asian-type *cagA* contain the EPIYA-A, EPIYA-B, and EPIYA-D segments, but not the EPIYA-C segment (Fig. 6.2). Individuals infected with East-Asian-type *cagA* strains were reported to have an increased risk of peptic ulcer or gastric cancer compared with those with Western-type *cagA* strains [9, 11].

However, there are limitations to explain the higher incidence of gastric cancer in East Asia using the concept of East-Asian-type *cagA* and Western-type *cagA* because the incidence of gastric cancer is also high in some regions where Western-type *cagA* strains are reported to account for the majority of *H. pylori* strains (e.g., in Peru and Columbia (age standardized rate [ASR] per 100,000 population 21.2 and 17.4, respectively)) [22]. In addition, in Africa the rate of *H. pylori* infection is high (e.g., 70–97 % of patients with dyspepsia are infected with *H. pylori*, as are 80 % of asymptomatic volunteers), but gastric cancer is generally uncommon; this seemingly contradictory situation is known as the "African enigma" [23]. The incidence of gastric cancer is extremely high in Mali, West Africa (ASR per 100,000 population 20.3), and the frequency of gastric cancer among women in this country is higher than it is among women in Japan (ASR per 100,000 population 19.3 vs. 18.2) [9]. Accordingly, these facts cannot be explained by the presence of East-Asian-type *cagA* versus Western-type *cagA* alone. In a study comparing the *cagA* gene repeat sequences found in Columbia (ASR per 100,000 population 17.4) with those found in the USA (ASR per 100,000 population 4.1) to explain the geographic difference in the incidence of gastric cancer, 100 *H. pylori* isolates from patients with simple gastritis (30 from Columbia and 70 from the USA) were analyzed; 57 % of the isolates from Columbia had two EPIYA-C segments, whereas only 4 % of the isolates from the USA had two EPIYA-C segments (Y. Yamaoka, unpublished data) [9, 11]. Overall, the number of EPIYA-C segments may explain, to some extent, the geographic difference in the incidence of gastric cancer in Western countries [9]. Further research is required to determine whether these subtypes are involved in the pathogenesis of gastric cancer.

In the meta-analysis of case control studies with age- and sex-matched controls, which provided raw data in East-Asian countries including Japan, Korea, and China, in eight studies, the pooled prevalence of CagA seropositivity was 71.6 % (1,019 out of 1,423) in cases and 62.7 % (1,595 out of 2,542) in controls. The estimated

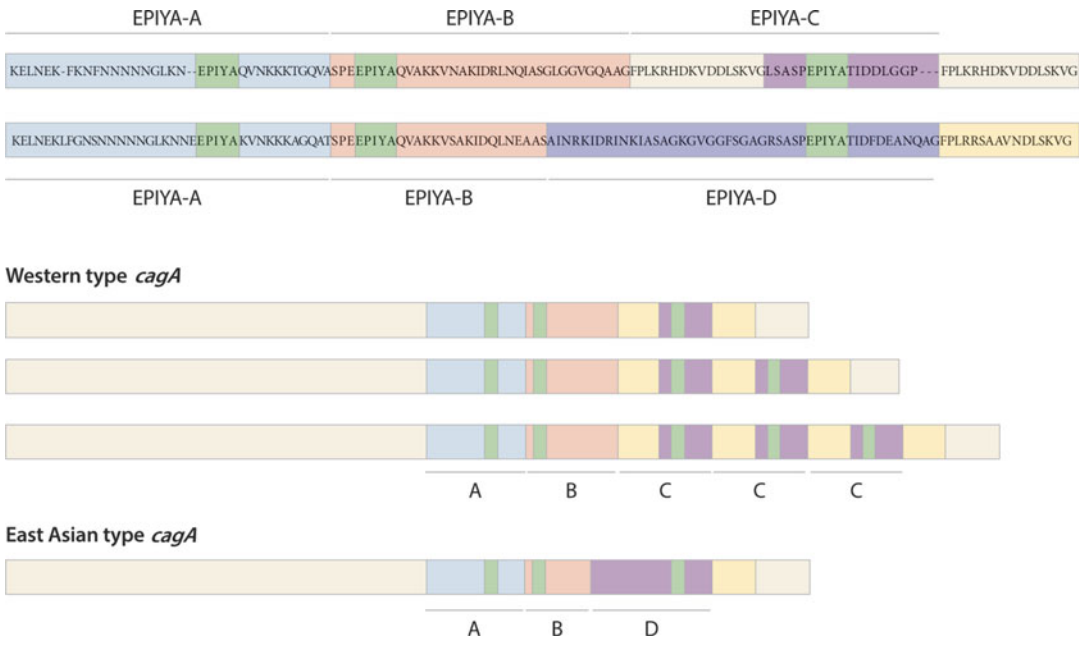

Fig. 6.2 Structural polymorphism in *cagA*. Western-type *cagA* contain EPIYA-A, EPIYA-B, and EPIYA-C segments. By contrast, East-Asian-type *cagA* contain the EPIYA-A, EPIYA-B, and EPIYA-D segments, but not the EPIYA-C segment. The EPIYA motif in each segment (shown in *green*) represents the tyrosine phosphorylation sites of *cagA*. The sequence flanking the tyrosine phosphorylation site of the EPIYA-D segment (EPIYATIDF), but not the EPIYA-C segment (EPIYATIDD), matches perfectly the consensus high-affinity binding sequence for the SH2 domains of SHP2. In Western countries, the incidence of gastric cancer is significantly higher in patients infected with strains containing multiple EPIYA-C segments than in patients infected with strains containing a single EPIYA-C segment (i.e., ABCCC vs. ABC). By contrast, almost all East-Asian strains contain a single EPIYA-D segment. *cagA* forms dimers in cells in a phosphorylation-independent manner, and the CagA multimerization (CM) sequence (also named the conserved repeat responsible for phosphorylation-independent activity [CRPIA] or MARK2/PAR1b kinase inhibitor [MKI]) in *yellow* was identified as the site responsible for dimerization, for inhibition of MARK2/PAR1b kinase, and for the interaction of *cagA* with activated c-Met (Adapted from Yamaoka [9])

overall OR was 1.50 (95 % confidence interval [CI], 1.30–1.72). In meta-analysis in a random effect model, overall OR was 1.81 (95 % CI, 1.30–2.11). This shows that the gastric cancer risk for *cagA*-positive cases was higher overall than in *H. pylori*-infected subjects; however, the OR in East-Asian countries was smaller than the result of the meta-analysis that included Western countries (1.81 vs. 2.64). In addition, the presence of anti-CagA antibodies increases the risk of gastric cancer in the *H. pylori*-negative population. The prevalence of anti-CagA antibodies ranged from 18.2 % to 81.8 % in gastric cancer patients and 9.8–60.2 % in controls [24]. The lower frequency of higher titer IgG antibody in advanced cancer may be due to the increasing extent of intestinal metaplasia associated with transition from the intestinal type of early gastric cancer to advanced cancer, such that the local environment is no longer ideal for the growth of *H. pylori* [9, 25]. CagA antibodies may be positive in patients who have a negative *H. pylori* serologic test since CagA antibodies can potentially remain positive for a longer period of time than the anti-*H. pylori* antibody [9, 24]. This evidence confirms that CagA antibodies can potentially remain positive for a longer period of time than the anti-*H. pylori* antibody [9]. Accordingly, anti-CagA antibody was related to gastric cancer in both *H. pylori*-positive and *H. pylori*-negative populations in East-Asian population [9]. However, it is necessary to evaluate the availability of anti-*H. pylori* antibody plus anti-CagA antibody for screening for risk of gastric cancer.

6.3 Vacuolating Cytotoxin (*vacA*)

All the *H. pylori* strains have a functional *vacA*, which encodes a vacuolating cytotoxin. However, there is significant sequence diversity in *vacA* genes across the many *H. pylori* isolate strains [26–30] (Fig. 6.3). There is variation in the vacuolating activity of different *H. pylori* strains, primarily due to differences in the *vacA* gene structure at the signal (s)-region (s1 and s2) and the middle (m)-region (m1 and m2) [9, 26]. In vitro experiments demonstrated that s1/m1 strains are the most cytotoxic, followed by s1/m2 strains, whereas s2/m2 strains have no cytotoxic activity and s2/m1 strains are rare [26]. *vacA* s1/m1 is the most common strain in East Asia including Korea [27].

6.3.1 Geographic Differences in *vacA* Genotypes

It has been known that there are geographic differences in the distribution of both the *vacA* s- and m-region subtypes [26] (Fig. 6.4). Subtype s1a is predominant in Northern Europe and Australia, whereas subtype s1b is prevalent in South America. Subtype s1c is the major subtype in East Asia, but is extremely rare in Western Europe. The most common *vacA* genotypes in Korea are s1c for the s-region and m1 for the m-region [26–28].

There have been many reports that individuals infected with s1 or m1 *H. pylori* strains have an increased risk of peptic ulcer or gastric cancer compared with individuals infected with s2 or m2 strains [26–28]. In East Asia including Korea, however, most *H. pylori* strains have an s1-type s-region; therefore, the pathogenic difference cannot be explained by the type of s region present [9].

The clear geographic differences in the distribution of both the *vacA* s- and m-region subtypes strongly suggest a geographic structure of *H. pylori* populations throughout the world [26]. The distribution of both s- and m-region allelic types in Central and South America is similar to that in the Iberian Peninsula. This distribution of *H. pylori vacA* genotypes may reflect the extensive cultural and socioeconomic relationships between these parts of the world during past centuries. Particular *H. pylori* strains may have been spread through Central and South America by colonization of these areas by Spanish and Portuguese descendants. Similarly, subtype s1a is more common in the northern European countries and its predominance in Commonwealth countries (Canada and Australia) again may reflect historic relationships. It is unknown whether the evolution of both subtypes occurred in Europe. Within Europe, the distribution gradient of subtypes s1a (northern and eastern Europe) and s1b (Iberian Peninsula) was highly consistent among all tested strains from 12 different countries. If subtypes s1a and s1b already existed for a long time in Europe and were freely movable, a more homogeneous distribution would have been expected. On the other hand, if transmission

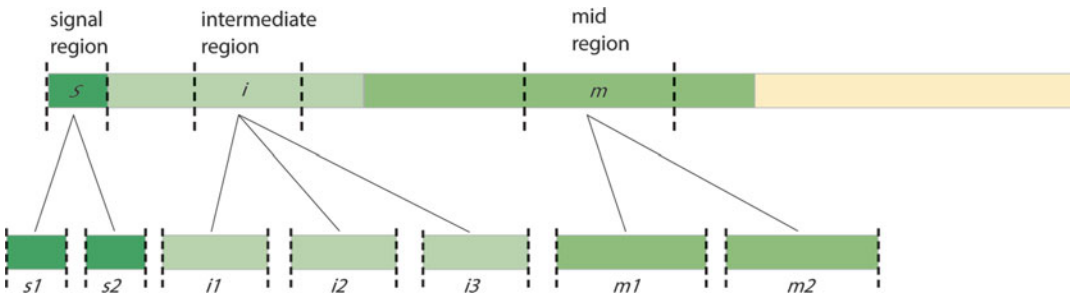

Fig. 6.3 *vacA* allelic diversity and structure. Significant allelic diversity exists in three regions of the *vacA* gene: the signal region (*s1* and *s2*), the intermediate region (*i1*, *i2*, and *i3*), and the mid-region (*m1* and *m2*) (Adapted from Palframan et al. [30])

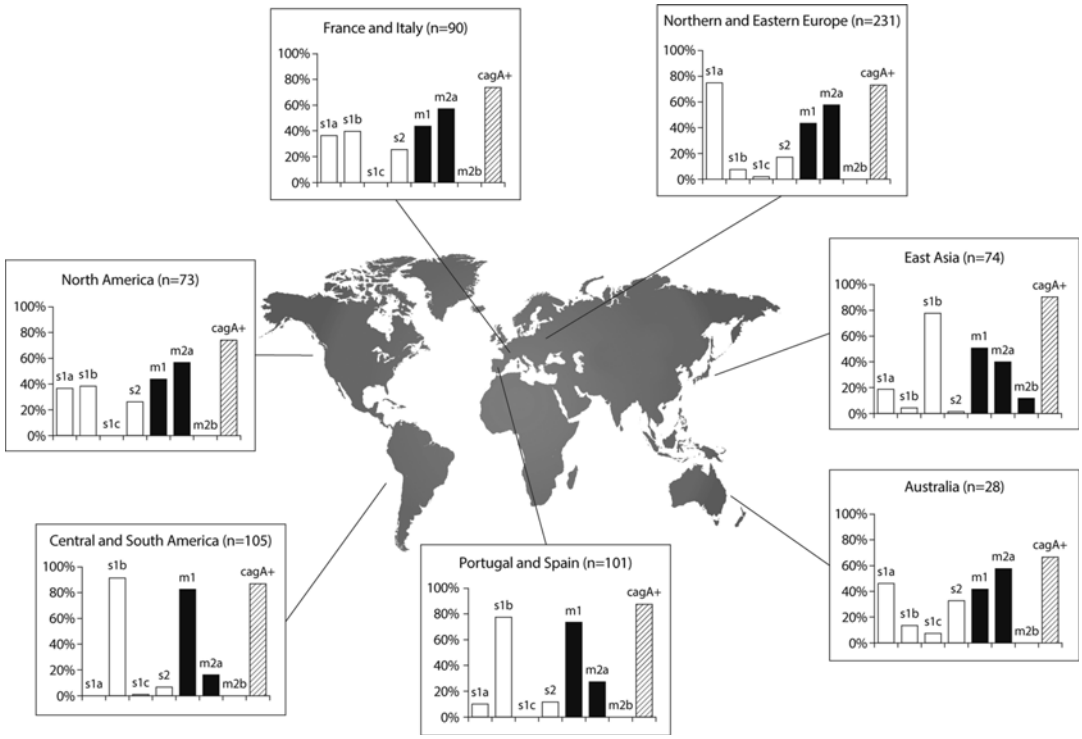

Fig. 6.4 Distribution of *vacA* s- and m-region and *cagA* genotypes of *H. pylori* strains from different parts of the world. For each region, the prevalence of each type (s1a, s1b, s1c, s2, m1, m2a, and m2b; *cagA* positive) is given as a percentage of the total number of strains (shown in *parentheses*). Only strains containing a single *vacA* genotype are represented (Adapted from Van Doorn et al. [26])

of *H. pylori* is highly local, only occurring over very short distances during childhood, this may have prevented a broader geographic distribution of each subtype. An alternative hypothesis would be that the distribution of different subtypes reflects particular adaptation of *H. pylori* to specific host populations [26–28, 31, 32].

In Korean *H. pylori* strain, a wide diversity has been observed in *vacA* s1-region. In the study performed by Kim et al. [12], *vacA* s1a–s1c was determined as the most common subtype in South Korea and considering the positivity of genotypes, *vacA* s1c and s1a were identified as the major genotypes. s1a–s1c was significantly frequent in benign gastric ulcer (73.2 %, $p=0.023$), gastric cancer (73.6 %, $p=0.018$), and dysplasia (71.9 %, $p=0.048$) than control (58. 7 %) (Tables 6.2 and 6.3).

On the other hand, a study performed in Korea for peptic ulcer and gastritis patients by Park et al. [31] showed that s1c, s1a, and s1b were found in 66.1 %, 35.6 %, and 0 % of *H. pylori* isolates, respectively. Another study [8] analyzed *H. pylori* isolates from Seoul, Korea; s1c and s1a were found in 90 % and 7 %, respectively. In both studies, s1 subtypes were not related to clinical outcome. This explanation may support the reason for the possible infection of multiple strains in Korea and other countries. However, further studies will be required to clarify these diversities.

Regarding the combination of the *vacA* s and m genotypes, Japanese strain from Okinawa showed that the *vacA* s1/m1 genotype was significantly higher in strains from gastric ulcer (79.2 %) and gastric cancer (87.5 %) than those from gastritis (59.2 %) ($p=0.002$ and 0.006, respectively). The prevalence of the *vacA* s1/m2 genotype tended to be higher in strains from patients with duodenal ulcer than those from

Table 6.2 Positivity of *vacA*, *cagA*, *iceA*, *oipA*, and *dupA* of *H. pylori* in 401 colonies from Korea

	Control ($n=75$[a])	BGU ($n=71$[a])	DU ($n=102$[a])	Stomach cancer ($n=121$[a])	Dysplasia ($n=32$[a])	Total ($n=401$[a])	p value
vacA s1 (%)	75/75 (100.0)	71/71 (100.0)	102/102 (100.0)	121/121 (100.0)	32/32 (100.0)	401/401 (100.0)	0.999
s1a	61/75 (81.3)	67/71 (94.4)*	84/102 (82.4)	108/121 (89.3)	31/34 (96.9)*	351/401 (87.5)	0.026
s1b	9/75 (12.0)	6/71 (8.5)	15/102 (14.7)	17/121 (14.0)	7/32 (21.9)	54/401 (13.5)	0.436
s1c	67/75 (89.3)	58/71 (81.7)	83/102 (81.4)	113/121 (93.4)	28/32 (87.5)	349/401 (87.0)	0.052
vacA m1	70/75 (93.3)	69/71 (97.2)	89/102 (87.3)	116/121 (95.9)	32/32 (100.0)	376/401 (92.9)	0.018
vacA m2	5/75 (6.7)	2/71 (2.8)	13/102 (12.7)	5/121 (4.1)	0/32 (0.0)	25/401 (6.2)	0.018
vacA i1	75/75 (100.0)	71/71 (100.0)	102/102 (100.0)	121/121 (100.0)	32/32 (100.0)	401/401 (100.0)	0.999
vacA i2	10/75 (13.3)	14/71 (19.7)	10/102 (9.8)	15/121 (12.4)	1/32 (3.1)	50/401 (12.5)	0.153
cagA	59/66 (89.4)	53/64 (82.8)	82/96 (85.4)	96/106 (90.6)	23/27 (85.2)	313/359 (87.2)	0.586
EPIYA-C	1/46 (2.2)	1/39 (2.6)	3/52 (5.8)	2/78 (2.6)	0/19 (0.0)	7/234 (3.0)	0.709
EPIYA-D	45/46 (97.8)	38/39 (97.4)	49/52 (94.2)	76/78 (97.4)	19/19 (100.0)	227/234 (97.0)	0.709
*iceA*1	74/75 (98.7)	68/71 (95.8)	96/102 (94.1)	114/121 (94.2)	32/32 (100.0)	384/401 (95.8)	0.359
*iceA*2	27/75 (36.0)	27/71 (38.0)	45/102 (44.1)	67/119 (56.3)*	16/32 (50.0)	182/399 (45.6)	0.036
oipA	63/75 (84.0)	63/71 (88.7)	96/100 (96.0)*	114/120 (95.0)*	27/32 (84.4)	363/398 (91.2)	0.015
dupA	12/72 (16.7)	52/64 (81.3)*	74/99 (74.7)*	22/95 (23.2)	10/24 (41.7)*	170/354 (48.0)	<0.001

Adapted from Kim et al. [12]
Missing values are not included. Each number behind the dash is the total number of colonies which were analyzed
BGU benign gastric ulcer, *DU* duodenal ulcer, *H. pylori Helicobacter pylori*, *EPIYA-C* Western-type *cagA*, *EPIYA-D* East-Asian-type *cagA*
*$p<0.05$, comparing with control group
[a]Total number of each group

patients with gastritis (27.2% vs. 17.3%), although the difference did not reach statistical significance ($p=0.08$). The prevalence of the *vacA* s2/m2 genotype was significantly higher in strains from gastritis patients than in those from gastric ulcer, duodenal ulcer, and gastric cancer patients (22.4% vs. 11.9%, 10.5%, and 4.2%, $p=0.04$, 0.01, and 0.04, respectively) [33–35]. These results suggested that diverse *vacA* genotypes contribute to the clinical outcomes in Okinawa and low incidence of gastric cancer in Okinawa [9, 11].

With respect to the m-region, there is variation within East Asia. Although m1 strains are common in parts of north East Asia, such as Japan and South Korea, m2 strains are predominant in parts of south East Asia, such as Taiwan and Vietnam [35–37]. As the incidence of gastric cancer is higher in the north than in the south of East Asia, the m-region may play a role in the regional difference in disease pattern [9]. Even within Vietnam, the incidence of gastric cancer is approximately 1.5 times higher in Hanoi in the north than in Ho Chi Minh in the south of the

Table 6.3 *vacA* and *iceA* subtypes of *H. pylori* in 401 colonies from Korea

	Control ($n=75^a$)	BGU ($n=71^a$)	DU ($n=102^a$)	Stomach cancer ($n=121^a$)	Dysplasia ($n=32^a$)	Total ($n=401^a$)
vacA s1 (%)						
a	8/75 (10.7)	9/71 (12.7)	12/102 (11.8)	4/121 (3.3)	1/32 (3.1)	34/401 (8.5)
b	–	–	–	–	–	–
c	14/75 (18.7)	4/71 (5.6)*	18/102 (17.6)	11/121 (9.1)	1/32 (3.1)*	48/401 (12.0)
a–b	–	4/71 (5.6)	7/102 (6.9)	2/121 (1.7)	3/32 (9.4)	16/401 (4.0)
a–c	44/75 (58.7)	52/71 (73.2)*	57/102 (55.9)	89/121 (73.6)*	23/32 (71.9)*	265/401 (66.1)
others	9/75 (12.0)	2/71 (2.8)	8/102 (7.8)	15/121 (12.4)	4/32 (12.5)	38/401 (9.5)
vacA m						
m1	70/75 (93.3)	69/71 (97.2)	89/102 (87.3)	116/121 (95.9)	32/32 (100.0)	376/401 (92.9)
m2	5/75 (6.7)	2/71 (2.8)	13/102 (12.7)	5/121 (4.1)	0/32 (0.0)	25/401 (6.2)
vacA i						
i1	65/75 (86.7)	57/71 (80.3)	92/102 (90.2)	106/121 (87.6)	31/32 (96.9)	351/401 (87.5)
i2	–	–	–	–	–	–
i1 and i2	10/75 (13.3)	14/71 (19.7)	10/102 (9.8)	15/121 (12.4)	1/32 (3.1)	50/401 (12.5)
vacA						
s1i1m1	60/75 (80.0)	56/71 (78.9)	83/102 (81.4)	103/121 (85.1)*	31/32 (96.9)*	333/401 (83.0)
s1i1m2	5/75 (6.7)	1/71 (1.4)	9/102 (8.8)	3/121 (2.5)	–	18/401 (4.5)
s1i1 and i2m1	10/75 (13.3)	13/71 (18.3)	6/102 (5.9)	13/121 (10.7)	1/32 (3.1)	43/401 (10.7)
s1i1 and i2m2	–	1/71 (1.4)	4/102 (3.9)	2/121 (1.7)	–	7/401 (1.7)
iceA						
1	48/75 (64.0)	44/71 (62.0)	57/102 (55.9)	51/119 (42.9)*	16/32 (50.0)	216/399 (54.1)
2		3/71 (4.2)	6/102 (5.9)	7/119 (5.9)	–	16/399 (4.0)
1 and 2	27/75 (36.0)	24/71 (33.8)	39/102 (38.2)	61/119 (51.3)*	16/30 (50.0)	167/399 (41.9)

Adapted from Kim et al. [12]
Missing values are not included. Each number behind the dash is the total number of colonies which were analyzed
BGU benign gastric ulcer, *DU* duodenal ulcer, *H. pylori Helicobacter pylori*
*$p<0.05$, comparing with control group
[a]Total number of each group

country. Comparison of two geographically distant cities in Vietnam, Hanoi and Ho Chi Minh, showed that the *vacA* m1 genotype, thought to be more toxic than the *vacA* m2 type, is more prevalent in Hanoi, where the incidence of gastric cancer is higher than in Ho Chi Minh [36, 37]. These data support the hypothesis that the *vacA* m1 type is closely associated with gastric carcinogenesis [9].

In 2007, a third disease-related region of *vacA* was identified between the s-region and the m-region; it was named the intermediate (i)-region [38]. Yamaoka et al. [9] reported that all s1/m1 strains were classified as type i1, and all s2/m2 strains were classified as type i2, but s1/m2 strains were classified as either type i1 or i2, and i1 strains were shown to be more pathogenic. Typing of the i-region was also reported to be more effective for determining the risk of gastric cancer in Iranian patients than typing of the s-region or m-region [39]. However, in a study of patients from East and Southeast Asia, there was no association between the i-region and disease [11, 40].

More recently, a fourth disease-related region – the deletion (d) region – was identified between the i-region and the m- region [9]. The d-region is divided into d1 (no deletion) and d2 (a 69–81 bp deletion). The study of Western strains demonstrated that

d1 was a risk factor for gastric mucosal atrophy; however, almost all East-Asian strains are classified as s1/i1/d1. Therefore, further researches are needed to clarify association between d- or i-region and clinical outcome [11, 39, 40].

6.4 Induced by Contact with Epithelium (*iceA*)

An initial series of studies showed that *iceA* has two main allelic variants, *iceA*1 and *iceA*2. The expression of *iceA*1 was upregulated on contact between *H. pylori* and human epithelial cells, and the *iceA*1 genotype was linked with enhanced mucosal IL-8 expression and acute antral inflammation [9, 11, 41]. The *iceA* type 1 allele is reported to be predominant in Japan and Korea, and the *iceA* type 2 allele in the United States and Colombia [8, 9, 35, 41].

In a meta-analysis [42] including 50 studies with a total of 5,357 patients to confirm the relationship between the *iceA* allelic type and clinical outcomes, the overall prevalence of *iceA*1 was significantly higher by 64.6 % (1,791/2,771) in Asian countries than in Western countries (64.6 % vs. 42.1 %), whereas the prevalence of *iceA2* was more prevalent in Western countries than in Asian countries (45.1 % vs. 25.8 %). Sensitivity analysis showed that the presence of *iceA1* was significantly associated with peptic ulcer (OR 1.25; 95 % CI, 1.08–1.44); however, the presence of *iceA2* was inversely associated with peptic ulcer (OR 0.76; 95 % CI, 0.65–0.89). These findings were significant in Western countries. And the presence of *iceA* was not associated with gastric cancer. Most studies examined the *cagA* status; however, only 15 studies examined the correlation and only 2 showed a positive correlation between the presence of *cagA* and *iceA1*. It is possible that *iceA* is a discriminating factor for peptic ulcer which is independent of *cagA* [9, 11]. However, it is a result that has not been confirmed in other countries, such as Japan and Korea [8, 11, 41]. Kim et al. [12] reported that the positivity of *iceA*1 in Korean *H. pylori* isolates was more than 95 %, and *iceA*2 was variable from 35 % to 55 % among clinical disease. In addition, strains expressed with *iceA*2 alone were only about 5 %, and most strains were detected with *iceA*1 along with *iceA*2. This result could support a wide diversity of *H. pylori* infection in South Korea.

In summary, despite numerous attempts to relate *vacA* genotypes to outcome or disease pathogenesis, no consistent associations or demonstrable biologic basis for the putative associations has appeared. Further studies are warranted.

6.5 Outer Membrane Protein

Outer membrane protein has been shown to act as an adhesion that facilitates bacterial attachment to the host epithelium. Approximately 4 % of the *H. pylori* genome is predicted to encode outer membrane proteins. There have been many studies that investigate the expression status of outer membrane protein such as OipA, BabA, or BabB in different clinical outcomes [9, 43–45].

6.5.1 Outer Inflammation Protein (*oipA*)

oipA was initially identified as a proinflammatory response-inducing protein based, in part, on the fact that *oipA* isogenic mutants reduced the production of IL-8 from gastric epithelial cell lines [9, 43–45]. Transcription of *IL-8* genes in both *oipA* and *cag* PAI dependent through interactions with different binding sites are involved, such as transcription factors within the IL-8 promoter for nuclear factor-κB (NF-κB), activator protein 1 (AP-1), and interferon-stimulated responsive element (ISRE)-like element [9]. *oipA* functional status was related to clinical presentation, *H. pylori* density, and gastric inflammation. *cag* PAI, *babA2*, or *vacA* status appears important only as surrogate markers for a functional *oipA* gene. It is also important to reconfirm that the presence of *cagA*, *vacA*, and *oipA* are linked such that typically *H. pylori* either produce all of these proteins or none of them and that clinical outcomes, such as peptic ulcer and

gastric cancer, are associated with strains with and without these virulence factors. However, strains with recognized virulence factors tend to produce more severe inflammation and are associated with higher risk of these important clinical outcomes [9, 11].

In a study [45] analyzed *H. pylori* isolates from the United States and Colombia, an independent univariate analysis, showed that the *oipA* "on," *cag* PAI-positive, *vacA* s1 genotype, and the *babA*-positive type were all related to a risk of duodenal ulcer. Importantly, a multiple logistic regression analysis showed that only the *oipA* "on" status was an independent determinant predictor of duodenal ulcer from gastritis. This finding was confirmed in another study [46] based on a non-overlapping cohort of 200 patients who were examined for four outer membrane proteins, OipA, BabA, BabB, and sialic acid-binding adhesion (SabA), by immunoblot, in which multiple logistic regression analysis showed that only *oipA*-positive status was an independent determinant predictor of gastric cancer vs. gastritis and duodenal ulcer vs. gastritis.

However, strains in Asia appeared to be different from those in Western countries in the aspect of outer membrane proteins and their actions [9, 11]. Kim et al. [12] reported that *oipA* was more frequently detected in duodenal ulcer and gastric cancer, but significant effect on gastroduodenal diseases was not found in Korean *H. pylori* isolates. These results could also be an evidence of the different distribution of virulence factors according to geographic differences.

The *H. pylori oipA* has been demonstrated to be a potential antigen for a vaccine. Recently, *oipA* have been tested in mice and vector-based approaches and/or multicomponent vaccines have been investigated [46]. The study showed that *H. pylori oipA* encoding construct is capable of inducing humoral and cellular responses in immunized mice. The antibody response profiles elicited by the DNA vaccine alone administered intradermally (the gene gun method) showed that it produced a Th2 immune response, while co-delivery of *IL-2* and *LTB* gene encoding constructs promoted a Th1-biased immune response. Further studies warranted for developing vaccination for *H. pylori*.

6.5.2 Duodenal Ulcer Promoting Gene A (*dupA*)

In 2005, the first disease-specific *H. pylori* virulence factor that induced duodenal ulcer and had a suppressive action on gastric cancer was identified and was named duodenal ulcer promoting gene A (*dupA*) [47, 48]. The presence of *dupA* was associated with elevated IL-8 production in the antrum (i.e., antrum-predominant gastritis – a feature of duodenal ulcer disease) and has been reported to induce IL-12 production from monocytes [49].

In an initial study of a total of 500 *H. pylori* isolates, including 160 from Japan, 175 from South Korea, and 165 from Colombia, the positive rate for the *dupA* gene was high in patients with duodenal ulcer and low in patients with gastric cancer, regardless of a patients' nationality (42% vs. 9% on average) [47]. In the study analyzed 401 Korean *H. pylori* isolates by Kim et al. [12], the prevalence of *dupA* was 48.0%. Infection by *dupA*-positive *H. pylori* showed an increased risk of gastric ulcer (OR 33.06; 95% CI, 11.91–91.79) and duodenal ulcer (OR 15.60; 95% CI, 6.49–37.49). More than 75% of colonies with gastric ulcer and duodenal ulcer expressed *dupA*, which suggests that *dupA* may be a fundamental factor for the development of peptic ulcer diseases in South Korea.

However, Brazil, Singapore, Malaysia, and Japan failed to demonstrate a correlation between the presence of the *dupA* gene and disease [9]. An academic report on Brazilian strains by Queiroz et al. [50] showed that a *dupA* gene mutation (deletion or insertion) was found in 50% of patients with gastric cancer, whereas it was found in only approximately 20% of patients with duodenal ulcer. As a result, the positive rate for the functional *dupA* gene was considerably higher in patients with duodenal ulcer than in patients with gastric cancer. Further investigation might clarify the effect of functional *dupA* on various gastroduodenal diseases.

6.5.3 Blood Group A Antigen-Binding Adhesion (*babA*)

babA-mediated adherence of *H. pylori* to the gastric epithelium plays a critical role in the efficient

delivery of bacterial virulence factors that damage host tissue [51, 52]. Interactions between BabA and Lewis b (Leb)-related antigens are the best characterized adhesion receptor interactions in *H. pylori*. The *babA* gene was initially cloned from strain CCUG17875, which contains a silent *babA*1 gene and an expressed *babA*2 gene [9, 51]. A number of studies have suggested a relation between *babA2*-positive *H. pylori* and increased cellular mucosal inflammations and an increased risk of developing clinical outcomes [9].

Gerhard et al. [51] reported that the presence of *babA2* could be regarded as a good indicator of the ability of strains to express the Lewis b antigen-binding adhesion and that *babA2* is significantly associated with duodenal ulcer in *H. pylori* isolated from a German population. The incidence of the *babA2* genotype was about 72 % in their study (duodenal ulcer 100 %, gastric cancer 77.8 %, and gastritis 51.4 %).

However, Kim et al. [53] reported that the incidence of *babA* was low and was not related to peptic ulcer disease in Korea. The presence of the *babA* genes in *H. pylori* isolates from peptic ulcer and gastritis patients was 27.3 % and 26.3 %, respectively ($p=0.578$). In addition, the four pathogenicity-related genes, *cagA*, *vacA* s1c/m1, *iceA1*, and *babA*, did not correlate with other genes. In a Japanese study by Fujimoto et al. [54], all strains from East Asia expressed BabA protein, and 24 (9.8 %) of the Western strains were *babA* negative. For these strains, the *babA*-negative status was correlated inversely with *cagA* or *vacA* s status (i.e., only 1 [4.2 %] and none [0 %] of these *babA*-negative strains were *cagA*- or *vacA* s1-positive, respectively). Most (91 %) Western strains were classified as either *cagA*-positive/*vacA* s1-positive/BabA-H (triple positive, 76 %), *cagA*-positive/*vacA* s1-positive/BabA-L (6.1 %), or *cagA*-negative/*vacA* s2-positive/*babA*-negative strains (9.4 %). However, there was no relationship between the triple-positive strains and clinical outcome. *babA*-negative status is associated with mild gastric injury and lower *H. pylori* density. *babA*-negative strains also are associated infrequently with duodenal ulcer or gastric cancer.

However, because *babA*-negative status is linked closely to *cagA*-negative/*vacA* s2 status, potential interactions between these different putative virulence factors cannot be ruled out.

In summary, it remains unclear how BabA expression is regulated or if expressing low levels of BabA has a direct role in the pathogenesis of duodenal ulcer or gastric cancer [11]. Further studies are warranted.

Conclusions

The *H. pylori* virulence factors are important for clarifying the role of *H. pylori* in the regional differences in the gastric cancer distribution and the pathogenesis of clinically significant diseases such as gastric cancer or peptic ulcer. If all the studies to data are put together, *cagA*, *vacA*, and *oipA* are the factors associated with gastric cancer, and *dupA* can be considered to be an important virulent factor for duodenal ulcer, but because different studies have showed different results and particularly results were different by geographic region, more research is needed.

The biggest reasons why there is a limit in clarifying the relationship between *H. pylori* and diseases only by the virulence factors are as follows: *H. pylori* is composed of nearly 1,600 genes; thus, it is possible that a pathogenic gene which has not yet been identified plays a critical role. Moreover, pathogenesis of gastroduodenal diseases including gastric cancer involves several factors such as diet, environmental changes caused by human movement, host factors, or duration of *H. pylori* infection, which should be taken into account along with the virulent factors in the pathogenesis of diseases. In addition, it is needed to better understand and interpret the research methods and terminology when the study results associated with *H. pylori* virulence factors are comprehended and analyzed. Tests for virulence factors are to be made through the use of the strains isolated from the host in vitro or an animal model; however, the actual results caused by these virulence factors in the human body can be different from the results obtained in the laboratory.

Therefore, it should be kept in mind that actual working mechanisms of the *H. pylori* virulence factors in the host can be much more complicated and divers than we imagine.

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Host Factor: Genetic Polymorphism

7

Jung Mook Kang

Abstract

Proinflammatory cytokines and anti-inflammatory cytokines are produced in gastric mucosa from inflammatory cells activated by *Helicobacter pylori* (*H. pylori*) infection. Polymorphisms in these genes are associated with individual differences in cytokine messenger RNA levels, which result in different gastric mucosal inflammation, different acid inhibition, and different gastroduodenal disease risks in response to *H. pylori* infection. Of the inflammatory cytokines, polymorphisms related to interleukin (IL)-1β and tumor necrosis factor (TNF)-α, IL-10, and IL-8 gene have been reported to be associated with the risk of gastric cancer. A functional polymorphism within toll-like receptor 4 (TLR4) has also been demonstrated to increase the risk for gastric atrophy and gastric cancer. However, the prevalence of cytokine gene genotypes differs between Western and Asian populations, and the role of genetic polymorphism on gastric cancer related to *H. pylori* might be different between race and geographic region.

Keywords

Genetic polymorphism • Cytokine • *Helicobacter pylori*

7.1 Introduction

Helicobacter pylori (*H. pylori*) infects over half of the world population, but there is variation in incidence among different geographic regions. Eighty-five percent of *H. pylori*-infected individuals remain lifelong asymptomatic, while only 1 % of these individuals develop gastric cancer and 10 % develop peptic ulcer [1]. Indeed, the clinical consequences of infection by *H. pylori* are determined by multiple factors, including genetic predisposition of the host, especially regarding certain cytokine, and receptor gene polymorphisms, gene regulation, environmental factors such as high dietary salt intake, and heterogeneity of *H. pylori* strains [2] (Fig. 7.1). Among them, host genetic factors that affect cytokine polymorphisms may determine why some individuals infected with

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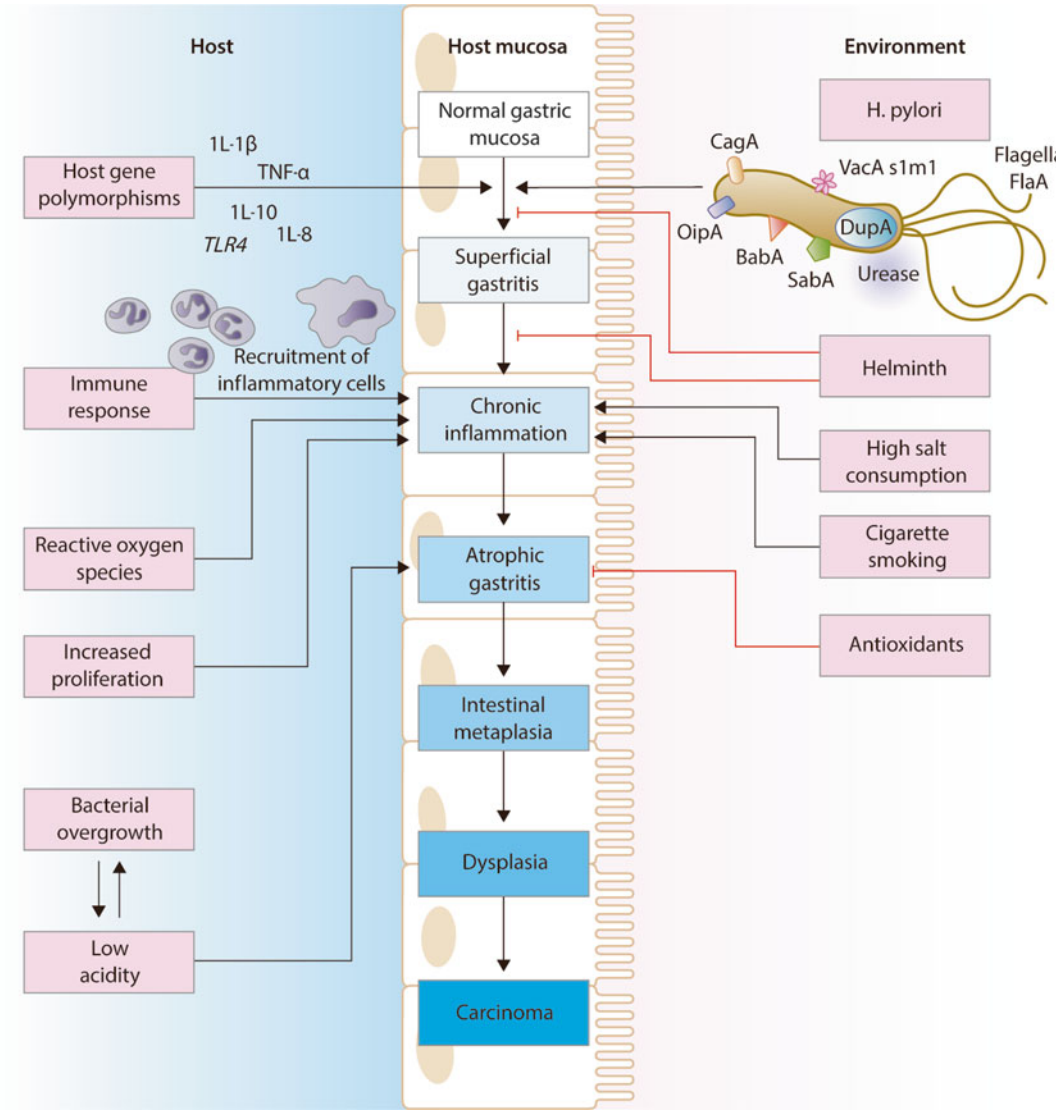

Fig. 7.1 Multifactorial pathway leading to gastric cancer. Many host, bacterial, and environmental factors act in combination to contribute to the precancerous cascade leading to development of gastric cancer (Adapted from Wroblewski et al. [2])

H. pylori develop gastric cancer while others do not. The inflammatory-related genes that have been most frequently studied in relation to gastric cancer, sometimes with conflicting results, are the gene-related interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-10, IL-8, and toll-like receptor 4 (TLR4). Single nucleotide polymorphisms (SNPs) within these and other functional cytokine regions that markedly influence expression and secretion profiles may modify the intensity of the inflammatory response to infectious agents, thereby contributing to variations in gastric cancer risk [3–19] (Table 7.1).

7.2 Interleukin-1β

IL-1β is a Th1 cytokine that inhibits acid secretion and is increased within gastric mucosa of *H. pylori*-infected persons [20]. IL-1β is 100 times

Table 7.1 Host genetic polymorphism that has been associated to the development of gastric cancer

Protein/effectors	Polymorphism	Effect	References
Interleukin 1β	*IL-1B*-31 C *IL-1B*-511 T *IL-1RN* *2	High-level expression of IL-1β, reduction of acid output, corpus colonization by *H. pylori* *IL-1B*-31 C, *IL-1B*-511 T, and *IL-1RN* *2 alleles are associated to an increased risk of gastric cancer	[3–8]
Tumor necrosis factor-α	*TNF*-α-308	High-level expression of TNF-α *TNF*-α-308A are associated to an increased risk of gastric cancer	[9, 10]
Interleukin-10	*IL-10*-1082 G/A *IL-10*-819 C/T *IL-10*-592 C/A *IL-10* ATA *IL-10* GCC	Low secretion of IL-10 is associated to high inflammation and high risk to gastric cancer Haplotype ATA is low IL-10 secreting and haplotype GCC is high IL-10 secreting	[11, 12]
Interleukin-8	*IL-8*-251	High IL-8 levels are found in gastric cancer *IL-8*-251 A allele is associated to a higher production of IL-8	[12–17]
Toll-like receptor 4	*TLR4* Asp299Gly *TLR4* Thr399Ile	TLR-4 is associated to hyporesponsiveness to LPS and therefore to *H. pylori*	[18, 19]

more potent inhibitor than a proton pump inhibitor (PPI) and 6,000 times more potent than histamine-2 receptor antagonists on a molar basis [21, 22]. In animal studies, decreased acid secretion is reported to be accompanied by an elevation of IL-1β mRNA levels in the *H. pylori*-infected gastric mucosa [23].

IL-1β is encoded by *IL-1B* on chromosome 2q13-14, which has polymorphisms at positions *IL-1B*-511 and *IL-1B*-31 base pairs (bp) from the transcriptional start site [24]. The gene for *IL-1 receptor antagonist* (*IL-1RN*) has a variable number tandem repeats (VNTR) of 86 bp in length in intron 2 [25, 26] (Fig. 7.2). The carriers of the *IL-1B*-511 T, *IL-1B*-31 C, and *IL-RN* *2 (2 repeats of 86 bp) allele have significantly higher IL-1β levels than carriers of the other genotype [3] (Fig. 7.3). Consistent with this difference, carriers of the *IL-1B*-511 T, *IL-1B*-31 C alleles, and *IL-1RN* *2 genotype show enhanced suppression of gastric acid secretion, which results in more rapid development of gastric atrophy and a consequently greater risk of developing gastric cancer than in those with the *IL-1B*-511 C, *IL-1B*-31 T, and *IL-1RN* *1 alleles [3–8]. The meta-analysis by Xue et al. [27] showed that *IL-1B*-511 T and *IL-1RN* *2 alleles were significantly associated with an increased risk of developing gastric cancer among Caucasians, but not in Asians. Another meta-analysis by Persson et al. [28] revealed a consistent negative association of *IL-1B*-31 C with gastric cancer in Asians. These suggest that this divergence may reflect the different genetic background related to ethnicity.

7.3 Tumor Necrosis Factor-α

TNF-α is a proinflammatory cytokine induced by *H. pylori* and inhibits gastric acid secretion [29, 30]. TNF-α mRNA and protein expressions were significantly increased in gastric cancer patients [31]. *TNF*-α encoding TNF-α is located on chromosome 6 (6p21). Among the many polymorphisms in *TNF-α* promoter, the *TNF-α*-308 G/A polymorphism were wildly studied, and there is functional study suggesting that the *TNF-α*-308 A allele is associated with increased TNF-α production [9, 10]. To date, several molecular epidemiological studies have been

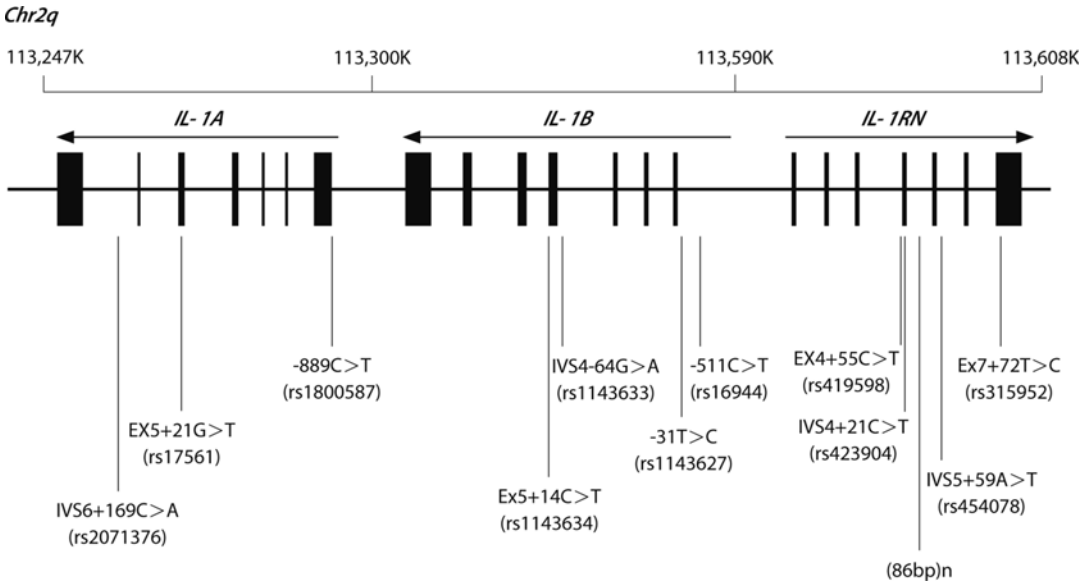

Fig. 7.2 *IL-1A*, *IL-1B*, and *IL-1RN*, showing polymorphic nucleotide sequences (Adapted from Kim et al. [26])

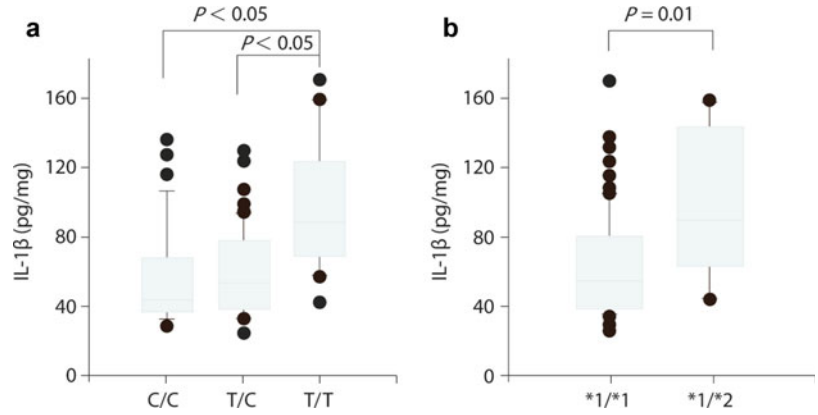

Fig. 7.3 Mucosal IL-1β levels in relation to the genotypes at (**a**) *IL-1B*-511 and (**b**) *IL-1RN* (Modified from Hwang et al. [3])

conducted to investigate the association between the *TNF-α*-308 G/A polymorphism and gastric cancer risk. However, the results from these studies remain inconclusive. There are two published meta-analyses on the associations between the *TNF-α*-308 A alleles and gastric cancer risk that have identified this polymorphism as a risk factor for gastric cancer in Caucasian populations but not in Asian populations [32, 33]. Actually, these polymorphisms are not found in Asian populations because most of their patients have lower producer alleles, *TNF-α*-308 G/G genotypes [8, 34, 35].

7.4 Interleukin-10

IL-10 is an anti-inflammatory cytokine that downregulates cell-mediated immune responses and cytotoxic inflammatory responses [36, 37]. *H. pylori* can lead to IL-10 upregulation as a way to suppress an efficient immune response, which then favors infection and parasite survival [38]. IL-10 levels are elevated in gastric mucosa infected with *H. pylori* and are higher in patients that have severe chronic inflammation [39]. Furthermore, IL-10 mRNA expression and serum levels are elevated in gastric cancer, particularly in the advanced stage [40].

The gene encoding IL-10 is located on chromosome 1 (1q31–32). There are three functional promoter SNPs in the *IL-10* locus: *IL-10*-1082 G/A, *IL-10*-819 C/T, and *IL-10*-592 C/A. It has been reported that these variants (GCC carriers) of IL-10-1082/*IL-10*-819/*IL-10*-592 are associated with increased IL-10 production [41]. The low IL-10 production is associated with increased gastric inflammation intensity and with an enhanced risk of gastric cancer in patients infected with *H. pylori* [2]. However, high IL-10 producer genotype was significantly increased in gastric cancer patients from Taiwan and China [11, 12]. In addition, recent meta-analyses suggested that Asian carriers of the promoter polymorphisms of *IL-10*-592 C and *IL-10*-1082 G alleles may be associated with an increased risk of gastric cancer [42, 43]. In contrast to Asian populations, mostly studies conducted in Western populations have reported a different association. That is there was an association identified between the low IL-10 producer (*IL-10*-592 A alleles) and the risk of GC in populations from the United States and Italy [44, 45]. Several meta-analyses have been performed to evaluate the association between IL-10 polymorphisms and cancer risk [46–49]. When stratifying the data by race, the low IL-10 producer was found to be a protective factor against the development of this neoplasm in Asians but not among Western populations suggesting a possible role of ethnic differences in genetic backgrounds and the environment they lived in.

7.5 Interleukin-8

IL-8, a member of the CXC chemokine family, which was originally identified as a potent chemoattractant for neutrophils and lymphocytes, induces not only cell proliferation and migration but also angiogenesis. The high expression of IL-8 has been demonstrated in gastric mucosa infected with *H. pylori* [50]. IL-8 causes chemotaxis and the activation of inflammatory cells in gastric mucosa infected with *H. pylori* [51]. In addition, IL-8 protein levels are tenfold higher in gastric cancer than in normal gastric tissue [52] and directly correlate with the vascularity of the tumors [53].

The *IL-8* gene, which is located on chromosome 4q12–21, exhibits SNP T-A base transition at 251 bp from the transcription start site [54]. *IL-8*-251 A alleles are associated with higher IL-8 production, and consequently with increased mucosal injury, by activating and recruiting neutrophils in response to infection by *H. pylori* [45, 55]. *H. pylori*-positive patients and carriers of the *IL-8*-251 A/A genotype at position had an increased risk of peptic ulcer disease [13, 14]. Most studies that have reported positive associations for gastric cancer risk have been conducted in Asian populations [12–17], whereas those that have reported negative findings were conducted in Western populations [56, 57]. Moreover, a recent meta-analysis suggests the potential influence of ethnicity in the association of *IL-8*-251 A/T polymorphism with gastric cancer, since it is generally stronger in Asian than in Caucasian population [58].

7.6 Toll-Like Receptor 4

TLR4, which constitutes one of the most active members of TLRs, promotes the transcription of genes involved in immune activation [59]. TLR4 was initially identified as the potential signaling receptor for *H. pylori* on gastric epithelial cells [60]. Two SNPs, located on chromosome 9, are reportedly associated with certain types of digestive cancer [61]. One is Asp299Gly (TLR4-896 A/G) and the second SNP is Thr399Ile (TLR4-1196 C/T) [18]. These mutations alter the extracellular structure of this receptor and are correlated with a blunted response to lipopolysaccharide (LPS) in vivo and in vitro [18, 19]. From a theoretical point of view, the activation of TLR4 may irritate the immune response that protects the organism against tumors, produce a proinflammatory environment, and, thus, may promote carcinogenesis. Several genetic association studies have linked these two SNPs with susceptibility to *H. pylori* infection, atrophic gastritis, and gastric cancer in Caucasian populations [62, 63]. Asp299Gly polymorphism was found to be a risk factor for non-cardia gastric cancer and its precursors including achlorhydria

in Poland, Scotland, and the United States population [62]. Recent meta-analysis including 12 case-control studies demonstrated a significant correlation between the G allele of the Asp299Gly polymorphism and increased risk of gastric cancer but not in Thr399Ile polymorphism [64]. However, there are significant differences in the intercontinental incidence of these two SNPs. Actually, in Asian populations at relatively high risk of *H. pylori* infection and its associated gastric cancer, such as Chinese, Japanese, and Korean populations, the two TLR4 polymorphisms are almost never found [65–67].

Conclusions

Currently, many epidemiologic studies have demonstrated that gastric cancer has a multifactorial etiology and is comodulated by different factors including *H. pylori* infection, lifestyle, socioeconomic status, and environmental factors. In addition, genetic factors are increasingly recognized as major contributors to gastric cancer risk. Recent studies have reported the association between *IL-1B*, *TNF-α*, *IL-10*, and *IL-8* polymorphisms and development of gastric cancer. Furthermore, a functional polymorphism within TLR4 has also been demonstrated to increase the risk for gastric atrophy and gastric cancer. However, the prevalence of cytokine gene genotypes differs between Western and Asian populations, and the role of genetic polymorphism on gastric cancer related to *H. pylori* might be different between race and geographic region. Thus, more large population studies for the analysis of genetic polymorphisms in relation to *H. pylori*-related disease are needed involving bacterial factor and environmental variables (lifestyle, dietary habits) in order to contribute to the understanding of gastric carcinogenesis and for the development of screening programs to prevent gastric cancer.

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Part III

Diagnosis

Serology

8

Nayoung Kim

Abstract

Serological diagnosis is noninvasive and relatively economic and has less possibility of showing false-negative results in the situations of hemorrhagic ulcer or taking medications like antibiotics. In addition, it performs mass epidemiological survey simultaneously. However, it is not recommended for monitoring changes after antimicrobial treatment because this diagnosis hardly differentiates past *Helicobacter pylori* (*H. pylori*) infection and current active infection. There are various types of serological diagnoses of *H. pylori* existence such as bacterial agglutination, complement fixation, indirect immunofluorescence test (IIF), enzyme immunoassay (EIA), and enzyme-linked immunosorbent assay (ELISA). Among those, ELISA is the most popular method in the world.

Keywords

Helicobacter pylori • Serology • Diagnosis • ELISA

8.1 Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative, spiral bacillus that the half of entire global population is suspected to be its carriers. There have been many researches on *H. pylori* since the 1980s because this bacterium has been remarked as a main reason of gastritis, peptic ulcer, and stomach cancer. Especially, the International Agency of Research on Cancer (IRAC) of WHO classifies *H. pylori* as a primary carcinogenic factor of gastric cancer [1], and there are many laboratorial results that support this categorization. Thus, *H. pylori* infection diagnosis is important. There are invasive diagnoses, such as rapid urease test and polymerase chain reaction (PCR), that require endoscopy and noninvasive diagnoses, such as serology and urea breath test (UBT), that do not need endoscopy. Among those diagnoses, serological diagnosis is used widely because it is noninvasive, relatively economic, and can be simultaneously applied to

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many patients for epidemiological survey [2]. This chapter will discuss on the advantages and the disadvantages of serological diagnoses, with emphases on classification of serological test, and its sensitivity and specificity for the diagnosis of *H. pylori* infection in the world.

8.2 Advantages and Disadvantages of Serological Diagnosis

Serological diagnosis can be a useful way to detect *H. pylori* preliminary infection because it is easy, fast, noninvasive, and relatively economic and tends to show less false-negative results under certain situations, such as taking antibiotics or hemorrhagic ulcer condition, compared with other diagnoses. However, the serological diagnosis could show false-positive results for several months or years on samples because it hardly differentiates current active infection from previous infection, which has been treated and no more infection is shown [3]. Thus, the serological diagnosis is not recommended after antimicrobial treatment because an average of 1 year was necessary until either antibody is undetectable or antibody titer is reduced to 50 % after antimicrobial treatment [3]. Rather, it is utilized as a preliminary, selective diagnosis for *H. pylori* infection, not after the antimicrobial treatment [3, 4]. For instance, the serological diagnosis is used for epidemiological survey, not as a diagnosis before or after antimicrobial treatment, in China [5]. In contrary, the diagnosis is recommended to be performed before or after antimicrobial treatment in Japan; *H. pylori* eradication is considered to be successful, if serological antibody titer that is performed from 6 to 12 months after antimicrobial treatment is 50 % less than the titer that is performed before the treatment [5].

8.3 Serological Diagnosis

There are numerous serological diagnoses, such as bacterial agglutination, complement fixation, indirect immunofluorescence test (IIF), enzyme immunoassay (EIA), and enzyme-linked immunosorbent assay (ELISA), and ELISA is the most prevalent diagnosis [4, 6]. This section will introduce these test methods, diagnostic kits, and sensitivity and specificity of each diagnosis.

8.3.1 Bacterial Agglutination, Complement Fixation, and Indirect Immunofluorescence Test (IIF)

Agglutination is a method when a particle with certain size, such as erythrocyte, is merged with suspension-form antibody that is made by absorption of antigen of fine particles like kaolin and latex and form immune complex. This method is used for either blood type determination or bacterial identification. Agglutination kits, which utilize agglutination reagent that binds to *H. pylori* antibodies in serum, are Hemkit® (LD-Diagnostika, Heiden, FRG), Pyloriset Dry (Orion Diagnostica, Espoo, Finland), and so on. Sensitivity and specificity of these agglutination diagnoses are reported to be 71–79 % and 81–82 %, respectively. Also, *H. pylori* strain status of this diagnosis was determined by one of three methods: histological analysis, ^{13}C-urea breath test (^{13}C-UBT), and rapid urease test [7, 8] (Table 8.1).

Next, complement system-related antigen-antibody reactions are cytolytic reaction and complement fixation reaction; the latter method is used for bacterial identification and serological diagnosis of *H. pylori* infection. Specifically, a complement system is added to a mixture that contains immune complexes due to antigen-antibody reaction. The complement system will be absorbed and metabolized, if the immune complex exists in the mixture. Then, antigen-antibody reaction can be analyzed by measuring the amount of reduced complement system compared with the amount of the added system at the beginning. According to the previous research, sensitivity and specificity of complement fixation test (CFT) for *H. pylori* (Institute Virion, Rüschlikon, Switzerland) were 86.6 % and

Table 8.1 Hemagglutination tests for detection of *H. pylori*

Product name	Sensitivity (%)	Specificity (%)	Diagnostic reference test	Reference
Pyloriset dry	91.1	87.5	Rapid urease test	[7]
			^{13}C-UBT, histology	
Hemki®	71–79	81–82		[8]

UBT urea breath test

Table 8.2 Complement fixation tests (CFT) for detection of *H. pylori*

Product name	Sensitivity (%)	Specificity (%)	Diagnostic reference test	Reference
CFT *H. pylori*	86.6	77.6	Histology	[9]
CFT *H. pylori*	71.0	90.0	Culture and histology	[10]

77.6 %, respectively, compared with stomach biopsy [9] (Table 8.2). In addition, another research reported that the sensitivity and the specificity of CFT reagents were 71 % and 90 %, respectively, compared with Giemsa stain [10] (Table 8.2).

Third, a labeled antibody method is defined as attaching a label on an immunoglobulin for performing antigen-antibody reaction, to find antigen existence and to measure the amount of antigen. IIF, EIA, and ELISA belong to labeled antibody method.

Immunofluorescence test utilizes fluorescent pigments, such as fluorescein isothiocyanate. Direct immunofluorescence test is a method that fluorescent substance-attached antibody solution reacts with a slide and then wash it off, and IIF is a technique that a slide reacts with antibody solution and then reacts with fluorescent substance-attached anti-immunoglobulin antibody to diagnose a result. When the slide passes through ultraviolet (UV) light, fluorescent antibody-attached region will be shown as fluorescent green, and the rest of region will be shown as dark. For instance, sensitivity and specificity of Immunfluoreszenz IgG Test (Bios, *Graefelfing, Germany*) were 62.8 % and 74.4 %, respectively, compared with stomach biopsy [9] (Table 8.3). Also, another research reported that the sensitivity and specificity of IIF were 95.9 % and 88.8 %, respectively, compared with culture, rapid urease test, and smears stained (carbolfuchsin) [11] (Table 8.3).

8.3.2 EIA and ELISA

EIA and ELISA are immunoserological method that measures either antigen or antibody; enzyme-linked antigen or antibody reacts with a sample to form an antigen-antibody-enzyme complex. Then, a substrate is added to this complex to induce colorization, to measure the degree of color change to extract, and to quantify either antigen or antibody. Color change can be measured by absorbance, fluorescence substance, or radioactive label, and all of these techniques belong to EIA. For ELISA, the antigen-antibody-enzyme complex is mixed with a substrate to induce coloration and then the color change is measured only with absorbance. ELISA also belonged to EIA. Commercialized EIAs are PYIoriset®-IgG EIA (Orion Diagnostica, Espoo, Finland), Immulite® 2000 *H. pylori* IgG system (Diagnostic Products, Los Angeles, CA), VIDAS® *H. pylori* IgG (BioMérieux, Marcy-l'Étoile, France), Radim® *H. pylori*-EIA-Well (Radim®, Rome, Italy), and so on. Sensitivities of EIA-based test are reported to be 68.0–100 % and 79.3–97.0 %; criteria of *H. pylori* diagnosis of each research are depicted in Table 8.4 [12–17]. Genedia® *H. pylori* (Green Cross Co., Seoul, South Korea), GAP® Test IgG (Bio-Rad, Milan, Italy), and Chorus *Helicobacter* IgG (DIESSE Diagnostica Senese, Siena, Italy) belonged to ELISA test, and their sensitivities and specificities were reported to be 93.2–100 % and 60.0–97.0 %, respectively, as in Table 8.5 [12, 16, 17].

Table 8.3 Indirect immunofluorescence test (IIF) for detection of *H. pylori*

Product name	Sensitivity (%)	Specificity (%)	Diagnostic reference test	Reference
Immunfluoreszenz IgG Test	62.8	74.4	Histology	[9]
IIF	95.9	88.8	Culture, rapid urease test, and stain with carbolfuchsin	[11]

Table 8.4 Enzyme immunoassay (EIA) for detection of *H. pylori*

Product name	Sensitivity (%)	Specificity (%)	Diagnostic reference test	Reference
Pyloriset®-IgG	82.4–92.0	71.4–84.0	Culture and histology	[13]
			Rapid urease test histology or ^{13}C-UBT	[14]
Immulite®	91.0	100	Histology, rapid urease test	[15]
VIDAS®	100	0	Rapid urease test, histology	[14]
Radim®	88.0–95.6	93.8–97.8	Culture, histology, rapid urease test	[12]
			Genedia®	[16]

UBT urea breath test

Table 8.5 Enzyme-linked immunosorbent assay (ELISA) for detection of *H. pylori*

	Enzyme-linked immunosorbent assay (ELISA)			
Product name	Sensitivity (%)	Specificity (%)	Detection of *H. pylori* presence	Reference
Genedia®	93.2–100	81.3–92	Culture, histology, rapid urease test	[12, 16]
			Rapid urease test	[17]
			histology or ^{13}C-UBT	
GAP®	60.0–93.8	79.3–87.5	Culture, histology, rapid urease test	[12]
			Rapid urease test	[17]
			histology or ^{13}C-UBT	

UBT urea breath test

8.3.3 Commercial Serological ELISA Kits Depending on *H. pylori* Antigen

When a comparison of 17 commercial ELISA serological kits was carried out in France, two to four of the ELISAs presented an excellent performance [2, 18]. When a line assay using six recombinant proteins corresponding to virulence factors (CagA, VacA, GroEL, gGT, HcpC, and UreA) was validated on a group of 600 patients (42 % *H. pylori* positive by histology) in Germany, it showed 97.6 % sensitivity and 96.2 % specificity which is an improvement on currently available serological tests [19]. The same group in collaboration with researchers in Iran was able to identify an *H. pylori* protein, FliD, essential in the assembly of the flagella [2]. The recombinant FliD protein was tested on a group of 618 patients (51.4 % *H. pylori* positive) with 97.4 % sensitivity and 99 % specificity using a line assay and 97 % and 96 % by ELISA, respectively [20]. Other attempts to select antigenic proteins of potential diagnostic value were made (CafI,

UreG, UreB) but have not been evaluated yet [21]. Interestingly, using Helicoblot 2.1 (Genelabs Diagnostics, Singapore), it was possible to identify a low molecular weight protein (35 kDa) associated with a low risk of gastric cancer (GC) (odds ratio (OR) 0.4; 95 % confidence interval (CI), 0.1–0.9) and the VacA protein associated with a high risk of GC (OR 2.7; 95 % CI, 1–7.1) among patients with GC ($n = 102$) and dyspepsia ($n = 122$) in Iran [22].

8.3.4 Genedia® *H. pylori* ELISA and Its Use on Nationwide *H. pylori* Epidemiological Survey in Korea

Genedia® *H. pylori* kit, which was made by Green Cross Co., is an ELISA reagent [17]. This reagent was made of ultrasound-treated antigens that are from MBRIHP 2 of Korean chronic gastritis patient's strains and from MBRIHP 8 Korean duodenal ulcer patient's strains, and it helps extracting anti *H. pylori* IgG antibody in patient's serum. This diagnosis was prepared under room temperature; a sample or a reference drug reacts with antigen-absorbed plate; wash it off and add dilution-concentration adhesive to wash again under 37 ± 1 °C and then add a substrate to react again. Absorbance is measured at 450 nm; if a sample's absorbance value is higher, then cutoff value is considered as positive and negative if absorbance value is lower than the cutoff value. In 1998, Kim et al. performed bacterial culture and rapid urease test and reported the sensitivity and specificity of Genedia® as 97.8 % and 92 %, respectively [23]. However, Yang et al. compared histological analysis (H&E, Giemsa stain) to Genedia® to report sensitivity, specificity, positive predictive value, and negative predictive value of Genedia® as 96.2 %, 56.8 %, 78.9 %, and 90.0 %, respectively [24], which are rather low values. The difference of sensitivity and specificity between researches probably depend on which test has been referenced. The nationwide mass epidemiological survey of *H. pylori*-positive rate by using this Genedia® kit was performed in 1999 [25], 2005 [16] and 2011 [26] in Korea, and the results showed the decrease of *H. pylori* positive as the socioeconomic status was improved.

Conclusions

Serological diagnosis is noninvasive, is relatively economic, has less possibility of showing false-negative results in the situation of hemorrhagic ulcer or taking medications like antibiotics, and can perform mass epidemiological survey at once. However, it is not recommended for monitoring change after antimicrobial treatment because it hardly differentiates past *H. pylori* infection and current active infection. ELISA method is the most prevalent method in the world. There are many kinds of serological kits with various results of sensitivity and specificity depending on its antigenic proteins of potential diagnostic value. Thus it is necessary to validate its efficacy and usefulness in each country.

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9 Histopathologic Diagnosis of *H. pylori* Infection and Associated Gastric Diseases

Hye Seung Lee

Abstract

Helicobacter pylori (*H. pylori*) can be diagnosed by histopathologic examination which is considered to be a standard method, since not only it observes *H. pylori* directly but also it evaluates the degree of gastric inflammation, gastritis-related intestinal metaplasia, atrophic gastritis, and tumor. Among various staining methods, hematoxylin and eosin (H&E) stain is a routine procedure in daily practice. However, if H&E stain is not enough for histological diagnosis, ancillary tests including special staining and immunohistochemistry (IHC) are needed for accurate diagnosis. Among various ancillary tests, Giemsa stain has high sensitivity, and silver stain and IHC have high sensitivity and specificity. The initial stage of *H. pylori* infection is mostly asymptomatic, but it can develop into acute and chronic gastritis. *H. pylori*-related chronic gastritis shows not only lymphocyte and plasma cell infiltration but also neutrophil infiltration inside epithelium. *H. pylori*-related chronic gastritis can lead to sequelae, such as atrophic gastritis, intestinal metaplasia, and lymphoid follicles. Moreover, *H. pylori* infection is a cause of mucosa-associated lymphoid tissue (MALT) lymphoma and gastric cancer, and especially the infection has a close relationship with extranodal marginal zone B lymphoma of MALT and intestinal-type gastric adenocarcinoma. Therefore, histopathologic diagnosis is necessary for gastritis patients, and it should include the degree of gastritis and the presence of *H. pylori*, sequela of gastritis, and malignant tumor.

Keywords

Gastric cancer • Gastritis • *Helicobacter pylori* • Histology • Pathology

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9.1 Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative bacteria that belongs to *Bacillus* genus. Diseases like acute and chronic gastritis, gastric ulcer, duodenal ulcer, and gastric cancer (gastric adenocarcinoma and malignant lymphoma) are caused by *H. pylori*, and the WHO has classified this bacteria as a definite or group I human carcinogen in 1994 [1, 2]. Either gastritis or gastric ulcer can be caused by nonsteroidal anti-inflammatory drug (NSAID), autoimmunity, infection other than *H. pylori*, and so on. Thus, *H. pylori* detection and clinical findings are important for accurate diagnosis and optimal treatment. This chapter is going to introduce histopathologic features of gastric diseases associated with *H. pylori* infection.

9.2 Histological Diagnosis of *H. pylori*

H. pylori diagnosis is divided into an invasive testing that requires endoscopy and a noninvasive testing that does not require endoscopy [3, 4]. Among diagnostic methods, histological examination that uses endoscopic biopsy samples is considered to be a standard method because not only it observes *H. pylori* directly but also it evaluates the degree of gastric inflammation, gastritis-related intestinal metaplasia, atrophic gastritis, and tumor.

H. pylori is a spiral or curved bacteria that has a length of 2–4 μm. It has flagella mainly at one side, but sometimes flagella can be seen on both sides. *H. pylori* can be observed on the thick mucus of the surface of the gastric mucosa via optical microscope under high magnification. These bacteria cannot be seen on the surface of erosion or ulcer but can be seen on surrounding mucosa of erosion or ulcer region. It is also hard to observe on intestinal metaplasia and atrophic gastritis regions.

9.2.1 Hematoxylin and Eosin (H&E) Stain

Hematoxylin and eosin (H&E) stain is routinely used for pathological diagnosis in daily practice, so additional stain is not necessary and can directly observe bacteria under high magnification (Fig. 9.1a). The sensitivity and specificity of *H. pylori* diagnosis with H&E stain has been reported as 69–93 % and 87–90 %, respectively [5, 6]. The sensitivity can be different based on types of reagent, intensity of the stain, and the concentration of bacteria in a sample. Especially, a sample with a low number of *H. pylori* leads to low sensitivity due to the difficulty of bacterial observation, and pathologists' experience and skill also affect on the accuracy of *H. pylori* diagnosis [7]. Thus, if *H. pylori* is not found in a histologically gastritis-positive sample, additional special stain or immunohistochemistry (IHC) is recommended for precise determination [8]. However, only a histopathologic diagnosis is enough, if *H. pylori* is clearly found and proper corresponding inflammatory features are also observed.

9.2.2 Special Stain and Immunohistochemistry (IHC)

Ancillary tests to diagnose *H. pylori* infection can be categorized into silver stain, special stains other than silver stain, and IHC. First of all, examples of silver stain are Warthin-Starry stain, modified Steiner stain, El-Zimaity stain, and Genta stain [9, 10]. An advantage of silver stain is that it shows high sensitivity and specificity like IHC but its disadvantages are its high price and inconsistent staining results due to blurry background caused by nonspecific stain [11].

Second, special stains other than silver stain are Diff-Quick stain, crystal violet stain, Giemsa stain, and so on [7, 12]. To begin with, Giemsa stain is easy, quick, and cheap and shows relatively consistent stains, so it is preferred by many pathology departments (Fig. 9.1b). However, this stain can show a false-positive result [13]. Next, IHC has higher accuracy in terms of diagnostic results than special stain because IHC has less possibility of showing false-positive result [14]. Both polyclonal and monoclonal antibodies are commercially available. IHC is helpful to differentiate *H. pylori* from other microorganisms,

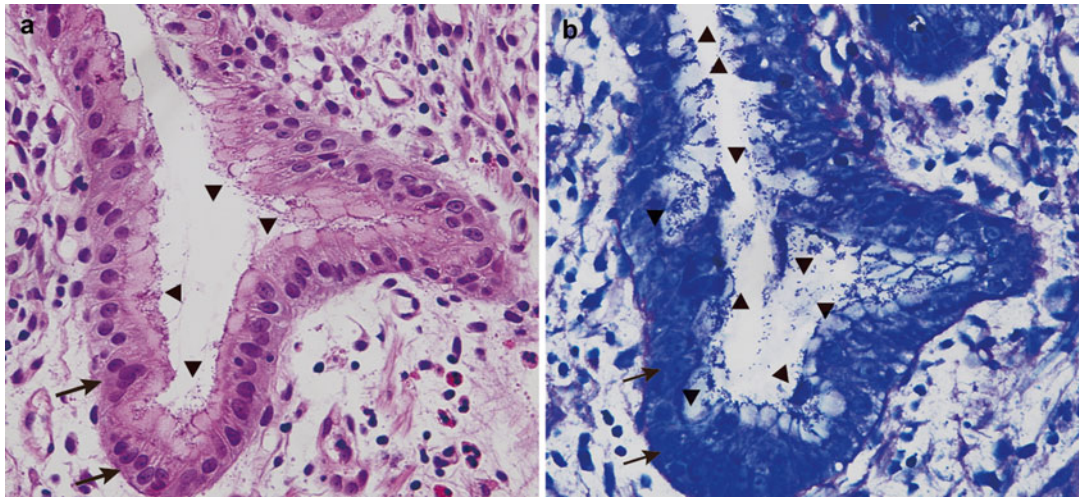

Fig. 9.1 Histopathologic findings of *H. pylori*. (**a**) Hematoxylin-eosin (H&E) staining. Morphological feature of epithelial and stromal cells is well preserved, but the sensitivity of the bacteria is low (*arrowhead*: *H. pylori*; *arrow*: nucleus of epithelial cell). (**b**) Giemsa staining. It has higher bacterial sensitivity than H&E staining, but it is difficult to recognize morphology of epithelial and stromal cells (*arrowhead*: *H. pylori*; *arrow*: nucleus of epithelial cell)

especially when a patient has atrophic gastritis or takes a proton pump inhibitor (PPI). Also, IHC is helpful to diagnose when *H. pylori* shows a nonselective form, not a spiral form; *H. pylori* can be in coccoid form and then it is difficult to distinguish from other coccoid form bacteria. There is a controversy on toxicity and survival ability of coccoid form of *H. pylori*, thus reporting it as coccoid form in pathologic diagnosis is recommended [15]. Nonetheless, it will be hard to observe the bacteria even with IHC, if there is a really small number of *H. pylori*. In addition, IHC can show a positive result on different *H. pylori* species (*H. heilmannii*) if polyclonal antibody is utilized [16].

Histological staining methods for *H. pylori* diagnosis are summarized in Table 9.1. A single perfect stain for *H. pylori* diagnosis does not exist; rather a proper stain method will be selected by each pathologist or pathology department. In addition, sensitivity and specificity can be increased, if histological features, such as degree of inflammation and morphologic feature and size of spiral bacteria, are considered at the diagnosis. However, site, number, and size of gastric biopsy can also lead to false-negative results on *H. pylori* histological diagnosis, so these factors should be considered when false negative is suspected.

9.3 Pathologic Features of *H. pylori*-Associated Gastritis

9.3.1 Acute Gastritis

The initial stage of *H. pylori* infection is mostly asymptomatic. Edema and mild neutrophilic infiltration in lamina propria are observed, like other acute infections. Acute inflammation is mostly observed in antrum and proceeds to chronic gastritis.

9.3.2 Chronic Gastritis

Histologic features of *H. pylori*-associated chronic gastritis are infiltration of various degrees of plasma cells, lymphocytes, and few eosinophils on lamina propria. These inflammatory cells are mostly distributed in superficial and subepithelial layer of gastric mucosa. Generally, in normal gastric mucosa, two to three lymphocytes and/or plasma cells can be

Table 9.1 Comparison of various staining methods for detecting *H. pylori*

Stain	Sensitivity	Specificity	Stain method	Cost	Note
H&E	Low	Low	Easy	Low	Other staining is necessary if false negative is suspected
Warthin-Starry	High	High	Difficult	High	Background stain can be blurry and H&E is necessary for diagnosis
El-Zimaity	High	High	Difficult	Intermediate	Efficient if low bacterial concentration
Genta	High	High	Difficult	High	Difficult method and takes long time
Diff-Quick/ crystal violet	Intermediate	Intermediate	Easy	Low	H&E is necessary for diagnosis
Giemsa	Intermediate	Low	Easy	Low	H&E is necessary for diagnosis
Immunostaining	High	Very high	Difficult	High	Staining takes long time and H&E is necessary for diagnosis

H&E hematoxylin-eosin staining

observed in lamina propria between foveolar glands, and approximately five intraepithelial lymphocytes are observed per 100 epithelial cells. If the infiltration of either lymphocyte or plasma cell is increased compared to the normal condition, the state can be diagnosed as chronic gastritis [8].

Neutrophilic infiltration is also observed; the infiltration is also mostly observed in superficial and subepithelial layer of the gastric mucosa and cause intraepithelial neutrophils and pit microabscess by entering epithelial cells and lumen of gastric gland. If neutrophils are observed inside epithelial cell or gastric gland lumen, it is diagnosed as chronic active gastritis [8]. Neutrophilic infiltration is a common feature of *H. pylori* infection, and the degree of infiltration is corresponded to the degree of *H. pylori* infection and mucosal damage. Also, neutrophilic infiltration is a crucial feature that indicates *H. pylori* infection.

Chronic gastritis is limited to the antral region during the initial stage, but it spreads to the body and fundus as the disease progresses (Fig. 9.2). In addition, inflammatory cell infiltration starts from the superficial layer of the gastric mucosa and then it enters deeper area to induce parietal cell and chief cell loss. As a result, the body mucosa becomes similar to the antral mucosa or progresses to atrophic gastritis.

9.4 Sequelae of Chronic Gastritis

9.4.1 Atrophic Gastritis

Atrophic gastritis is defined as the loss of appropriate glands in the gastric mucosa due to persistent chronic inflammation [17, 18]. Normally, deep portion of gastric mucosal biopsy is composed of different cell types based on regions; the antral region is made of mucous glands, and the body is composed of parietal cells and chief cells that produce gastric juice and enzymes, which takes part in gastric physiologic function. The loss of gastric deep glands is the diagnostic feature of atrophic gastritis. The superficial layer of the gastric mucosa is made of foveolar epithelium, and if intestinal metaplasia is limited to foveolar epithelium, then it is not diagnosed as atrophy.

Atrophy can be classified into metaplastic type that is due to intestinal metaplasia or pseudopyloric metaplasia (Fig. 9.3a) and nonmetaplastic type that is due to inflammation and/or stromal fibrosis. If a gastric biopsy specimen does not include full thickness of mucosal layer or difficult to evaluate atrophy due to lymphoid follicle, it is diagnosed as "indefinite." The degree of atrophic gastritis is categorized into mild, moderate, and severe based on the percentage of atrophic area/total area.

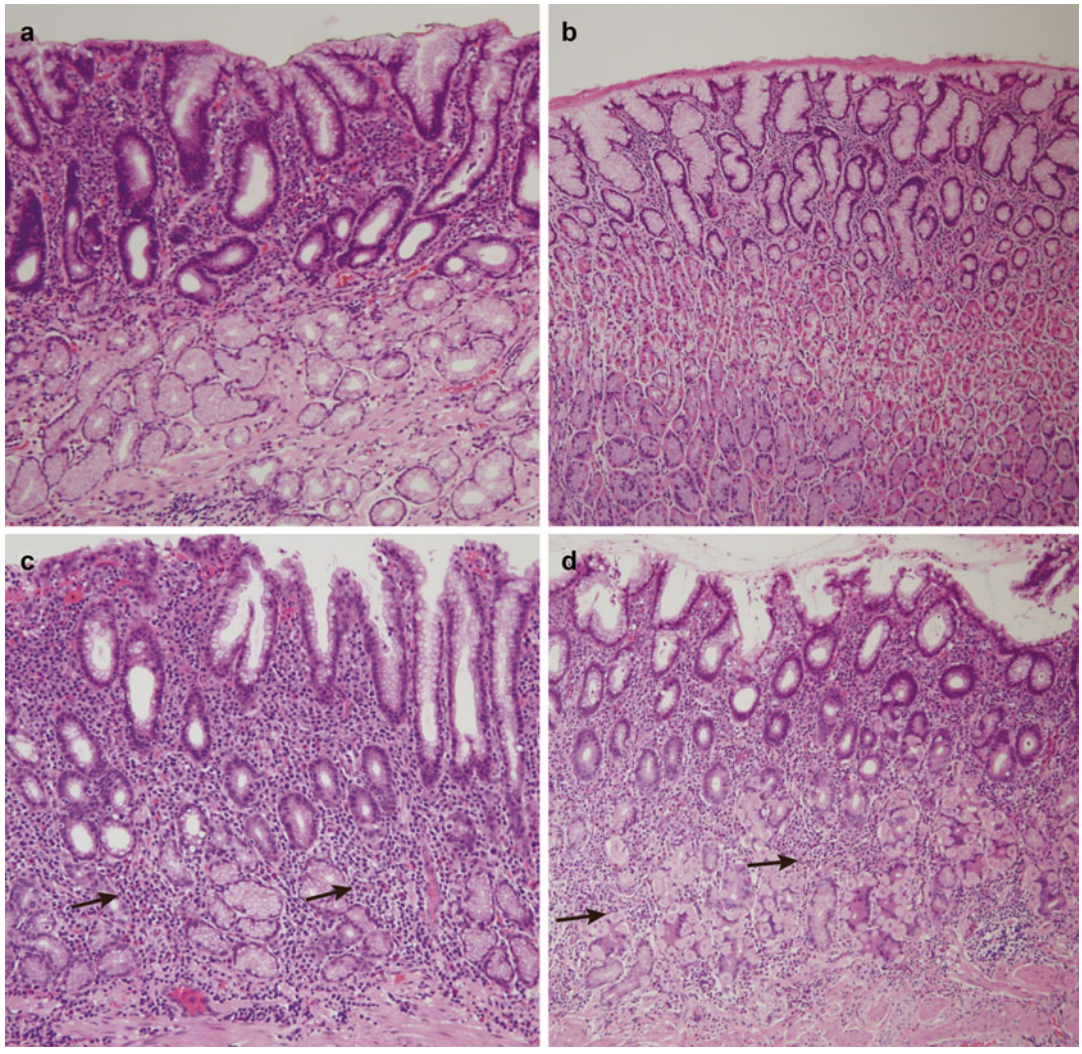

Fig. 9.2 Histopathologic features of chronic gastritis. (**a**) Superficial gastritis on gastric antral mucosa. (**b**) Superficial gastritis on gastric body mucosa. (**c**) Inflammatory cells infiltrate into deep layer with progression of gastritis on antral region and destroy the glands (*arrow*). (**d**) Inflammatory cells infiltrate into deep layer with progression of gastritis on body mucosa and destroy the glands (*arrow*)

9.4.2 Intestinal Metaplasia

Intestinal metaplasia can be divided into two types [19–21]. First of all, complete type shows similar histologic features as small intestine and positive to MUC2 by IHC, which is the mucin that is normally produced in the intestine. In comparison, incomplete type shows similar histologic feature as large intestine and positive to not only MUC2 but also MUC5AC and MUC6, which are normally produced in the stomach. In addition, high iron diamine-alcian blue (HID-AB) staining is a special stain that is used to distinguish those two types. After HID-AB stain, sialomucin is stained in bright blue in complete type, while sialomucin (bright blue) and sulfomucin (black) are both stained in incomplete type. Intestinal metaplasia usually occurs with atrophic gastritis and it is precancerous lesion. There is a higher possibility of gastric cancer under incomplete type of intestinal metaplasia than complete type.

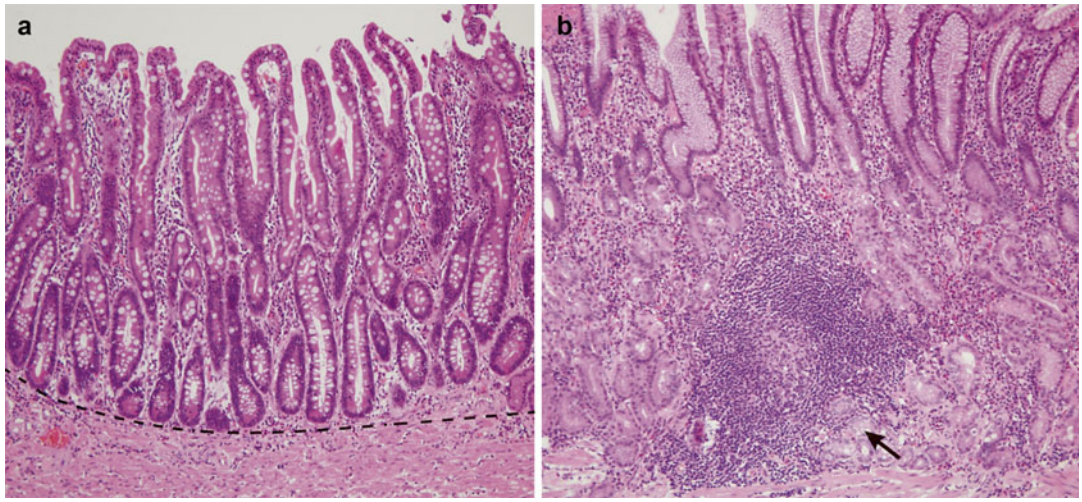

Fig. 9.3 Sequelae of chronic gastritis. (**a**) Atrophic gastritis accompanied with intestinal metaplasia (*dotted line*, *upper border of muscularis mucosa*). (**b**) Mucosa-associated lymphoid tissue (MALT). A lymphoid follicle with central germinal center is observed at the bottom of mucosal layer (*arrow*)

9.4.3 Mucosa-Associated Lymphoid Tissue (MALT)

Well-formed lymphoid follicles are not observed in normal gastric mucosa, unlike small or large intestine. Sometimes, lymphocytes can be accumulated on the upper portion of muscularis mucosa. For *H. pylori*-positive patients, lymphoid follicles that are similar to intestinal MALT are observed (Fig. 9.3b). If lymphoid follicles are observed from gastric mucosal biopsy, *H. pylori* infection is highly suggested [8].

Antral nodular gastritis indicates that multiple small nodules are observed in gastric antrum by esophagogastroscopy but its histopathologic definition is still unclear. However, there are several reports that lymphoid follicle formation is observed as antral nodular gastritis [22, 23]. According to the recent research, small, circular, and elevated nodules are mainly observed on the gastric antrum via esophagogastroscopy, those nodules are flat on the body and fundus, and the nodules contain lymphoid follicles by histopathologic examination [24].

Moreover, MALT in the gastric mucosa is a cause of marginal zone B-cell lymphoma of MALT. There was a report in early 1990s that 92 % of MALT lymphoma patients had *H. pylori* infection on their stomachs [25], and a recent research has reported that 70–95 % of MALT lymphoma patients have *H. pylori* infection [26]. The histopathologic feature of low-grade MALT lymphoma shows that marginal zone B-cell-oriented tumor cells, which have irregular-shaped nucleus and pale cytoplasm (Fig. 9.4), infiltrates into the surrounding areas of lymphoid follicles. Remarkably, lymphoepithelial lesion is observed, and this is a diagnostic finding of MALT lymphoma. Lymphoepithelial lesion is defined as infiltration of more than three neoplastic lymphocytes into surrounding gastric glands and destroys them. Ancillary IHC testing can verify destruction of cytokeratin-positive glands and infiltration of CD20-positive neoplastic B cells. Intraepithelial lymphocytes can be also observed inside gastric glands in lymphocytic gastritis. In lymphocytic gastritis, T lymphocytes infiltrate into gastric glands, and those lymphocytes are scattered, not accumulated in more than three cells.

9.4.4 Gastric Cancer

The relationship between *H. pylori* infection and gastric cancer can be accepted by (1) a correlation between *H. pylori* infection and gastric can-

cer by epidemiologic studies, (2) sequential alterations of gastric mucosal lesion from *H. pylori* infection to cancer emergence with correa cascade, and (3) animal research model established [27]. In 1965, Lauren classified the gastric cancer into intestinal type and diffuse type [28]. Lauren classification is still used in clinical practice along with WHO classification because it is easy to classify and related to carcinogenesis and molecular genetic feature (Fig. 9.5). Intestinal type is when tumor cells form gland structure that shows similar morphological features to normal gastrointestinal epithelium, while diffuse type is when tumor cells are scattered and rarely form gland structure [29]. *H. pylori* infection and associated chronic gastritis cause atrophic gastritis and intestinal metaplasia and then result in intestinal-type gastric cancer, but diffuse-type gastric cancer can also occur in correlation of *H. pylori* infection [27].

9.5 Pathologic Findings of Peptic Ulcer

Peptic ulcer is also an *H. pylori* infection-associated disease. Histologically, erosion indicates a tissue damage that is limited to mucosal layer, and ulcer indicates that the depth of tissue damage is below submucosa layer. The peptic ulcer is composed of four layers based on histological observation: (1) fibrinoid ulcer at the surface layer, (2) mainly neutrophils but nonselective inflammatory cell infiltration layer, (3) monocytic infiltration and capillary proliferation, and (4) fibrosis in deepest layer.

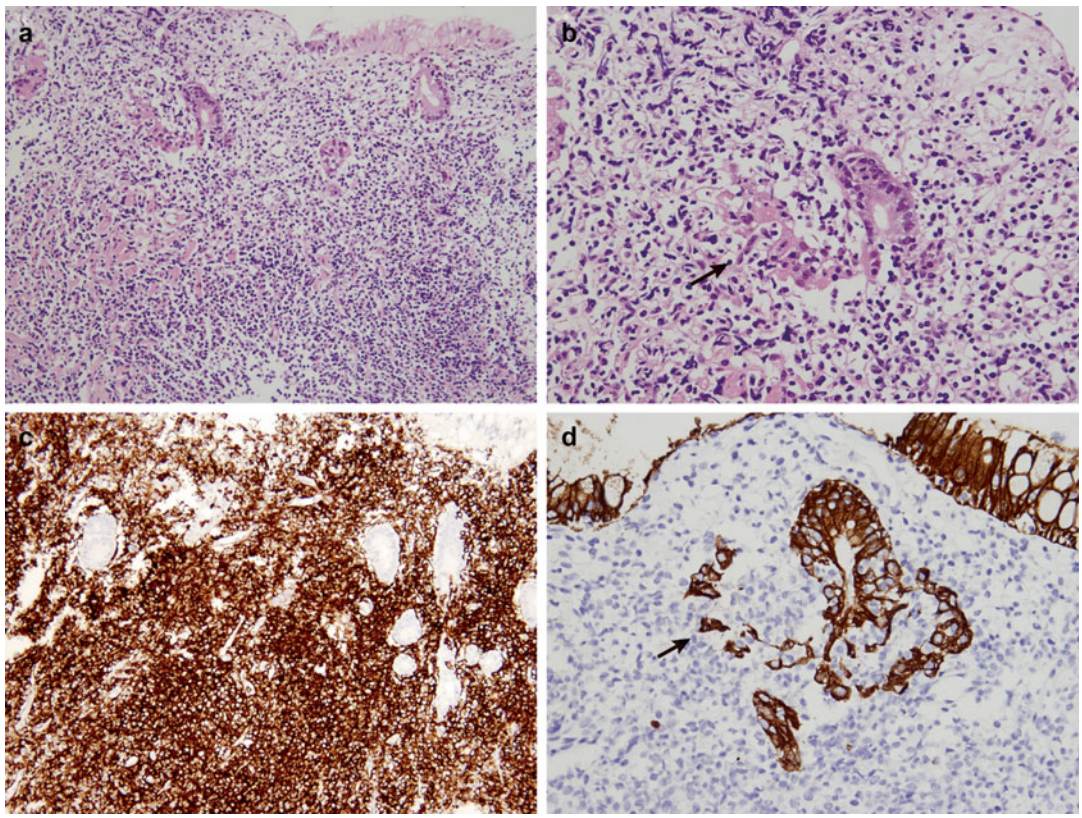

Fig. 9.4 Marginal zone of B-cell lymphoma of MALT (MALT lymphoma). (**a**) Diffuse infiltration of atypical lymphocyte is found in mucosal layer of gastric biopsy specimen by H&E staining. (**b**) Lymphoepithelial lesion. Atypical lymphocytes are infiltrated into the gastric epithelium (*arrow*). (**c**) B-cell infiltration is confirmed by immunohistochemistry for CD20. (**d**) Destroyed gastric epithelium is easily observed by immunohistochemistry for cytokeratin (*arrow*)

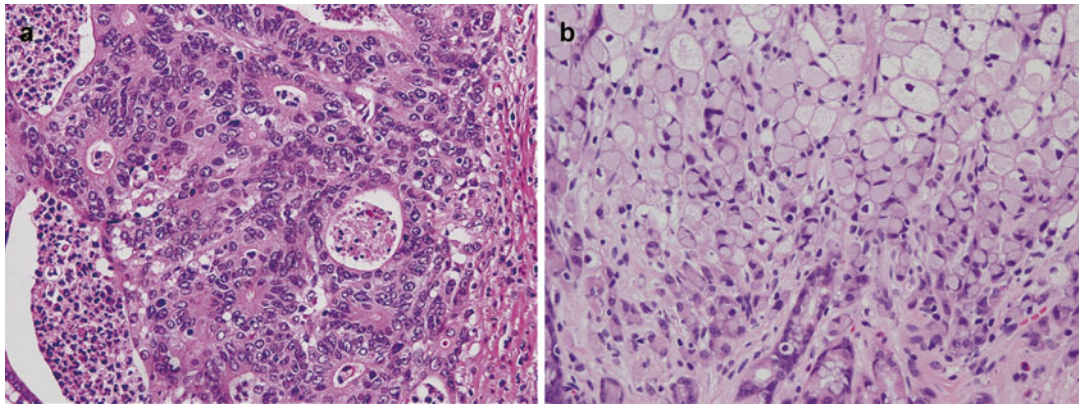

Fig. 9.5 Lauren classification in gastric cancer. (**a**) Intestinal type of gastric cancer. Tumor cells form gland-like structure, which is similar to normal gastrointestinal glands. (**b**) Diffuse type of gastric cancer. Tumor cells scattered without forming gland structure

Conclusions

H. pylori can be detected by several diagnostic methods, and histopathologic examination is one of those. H&E stain is a commonly used method in histopathologic diagnosis that does not require additional staining procedure and can obtain other histological findings, but it has low sensitivity and specificity in terms of *H. pylori* detection. Intraepithelial neutrophilic infiltration and pit abscess are distinctive features of *H. pylori*-associated gastritis, and special stain or IHC is helpful if there is significant inflammation but no *H. pylori* detection. Giemsa stain is a quick and easy method with high sensitivity, and silver stain and IHC have high sensitivity and specificity but labor intensive and time consuming. Each pathologic laboratory is recommended to choose proper staining method based on its facility and human resource.

Persistent chronic gastritis due to *H. pylori* infection results in atrophic gastritis, intestinal metaplasia, and MALT as sequelae. Most of them are precancerous lesion that should be reported in histopathologic diagnosis. In addition, *H. pylori* infection is related to MALT lymphoma or gastric cancer. Histopathologic diagnosis of *H. pylori* should include the degree of gastritis, sequela, and malignant tumor detection; therefore, histological diagnosis is necessary for gastritis patients.

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Culture

10

Jaeyeon Kim

Abstract

Culture of *Helicobacter pylori* (*H. pylori*) is a comprehensive method for typing and antimicrobial susceptibility testing. In the optimal culture condition, 35–37 °C in microaerophilic condition (85 % N_2, 10 % CO_2, 5 % O_2), colonies may appear in 3–4 days. However, in the case of negative culture, 7–10 days incubation is recommended to make sure that the result is negative. After culture of *H. pylori*, to asses antimicrobial susceptibility, agar dilution method, disk diffusion method, broth dilution method, and E-test are performed. Expensive and fastidious, culture study is limited to epidemiology and researches recently. Clinically it is used to test antimicrobial susceptibility after second failure of eradication. Increasing antimicrobial resistance, culture study may be essential in the future.

Keywords

Helicobacter pylori • Culture

10.1 Introduction

Bacterial culture is a comprehensive method for antimicrobial susceptibility testing and typing of strains [1]. In the optimal condition, the specificity is 100 % and the sensitivity is more than 90 %. However, *Helicobacter pylori* (*H. pylori*) is a fastidious bacteria that isolation rate varies according to collection and transport of specimen and selection of culture medium; the rather low sensitivity has been reported in 50–70 % according to the researchers [2–5]. Especially in patients with gastrointestinal bleeding, it has been reported in 40 % [6]. The sensitivity is 95.8 %, and specificity is reported 96.4 % in children [7].

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10.2 Culture Method

10.2.1 Specimen Collection

The proper specimen for culturing *H. pylori* is biopsy specimens obtained during endoscopy. Since proton pump inhibitors (PPI) may change

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distribution of mucosal bacteria [8], subjects who are scheduled to undergo endoscopy should quit PPIs or antibiotics for 2 weeks or more.

There is controversy over the number of tissue samples required for the diagnosis of *H. pylori*. A single biopsy at antrum (2 cm from the pylorus) is sensitive but it is not sufficient for a reliable diagnosis [9]. As *H. pylori* may have patch distribution, the larger number of samples taken increases the sensitivity. Therefore, it is recommended taking two specimens each on antrum and body [9].

10.2.2 Transport of Biopsy Specimens

H. pylori is fragile in room temperature and air. It is obligatory to avoid air exposure of the specimen and to place them in saline or transport media, such as Stuart's transport medium if transport time is more than 4 h [9, 10]. If these transport condition cannot be available, it is better to freeze the specimens at −70 °C. Storing at 4 °C in media containing 20% glycerol, it is known that 81% of *H. pylori* recovered [11].

10.2.3 Incubation

H. pylori is not distributed evenly in most cases; more colonies appear in the grinded specimens. In addition, direct plating to solid medium is used as *H. pylori* is difficult to grow in the broth culture.

The media include an agar base, growth supplements, and selective supplements. Blood or serum components are examples of growth supplements, which promote the growth of *H. pylori*, and the proportion is 5%, 7%, or 10%. Other growth supplements include yolk, charcoal, starch, bovine serum albumin, catalase, and so on [9]. Selective supplements are crucial due to the presence of contaminating bacteria. It consists of antibiotics and antifungal: antibiotics which inhibit the growth of gram-positive bacteria such as vancomycin or teicoplanin, antibiotics targeting gram-negative bacteria such as nalidixic acid colistin or trimethoprim, and antifungal such as nystatin or amphotericin B.

To increase sensitivity of the test, selective medium or the blood containing nonselective medium is used [12]. Selective medium such as Pylori agar and Skirrow agar and nonselective medium such as blood agar and Columbia agar are frequently used [13]. Incubating the bacteria cultures will be made in microaerobic conditions of 35–37 °C (85% N_2, 10% CO_2, 5% O_2), and the colonies appear within 3–4 days in optimal conditions. However, 7–10 days of incubation is recommended to make sure that the result is negative [14].

10.2.4 Identification

After 3–4 days in the culture medium, transparent and smooth colonies of small round *H. pylori* can be identified [9] (Fig. 10.1). If the bacteria are separated from the gastric biopsy samples, it is sufficient to identify the phenotype but not from a stool or salivary specimen. To differentiate other bacteria with similar characteristics, molecular biological method is used. For biochemical tests to confirm this, cytochrome oxidase, catalase, and urease are used [15].

10.3 Antimicrobial Susceptibility Test

After isolation of *H. pylori* (Fig. 10.2), to assess antimicrobial susceptibility, agar dilution method, disk diffusion method, broth dilution method, and E-test are performed [16–18]. Clinical Laboratory Standard Institute (CLSI) has recommended agar dilution test as standard for testing antimicrobial susceptibility of *H. pylori* [19].

10.3.1 Agar Dilution Method

Add 5% of blood or serum of sheep or horses based on Mueller-Hinton agar (MHA) with prepared twofold dilutions of antibiotics. Then, inoculate the isolated *H. pylori* on agar in 35–37 °C with 72 h of incubation [19, 20] (Fig. 10.3). The minimum concentration that *H. pylori* do not grow is determined as minimal inhibitory concentration (MIC). The method is useful in researches by observing antibiotic resistance of many strains at the same time in the laboratory. However, it is not frequently used in clinic.

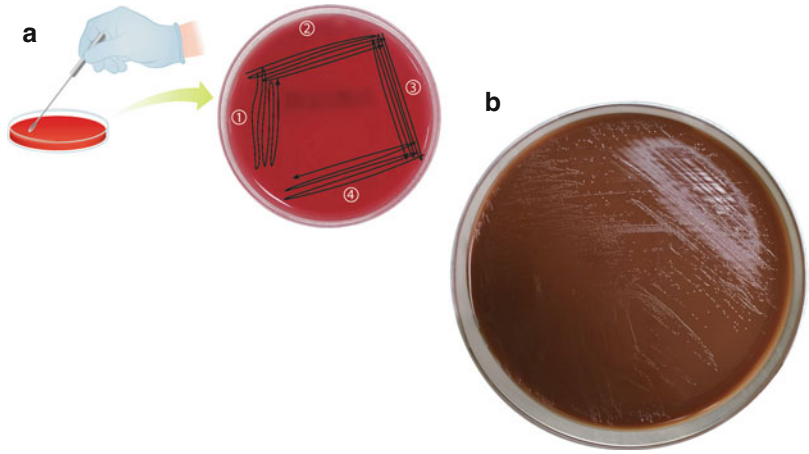

Fig. 10.1 *H. pylori* culture. (**a**) (1) Add 200 uL of *Brucella* broth to petri dishes. (2) Put two pieces of biopsy specimens from the gastric antrum and body on each petri dish. (3) After heating the end of the microtip, put it flattened on petri dishes. (4) With a flattened microtip, grind biopsy specimens and then streak from ① to ④ direction over the Pylori agar. (5) Incubate at 37 °C, 10 % CO_2, 5 % O_2 condition for 3–5 days. (**b**) After 3–4 days in the culture medium, transparent and smooth colonies of small round *H. pylori* can be identified on the Pylori agar

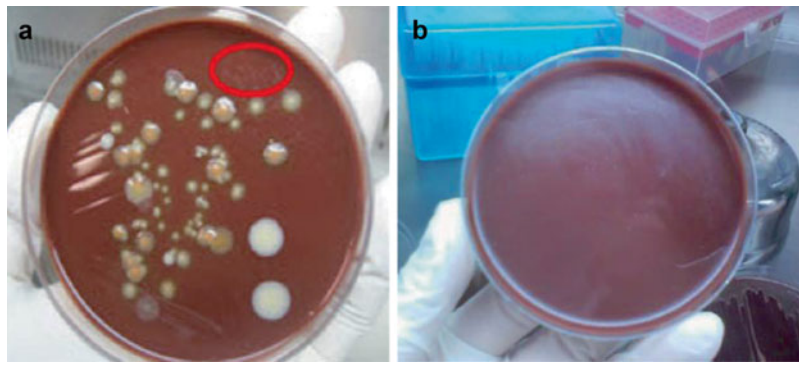

Fig. 10.2 Isolation of *H. pylori*. (**a**) Following the procedure on Fig. 10.1. The culture was considered to be positive for *H. pylori* by observing small, translucent, tiny colonies (*red oval*). (**b**) Repeat this step of inoculating and isolation for three times. Mass culture the forth isolated *H. pylori* after confirmed urease positive and gram negative

10.3.2 Disk Diffusion Method

The disk diffusion method is applicable only to bacteria which can form a colony in 1 day; slow-growing bacteria should check the broth dilution method. This method has not been validated for the other antibiotics except macrolide, but a good correlation is usually found with the other methods [9, 20].

10.3.3 Broth Dilution Method

The antibiotics diluted in twofold serial manner are added in the liquid culture medium by inoculating the microorganism. The lowest antibiotic concentration in a test tube that bacteria do not grow is an MIC. Strains should be used as a known MIC for quality control. As *H. pylori* are difficult to grow in the liquid medium, *Brucella* is a helpful

Unit: μg/mL					
Amoxicillin	2	1	0.5	0.25	0.125
Azithromycin	4	2	1	0.5	0.25
Ciprofloxacin	4	2	1	0.5	0.25
Clarithromycin	4	2	1	0.5	0.25
Metronidazole	32	16	8	4	2
Tetracycline	16	8	4	2	1
Levofloxacin	2	1	0.5	0.25	0.125
Moxifloxacin	2	1	0.5	0.25	0.125

Fig. 10.3 Antimicrobial susceptibility testing. Testing antimicrobial susceptibility of *H. pylori* with agar dilution method. Adding 5% of blood on Mueller-Hinton agar with prepared twofold dilutions of antibiotics. Then, inoculate the isolated *H. pylori* on agar in 35–37 °C with 72 h of incubation. The concentration range is made up of 1–32 μg/mL or 0.125–2 μg/mL for each antibiotic, and the minimum concentration that *H. pylori* do not grow is determined as minimal inhibitory concentration (MIC)

supplementation. It shows relative good correlation with the E-test except metronidazole [18].

10.3.4 E-Test

E-test strip is a thin inert, width 5 mm and length 50 mm, of plastic that do not have holes. The antibiotic concentration is recorded on one side of the strip, and the concentration range is made up of 1–32 μg/mL or 0.125–2 μg/mL for each antibiotic. Put the three to five colonies of bacteria to be tested in a liquid medium, and coat the bacteria on MHA according to McFarland standard 0.5. Then put the strip on MHA. When cultured in constant temperature of 35 °C in the absence of CO_2, there will be symmetrical inhibition zone elliptical around the strip. The edge of strips of oval intersection is the MIC values. E-test of antibiotic sensitivity testing of *H. pylori* and the agar diffusion method were reported to have relatively good correlation except metronidazole [18].

Conclusions

In summary, culture is a useful tool in the future. Even though it is influenced by experiences of microbiologists, quality of specimen, and transportation media, culture is the most sensitive and specific method to diagnose *H. pylori*. It has limited to epidemiology and researches. Clinically its role is limited to check antimicrobial susceptibility in patients who have failed more than twice in eradication. However, considering increasing antibiotic resistance, it will play a more important role in the future [9].

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Urea Breath Test

11

Yong Hwan Kwon

Abstract

The urea breath test (UBT) is a noninvasive, simple, and safe test which provides excellent accuracy both for the initial diagnosis of *Helicobacter pylori* infection and for the confirmation of its eradication after treatment (high sensitivity, 90–100 % and specificity, 90–100 %). This test is based on the organism's urease activity, which liberates carbon dioxide (CO_2) from urea and produces ammonia to buffer its acidic environment. Ingestion of labeled urea results in the production of labeled CO_2 which then can be detected in the breath. Urea can be labeled with two different isotopes, ^{14}C (the radioactive isotope) or ^{13}C (the nonradioactive stable isotope). The methodology of the UBT have been changed including the dose of labeled urea used, the type of test meal, the time of breath collection, the cutoff values, and the equipment adopted to measure isotope enrichment. However, a definitive standardization of this test does not yet exist, and a unique and generally proposed cutoff level is not possible because it has to be adapted to different factors.

Keywords

Diagnosis • Eradication • *Helicobacter pylori* • Urea breath test

11.1 Introduction

For detecting *Helicobacter pylori* (*H. pylori*) infection, several invasive and noninvasive diagnostic methods have been developed. Endoscopic biopsy is an invasive method and the gold standard for detecting *H. pylori* infection, but it has the potential for sampling error because of the heterogeneous localization of *H. pylori* in the stomach [1]. Serological examination is a noninvasive and convenient method, but it does not necessarily reflect the current status of infection [1]. To overcome this limitation of serology, the urea breath test (UBT) using ^{13}C- or ^{14}C-labeled urea has been developed [2–5]. In 1987, the ^{13}C-UBT was introduced for the detection of

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H. pylori infection [6]. The UBT is a noninvasive test based on the potent urease activity of *H. pylori* in the gastric mucosa. This test has been widely used because it was reported to have a sensitivity and specificity greater than 90 % for detecting *H. pylori* infection and to be a more convenient and safer method for the patients [3, 7]. Therefore, the ^{13}C-UBT has become the gold standard for the diagnosis of *H. pylori* infection [3–7]. Breath tests are used in gastroenterology practice to study pathophysiological and metabolic processes in an indirect way [8]. These tests have been used to measure gastric emptying, small bowel bacterial overgrowth, exocrine pancreatic function, liver metabolic capacity, and, finally, the presence of *H. pylori* in the stomach [8]. This test has become, during recent years, increasingly popular in clinical practice both for screening patients before endoscopy and for assessing the success of *H. pylori* eradication therapies. The UBT is simple, innocuous, easy to repeat, and highly accurate [3]. It is particularly suitable in all clinical conditions where endoscopy is not strictly necessary and to check the success of eradication regimens.

11.2 The Principle of the Urea Breath Test

The $^{13/14}$C-UBT exploits the copious amounts of urease produced by *H. pylori* which hydrolyzes urea to form ammonia and soluble carbon dioxide which is expired in the exhaled breath [9]. Labeling of urea with either isotope allows the $^{13/14}CO_2$ to be detected in the expired breath. Isotope enrichment can be measured by various methods in breath samples collected at appropriate times. A particular biochemical property of *H. pylori* facilitates the use of the ^{13}/^{14}C-UBT to diagnose the presence of the organism in gastric or duodenal mucosa. This microbe produces relatively high concentrations of urease, an enzyme that hydrolyzes urea into the alkaline buffers ammonium and bicarbonate (Fig. 11.1). Bicarbonate generated in gastric mucosa enters the blood stream, is transported to the lungs, and is rapidly excreted from the lungs as volatile carbon dioxide. In the ^{13}/^{14}C-UBT, a capsule of urea tagged with radioactive carbon is administered orally to a patient; the patient's exhaled carbon dioxide is then collected in airtight balloons. Detection of elevated quantities of exhaled carbon dioxide tagged with $^{13/14}CO_2$ indicates marked bacterial urease activity in the patient's stomach. Production of ammonium and bicarbonate could allow local buffering of highly acidic (pH 1.5) gastric secretions and confer on *H. pylori* its unique microbiologic ability to survive in the hostile gastric lumen [10]. Vigorous urease activity, then, might be vital to the organism's ecologic niche, and the elevated $^{13/14}CO_2$ measurements of exhaled carbon dioxide labeled with $^{13/14}CO_2$ from intense urea metabolism is a biologically plausible indirect measure of *H. pylori* presence [11] (Fig. 11.2).

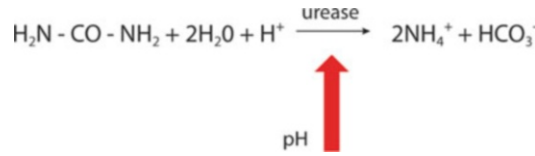

Fig. 11.1 The reaction of urea hydrolysis in which urea is broken down to ammonia and carbon dioxide by *H. pylori*

11.3 Urea Substrate and Measuring Equipment of the Urea Breath Test

While the first UBT was performed with 350 mg of urea [6], UBT may be performed with relatively low doses of urea: 100 mg, 75 mg (mean sensitivity and specificity, 97 %), or even 50 mg (mean sensitivity and specificity, 98 % [12, 13]. With the most widely used protocols (100 mg without citric acid and 75 mg with citric acid), excellent accuracy is obtained when breath samples are collected as early as 10–15 min after urea ingestion with a mean sensitivity and specificity of almost 100 % [14]. Urease-producing oropharyngeal bacteria may theoretically cause false-positive UBT results. As previously mentioned, to overcome this problem, some investigators have encapsulated the urea or administered it as a

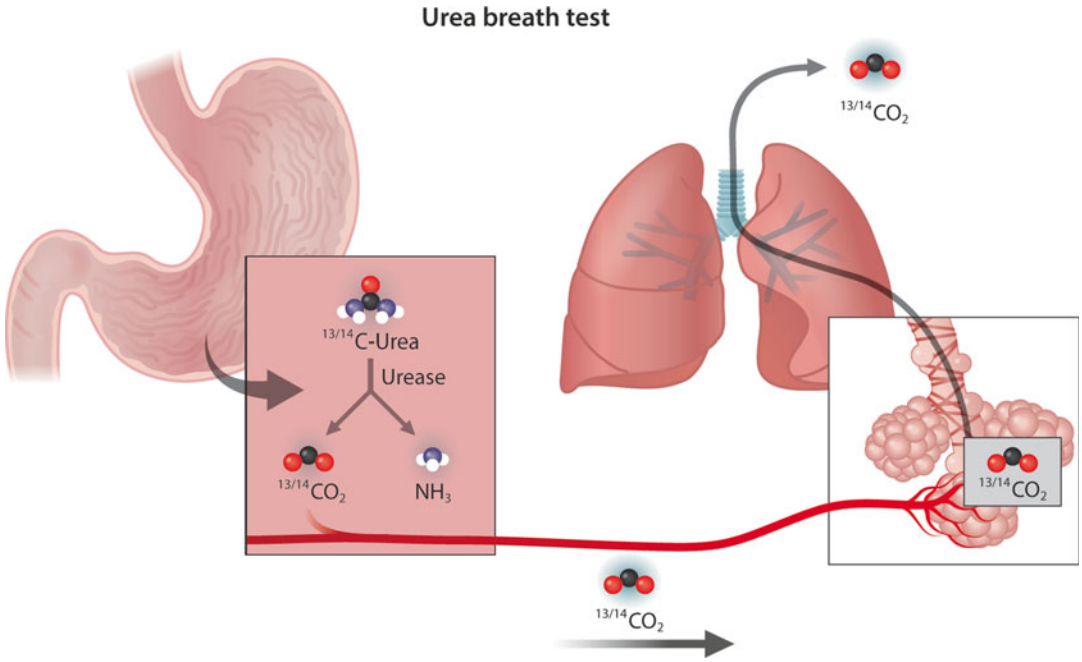

Fig. 11.2 The principles of the urea breath test. On an empty stomach, the patient swallows carbon-labeled urea. In the presence of *Helicobacter pylori*, the labeled urea is metabolized into carbon dioxide and ammonia by the enzyme urease, produced by *Helicobacter pylori*. The carbon dioxide produced diffuses into the blood vessels, and from there, it is transported as bicarbonate into the lungs and expelled as CO_2 with exhaled air to be captured during sampling. A positive answer offers conclusive evidence that the patient is infected with *Helicobacter pylori*. In the absence of *Helicobacter pylori*, the administered urea is absorbed from the gastrointestinal tract and subsequently voided (Adapted from http://www.kibion.se/scientific/info-diagnostic-methods/about-breath-tests/)

tablet [13, 15–18]. Urea can be labeled with two different carbon isotopes: ^{14}C and ^{13}C. The main difference between them is that the former is radioactive, whereas the latter is stable [19]. The advantages of using ^{14}C-urea are that it is cheap, so rapid that administering ^{14}C-urea in a gelatin capsule allows an accurate response to be obtained from a single 10 min breath sample [12, 20], and does not require any test meal. However, although the dose of ^{14}C has become progressively smaller and the test can now be performed with 1 μCi, which is equal to the natural background radiation received in 1 day [9, 20], the main problems are still the availability of a nuclear medicine department or centers licensed for storage and disposal of radioactive substrates, shipping difficulties, and the copious amounts of labeled tracer needed to perform large-scale epidemiological studies. In contrast, ^{13}C is a nonradioactive isotope that can be used safely for repeated testing, which is frequently required in clinical practice, and for detecting *H. pylori* infection in children and women of childbearing age [19]. However, the major drawbacks of ^{13}C-urea are the higher cost compared with ^{14}C-urea and the need for expensive mass spectrometry, which is the most preferable device for measuring ^{13}C enrichment in breath samples of subjects infected with *H. pylori* [19].

After hydrolysis of ^{13}C-labeled urea by *H. pylori* urease in the stomach and the formation of $^{13}CO_2$, which is transported to the lungs, ^{13}C must be detected in exhaled air by specific measuring equipment. ^{13}C is measured as the $^{13}CO2/^{12}CO_2$ isotope ratio and is expressed as delta over baseline (DOB, δ) per mil (‰) (Fig. 11.3) with respect to the international reference standard represented by the Pee Dee Belemnite limestone [19]: isotope ratio mass spectrometer (IRMS), non-dispersive isotope selective infrared spectro-

scope (NDIRS), and laser-assisted ratio analyzer (LARA) equipment [21] (Table 11.1). The advantage of IRMS is that it processes the highest number of breath samples and works in an automated manner. However, it is the most expensive and requires the longest analysis time. NDIRS does not require helium as the carrier gas and has the lowest weight and price, but it can analyze only a small number of samples and so does not need multitasking software. The LARA system has the quickest analysis time; the other characteristics are average compared with the other two machines, especially with regard to the number of samples it can process and the cost. Integrated bar code readers and the use of multitasking programs have made running IRMS instruments much easier; however, they do require more maintenance than NDIRS and LARA systems. IRMS and LARA are more suitable for gastroenterological centers requiring large quantity, automated analysis, whereas NDIRS is more suitable for small laboratories in which the daily number of assays is not high. In this light, a small, new, cheap, infrared device for examination of only two breath samples has been developed to be used exclusively in the doctor's office [19].

$$\delta^{13}C = \left(\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{sample}} - 1 \right) * 1000‰$$

Fig. 11.3 The definition of Delta over base line (DOB, δ-value). International standard, the Pee Dee Belemnite (PDB)

11.4 Test Meal

The original ^{13}C-UBT employed a test meal designed to slow gastric emptying and to maximize the distribution of the substrate within the stomach so as to increase the area and time of contact between the bacteria and the substrate [6]. Test meals containing fat are usually chosen due to the well-known ability of this nutrient to delay gastric emptying. Recently, citric acid, which acts by lowering duodenal pH, which in turn reduces antral motility and relaxes the gastric fundus, has been shown to be an optimal test meal [19]. Citric acid test meals have also been shown to enhance the amount of urea hydrolysis compared with traditional nutrient meals without increasing the rate of false-positive results [22–24]. The citric acid test meal also produced a more rapid increase in labeled CO_2 in the breath, suggesting that a post dose breath collection period as short as 10 min might be used for categorization of *H. pylori* status [23]. The effect was not due to pH as neither pentagastrin stimulated increase in acidity nor administration of ascorbic acid at a similar pH showed an increase in urea hydrolysis equal to that seen with citric acid [23, 25]. In addition to citric acid, other test drinks like orange or apple juice have been used because of a better taste.

Table 11.1 Comparison of the main characteristics of three different types of equipment for measuring the $^{13}CO_2$ to $^{12}CO_2$ ratio in breath samples

Characteristics of the instruments	Mass spectrometer (IRMS)	Infrared spectrometer (NDIRS)	Laser-assisted ratio analyzer (LARA)
Weight	90 kg	12 kg	350 kg
Reference gas	Necessary	Enclosed	Necessary
Carrier gas	Helium supply	None	None
Analysis time	120 s	90 s	60 s
Automation	For 220 samples	For 16 samples	For 60 samples
Breath sample	10 ml glass tube	1,200 breath bag	12 ml plastic tube
Sample mailing	Favorable	Not practicable	Favorable
Multitasking software	Commonly available	Unnecessary	Planned
Technical expertise	Easy to operate	Easy to operate	Easy to operate
Reported reliability	90–100 %	90–98 %	Limited experience

Adapted from Savarino et al. [19]

Several studies have included orange juice as a test meal, but the diagnostic accuracy of the UBT with either orange juice or citric acid solution debated [16, 26–28]. However, some authors have also evaluated and demonstrated equivalence results without using citric acid (or any other test meal) in UBT [14, 29–35].

11.5 Time of Breath Collection

Breath samples of UBT were collected before (baseline) and after substrate ingestion. In the original protocol by Graham et al. [6], breath samples were collected every 10 min for 3 h. Subsequently, the test has been progressively simplified using the protocol by measuring $^{13}CO_2$ at 20–30 min after urea ingestion. It has been shown that sampling too early may produce false-positive results because of urease activity of oral bacteria [6, 36]. Conversely, sampling too late may produce false-negative results because of the emptying of urea from the stomach. Accuracy with these simplified protocols was very high, with both sensitivity and specificity of 97%. In conclusion, when UBT is performed following the most widely used protocol (i.e., with citric acid and 75 mg of urea solution), excellent accuracy results (sensitivity of 99% and specificity of 98%) are obtained when breath samples are collected as early as 10–15 min after urea ingestion [3].

11.6 Diagnostic Accuracy and Appropriate Cutoff Point of the Urea Breath Test

The sensitivity and specificity of the UBT exceed 90% in most studies. The precise choice of the cutoff point to define whether the UBT is positive or negative still represents a controversial issue. A unique and generally proposed cutoff level is not possible, because it has to be adapted to different factors, such as the test meal, the dose and type of urea, or the pre-/posttreatment setting [13, 14, 16–18, 22, 24, 27, 31, 33, 35, 37–49] (Table 11.2). Previously, Logan et al. [9] suggested that the cutoff value for the ^{13}C-UBT was 5.0‰ based on the normal distribution of excess $\delta^{13}CO_2$ values for *H. pylori*-negative subjects who have never been infected. Later studies reported that the cutoff value of the ^{13}C-UBT could be lowered from 3.5‰ to 3.0‰ [50], and the cutoff value was even lower at about 2.5‰ with the use of lower doses of urea, and this value was generally accepted as the best cutoff point [13, 14, 16, 22, 24, 27, 31, 33, 37–44]. Several studies had set up the cutoff point between 1.3‰ and 7.4‰ with a high sensitivity and specificity of the ^{13}C-UBT after *H. pylori* eradication [2, 3]. However, many reports suggested that a ^{13}C-UBT value between 2.0‰ and 5.0‰ could fall into the "gray zone," in which the results are inconclusive due to many variant factors affecting the ^{13}C-UBT [2, 50]. By contrast, recent Spanish study reported that the ^{13}C-UBT (UBit 100 mg, Otsuka Pharmaceutical Europe) was associated with a very low specificity (60%) with the previous cutoff value (2.5‰) [51]. They have applied for a higher cutoff value (8.5‰) of the ^{13}C-UBT and considerably acquired the reliability in the healthy volunteers, although the sensitivity and specificity remained near 90% when UBiTkit 100 mg was applied for this test. In a Korean retrospective study [52], the prevalence of the false-negative result in the ^{13}C-UBT value in the 2.0–2.5‰ range was very low (1.5%). In contrast, the prevalence of the false-positive rate in each value of the ^{13}C-UBT between 2.5‰ and 10.0‰ range was from 6.7% to 77.3%. These occurred for results that were near the cutoff value, such as between a DOB between the cutoff values. The problem is most common for patients with atrophic gastritis, likely owing to the presence of non-*H. pylori* urease-containing organisms [25, 52]. Thus, the addition of citric acid, which suppresses the delta over baseline value in uninfected individuals and increases the value in subjects with *H. pylori* infection, should reduce the problem, but this strategy requires direct testing. False-positive results lead to the erroneous conclusion that antibiotic treatment failed. This is one cause of what appears to be multiple treatment failures [53]. False-negative UBT results have been reported to occur in as many as 40% of individuals taking proton pump inhibitors [16,

Table 11.2 Diagnostic accuracy of the ^{13}C-urea breath test

Author	Indication	Measuring equipment	Urea dose (mg)	Test meal	Cutoff point (δ ‰)	Sensitivity (%)	Specificity (%)
Braden et al. [39]	Pre	IRMS	75	None	5	99	100
Dominguez et al. [24]		IRMS	75	Citric acid	4	100	100
Gatta et al. [13, 16]	Pre/post	IRMS	50, 75, 100	Citric acid	1.6–7.4	100	100
Gisbert et al. [31, 40]	Pre/post	IRMS	100	None or citric acid	3.3–4.6	100	100
Hamlet et al. [41]	Pre/post	IRMS	100	Citric acid	1.8–4.6	95–100	97–100
Kato et al. [42]	Pre/post	IRMS	100	None	2.5–3.5	67–100	96–100
Klein et al. [22]	Pre/post	IRMS	125, 250	Milk	2.4–4.2	100	98–100
Leodolter et al. [27, 43]	Pre/post	IRMS	75	Citric acid	4	92–100	98–100
Liao et al. [44]	Pre	IRMS	50	Milk	2.5	99	95–97
Malaty et al. [14]	Pre	IRMS	125	None	2.4	96	100
Miwa et al. [33]	Pre/post	IRMS	100	None	5	96	97
Ng et al. [45]	Pre	IRMS	75	None or citric acid	3.5–5	93–97	94–97
Savarino et al. [46, 47]	Pre/post	IRMS	75	Citric acid	5	98–100	97–100
Sheu et al. [48, 49]	Pre/post	IRMS	50, 100	None or citric acid	2.5–4	94–97	95–99
Wong et al. [17, 18, 35]	Pre/post	IRMS	50, 75	None or citric acid	1.2–7	96–100	90–100

Modified from Gisbert et al. [3]
Pre before eradication treatment, *Post* after treatment to assess *H. pylori* eradication success, *IRMS* isotope ratio mass spectrometer

38]. One is related to the effect of the proton pump inhibitor on intragastric pH, which could make the intragastric environment unattractive for *H. pylori* and thus indirectly reduce the bacterial load. Alternatively, the pH could be increased sufficiently to close the postulated urea channel, UreI, and thus reduce urea's access to *H. pylori* urease [2]. Besides the effect on pH, the known direct anti-*H. pylori* antimicrobial effect of proton pump inhibitors could result in a direct reduction in bacterial load below the critical threshold of urea hydrolysis required for a positive test. Graham et al. [38] reported that acidification of the stomach did not prevent false-negative UBT results. Three days is likely the minimum delay from stopping PPI until one should perform a test for active infection. A delay of 14 days is preferred [19]. Moreover, the UBT has shown heterogeneous accuracy in the pediatric population, especially in young children, with values of sensitivity and specificity ranging from 75 % to 100 %, before and after treatment (using several protocols), despite being a simple and safe noninvasive test in children older than 6 years old [54]. Although several modifications have been proposed since the original description by Graham of the ^{13}C-UBT to diagnose *H. pylori* infection [6], in children, performance criteria are not yet sufficiently established [55].

Few studies using UBT have been performed in patients subjected to a partial gastrectomy, a specific group in which the identification of *H. pylori* infection is mostly relevant. After partial gastrectomy, the gastric anatomy is altered and the test urea might be expected to pass through the stomach faster, giving different reactant percentages

in reaction time, or the ^{13}C-UBT results could be influenced by bile acid reflux. In a hypochlorhydric state of the remnant stomach, it accelerates the colonization and the overgrowth of non-*H. pylori* urease-positive bacteria (*Streptococcus*, *Staphylococcus*, *Gardnerella*, *Lactococcus*, and *Enterococcus*) [56], and elevation of intragastric pH removes the neutralizing action of hydrochloric acid on local ammonia production by *H. pylori* urease, leading to the ultimate death of the bacterium as a result of overalkalization [57–59]. Several reports have shown that ^{13}C-UBT provides lower diagnostic accuracy when using histology as a reference in the remnant stomach after partial gastrectomy [60, 61].

Conclusions

Since the discovery of *H. pylori* in 1983, many different methods have been used for detection of *H. pylori* infection, either noninvasive or invasive. The ^{13}C-UBT is the ideal test for those in whom endoscopy is not required because it offers the combination of simplicity, accuracy, absence of exposure to radioactivity, and reliability. However, definitive standardization of the protocol for UBT does not yet exist (sufficient urea dose, use of test meal, and appropriate cutoff value for UBT result). Future studies should be provided for reducing the frequency of false-positive and false-negative UBT results.

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H. pylori Stool Antigen Test

12

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Abstract

Helicobacter pylori (*H. pylori*) stool antigen (HpSA) test is a reliable noninvasive diagnostic method for the detection of *H. pylori* infection in both adults and children. According to recent data, the HpSA test using an enzyme immunoassay is well validated as a noninvasive test to determine the status of *H. pylori* infection with high diagnostic accuracy before eradication therapy as well as after therapy. The HpSA test using a monoclonal antibody is more accurate than a polyclonal antibody test. The rapid HpSA test is also a reliable test and easy to perform in clinical practice. When the HpSA test is applied, some factors that can affect the diagnostic accuracy such as drugs, temperature, and gastrointestinal bleeding and diarrhea as accompanying symptoms should be taken into consideration to interpret the results of the HpSA test.

Keywords

Helicobacter pylori • Stool antigen test • Noninvasive • Diagnosis

12.1 Introduction

Helicobacter pylori (*H. pylori*) stool antigen (HpSA) test is a well-known noninvasive method for diagnosing *H. pylori* infection. Since it was developed and introduced in 1997, the HpSA test has been applied in clinical practice. When compared to other noninvasive diagnostic tests to detect *H. pylori* infection, the HpSA test has some advantages because it can be easily carried out at all ages and is relatively cheap despite its high diagnostic accuracy.

Initially, the HpSA test was based on the enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) methods using polyclonal antibodies to detect *H. pylori* antigens in the feces. Later, HpSA tests using monoclonal antibodies with higher diagnostic accuracy were also developed. Recently, rapid HpSA test based on immunochromatography methods was also developed and has been validated.

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In this chapter, updates on the HpSA tests for the diagnosis of *H. pylori* infection will be reviewed systematically.

12.2 Diagnostic Accuracy of *H. pylori* Stool Antigen Test

12.2.1 Diagnostic Accuracy of *H. pylori* Stool Antigen Test in Untreated Patients

The HpSA test using an EIA method is a noninvasive diagnostic method to identify *H. pylori* infection with high diagnostic accuracy in both adults and children of any age [1]. According to recent studies, its diagnostic accuracy is similar to the urea breath test and higher than both serologic and urine antibody tests [2].

In adults, the diagnostic accuracy of the HpSA test for initial diagnosis of *H. pylori* infection was known to be high with a sensitivity of 89–98 % and specificity of 78–100 % [3–5]. Overall diagnostic accuracy of the HpSA test based on EIA, of both polyclonal HpSA and monoclonal HpSA tests, as reported by a systemic review in 2004, revealed mean sensitivity of 91 %, mean specificity of 93 %, positive predictive value (PPV) of 92 %, and negative predictive value (NPV) of 87 % [6], and pooled sensitivity of 94 %, specificity of 97 %, positive likelihood ratio (LR+) of 24, and negative likelihood ratio (LR−) of 0.07 in a meta-analysis reported in 2006 [7]. According to this meta-analysis, the sensitivity of the monoclonal HpSA test (95 %) was higher than that of the polyclonal HpSA test (83 %) [7].

In children, diagnostic accuracy of the HpSA test is similarly high as that in adults. Recent meta-analysis study on the HpSA test reported that the HpSA test using ELISA polyclonal antibodies had the sensitivity of 92 %, the specificity of 93 %, LR+ of 16.2, and LR− of 0.09. The monoclonal study also revealed higher diagnostic accuracy in children with sensitivity of 97 %, specificity of 97 %, LR+ of 29.9, and LR− of 0.03 [8]. Additionally, another recent meta-analysis in 2014 reported similar diagnostic accuracy of the EIA-based HpSA test in children, reporting an overall pooled sensitivity of 92.1 %, specificity of 94.1 %, LR+ of 17.01, and LR− of 0.08 [9]. In this meta-analysis performed in children, mean sensitivity and specificity were 92.6 % and 93.8 %, respectively, for initial diagnosis of *H. pylori* infection [9].

12.2.2 Diagnostic Accuracy of *H. pylori* Stool Antigen Test After Eradication

The HpSA test is a useful follow-up method after eradication therapy in both adults and children [10–13]. After the eradication of *H. pylori* infection, diagnostic accuracy is still high with sensitivity of 70–96 % and specificity of 81–95 % [10–13]. In a recent meta-analysis on the accuracy of the HpSA test in children, mean sensitivity and specificity were 80.9 % and 97.2 %, respectively, with LR+ of 19.71 and LR− of 0.171 for the diagnosis of *H. pylori* infection after eradication therapy in children [9]. Recent guidelines from ESPGHAN (European Society for Paediatric Gastroenterology, Hepatology, and Nutrition) and NASPGHAN (North American Society for Paediatric Gastroenterology, Hepatology, and Nutrition) for the diagnosis of *H. pylori* infection in children recommended the HpSA test based on EIA as a reliable diagnostic test to identify the eradication of *H. pylori* infection [14].

When comparing the monoclonal HpSA and the polyclonal HpSA tests, the former is more accurate not only for the initial diagnosis of *H. pylori* infection, but also for follow-up after eradication therapy, with a reported pooled sensitivity of 93 %, specificity of 96 %, LR+ of 17, and LR− of 0.1 [7]. In particular, the HpSA test using EIA monoclonal antibody has higher sensitivity (91 %) than the polyclonal test (76 %) after eradication of *H. pylori* infection, according to the meta-analysis [7].

12.3 Types of *H. pylori* Stool Antigen Test

12.3.1 *H. pylori* Stool Antigen Test Based on EIA

There are two types of HpSA test for the diagnosis of *H. pylori* infection according to the basic techniques: one based on EIA and the other based on immunochromatography. Both of them have some advantages in clinical practice because they are inexpensive and noninvasive methods to identify *H. pylori* infection with relatively high diagnostic accuracy. These HpSA tests can be performed just by collecting stool specimens from the patients at anytime because stool samples can be stably stored in the freezer before testing.

Most HpSA tests used in clinical practice are EIA-based tests using polyclonal or monoclonal antibodies against *H. pylori* antigens from stool specimens which are coated on microplates and undergoes a sandwich enzyme immunoassay technique [15]. This EIA-based HpSA test is easily performed in all age groups, even in infancy, by collecting stool samples noninvasively without any specialized techniques but requires a spectrophotometer to interpret the test results.

12.3.2 *H. pylori* Stool Antigen Test Based on Immunochromatography

Rapid HpSA test using monoclonal antibody based on immunochromatography was more recently developed and has been applied for the diagnosis of the infection before and after therapy [13]. The test is very easy to perform and suitable as a one-step in-office test because it takes only 5–15 minutes to interpret the test results grossly without any expensive equipment or skilled personnel.

However, diagnostic accuracy of the rapid HpSA test is similar or a bit lower than the conventional HpSA test [16], with sensitivity of 88 %, specificity of 93 %, LR+ of 10.6, and LR− of 0.11, according to a recent meta-analysis study [8]. Therefore, this rapid HpSA test has some limitations in clinical practice despite its convenience.

12.4 *H. pylori* Stool Antigen Test in Specific Conditions

When applying the HpSA test clinically, additional attention is required since some factors can affect the test results. Diagnostic accuracy of the HpSA test using loose or watery stool specimen can be relatively lower because *H. pylori* antigens in stool samples are diluted. Thus, HpSA testing is not recommended when the patient has symptoms of diarrhea, especially in the posttreatment setting [17]. However, in another study, it was reported that the false-negative results were not brought on if the dilution was by less than ten fold [18].

When there is upper gastrointestinal bleeding, it has been reported that the diagnostic accuracy of the HpSA test is relatively low [19]. In clinical practice, monoclonal HpSA test based on ELISA is recommended in such patients [19].

The temperature also affects the diagnostic accuracy of the HpSA test. Although stool specimens can be stored for up to 3 days at 2–8 °C, it is recommended that the test be carried out as soon as possible when the stool was collected. If stool specimens should be saved for a long time, it is good to be stored at −80 °C to maintain its antigenicity [17].

Proton pump inhibitors also can affect the diagnostic accuracy of the test, thus it is recommended to test after a lapse of 1 or 2 weeks after discontinuing the drug [6].

Conclusions

HpSA test is useful for noninvasive diagnosis of *H. pylori* infection by using stool specimen for testing. HpSA test can be applied to both adults and children, even infants, with excellent diagnostic accuracy before and after eradication therapy of *H. pylori*, although posttreatment accuracy is relatively low after therapy. HpSA test based on ELISA monoclonal antibody, rather than polyclonal test may

be the more accurate noninvasive method to detect *H. pylori* infection in clinical practice. The rapid HpSA test using monoclonal antibody based on immunochromatography may be a good alternative as an in-office one-step test.

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Specific Conditions: Children

13

Hye Ran Yang

Abstract

Helicobacter pylori (*H. pylori*) infection is usually acquired during childhood, causing various diseases even in children. When applying diagnostic tests to children suspected of *H. pylori* infection, there are some differences between children and adults, although endoscopy with biopsies is also recommended for the initial diagnosis of *H. pylori* infection in children. On endoscopy, nodular gastritis is a pathognomonic gross finding of childhood *H. pylori* infection. On histopathology of gastric tissues, infiltration of lymphocytes and plasma cells and formation of mucosa-associated lymphoid tissue are the prominent features in children. Noninvasive diagnostic methods are also available and well validated in children. Both the ^{13}C-urea breath test (UBT) and the *H. pylori* stool antigen test based on the enzyme immunoassay using monoclonal antibody are reliable in identifying *H. pylori* status before and after therapy. However, when applying UBT to children younger than 6 years, test results should be interpreted cautiously because they tend to have high false positives. Serologic tests or urine tests are not recommended in children because of low diagnostic accuracy.

Keywords

Child • Diagnosis • Endoscopy • *Helicobacter pylori* • Noninvasive

13.1 Introduction

Helicobacter pylori (*H. pylori*) infection is a common infectious disease of the gastrointestinal tract in children, although its prevalence is decreasing [1]. *H. pylori* infection is mainly acquired during early childhood [2], and most of the infected children are asymptomatic initially. However, it can cause various gastrointestinal diseases such as gastritis, gastric or duodenal

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ulcer, and mucosa-associated lymphoid tissue (MALT) lymphoma, even in children, as well as extraintestinal diseases such as growth retardation and iron deficiency anemia [3].

When applying diagnostic tests to children suspected of *H. pylori* infection, it is obvious that there are some differences between children and adults. Endoscopy with biopsies is basically recommended for initial diagnosis of *H. pylori* infection even in children. However, endoscopic investigation is somewhat difficult to apply to young children because of its invasiveness. Thus, noninvasive diagnostic tests are preferred in pediatric population in clinical practice. With the development of equipment and techniques, endoscopy is now available for all ages. Moreover, a variety of noninvasive methods, including the urea breath test (UBT) and the *H. pylori* stool antigen (HpSA) test, have been validated to diagnose *H. pylori* infection in children. Hence, proper diagnostic strategies for *H. pylori* infection are required in children.

In this chapter, updates on the diagnostic approach to *H. pylori* infection in children will be systematically reviewed.

13.2 Endoscopic Diagnosis of *H. pylori* Infection in Children

13.2.1 Application of Endoscopy with Biopsy in Children

In children, endoscopy is not recommended to solely identify the status of *H. pylori* infection, even in an individual with functional abdominal pain [3]. When a child has a first-degree relative with the history of gastric cancer or the child suffers from iron deficiency anemia refractory to long-term iron supplementation therapy without any other causes of anemia, diagnostic approach using endoscopy can be considered as per the guidelines from ESPGHAN (European Society for Paediatric Gastroenterology, Hepatology, and Nutrition) and NASPGHAN (North American Society for Paediatric Gastroenterology, Hepatology, and Nutrition) for *H. pylori* infection in children published in 2011 [3]. According to these guidelines, diagnostic testing such as endoscopy should only be used to investigate the underlying cause of the gastrointestinal symptom itself and not to detect the presence of *H. pylori* infection [3].

Basic investigations for the initial diagnosis of *H. pylori* infection in children are rapid urease test and gastric tissue staining or culture for *H. pylori* using gastric tissues obtained during upper gastrointestinal endoscopy, just as in adults [4]. When both the rapid urease test and histology are positive or when culture for *H. pylori* is positive, it is reasonable to confirm *H. pylori* infection in children, according to the evidence-based guidelines from ESPGHAN and NASPGHAN [3].

For histological examination, at least two gastric tissues from the antrum and the body are required [3]. These tissues are stained with hematoxylin-eosin to evaluate the severity of acute and chronic inflammation, atrophy, and intestinal metaplasia, as per the updated Sydney classification for the histopathological findings of gastritis [5]. Special staining including the Warthin-Starry silver stain, Giemsa stain, or modified Genta stain and additional immunohistochemistry may aid in the diagnosis of *H. pylori* infection [5].

If the test results of the rapid urease test and histology do not match at all, additional noninvasive diagnostic tests such as the UBT or the HpSA test may be helpful [3].

13.2.2 Endoscopic Findings in *H. pylori*-Infected Children

When mucosal nodularity in the gastric antrum or ulceration in the duodenal bulb is noted on upper gastrointestinal endoscopy, *H. pylori* infection can be suspected in children [3].

Nodular gastritis or follicular gastritis has a gross appearance of numerous gooseflesh-like small nodules on the gastric mucosa, which is a common endoscopic finding in children with *H. pylori* infection [6, 7]. During the chronic course of *H. pylori* infection, antral nodularity (gastric lymphoid hyperplasia) was observed in 44–67 %

of *H. pylori*-infected children, in contrast to 0.19% of *H. pylori*-infected adults [7, 8]. Additionally, when nodular gastritis is observed on endoscopy, *H. pylori* can be present in approximately 90% of pediatric patients. Thus, endoscopic manifestations of gastric mucosa in children with *H. pylori* infection are somewhat different from those of adults, and nodular gastritis can be a pathognomonic endoscopic finding of childhood *H. pylori* infection with relatively high specificity, but low sensitivity [7]. Since *H. pylori* infection is acquired during childhood, it can cause chronic gastritis, mainly in the form of nodular gastritis.

Gastric cancer or MALT lymphoma is extremely rare during childhood. However, as reported in a previous study in adults, histopathological findings of antral nodularity caused by *H. pylori* infection are similar to those of early-stage gastric MALT lymphoma [9]. Moreover, a recent study in children also reported that severe nodular gastritis in the antrum on endoscopy can indicate gastric MALT on histology even in *H. pylori*-infected children [6]. Because the degree of nodularity on endoscopy may correlate with the grades of gastric MALT, histopathological evaluation for gastric MALT may be recommendable in cases of *H. pylori*-infected children with severe nodular gastritis on endoscopy.

13.2.3 Histopathologic Findings in *H. pylori*-Infected Children

For the diagnosis of *H. pylori* infection in children on the basis of histopathological investigation, along with the rapid urease test or culture for *H. pylori*, the updated Sydney classification should be applied to determine the presence and the severity of acute and chronic inflammation, atrophy, and intestinal metaplasia [3, 5].

Nodular gastritis, a pathognomonic endoscopic finding in *H. pylori*-infected children, indicates a high grade of *H. pylori* colonization and chronic active gastritis with lymphoid follicles with germinal center or MALT, exaggerated by inflammatory reaction caused by *H. pylori* infection [10, 11], and its severity may deteriorate as time passes [12]. In previous studies, antral nodularity in children were correlated with higher histological scores on the gastric mucosa, indicating higher severity of gastritis, and was also associated with higher density of *H. pylori* and *cagA*-positive strains [13, 14].

Histological findings of *H. pylori*-associated chronic gastritis in children are somewhat different from those in adults because lymphocytes or plasma cell infiltration is more prominent and neutrophil infiltration is less prominent in children [15, 16].

Atrophy or intestinal metaplasia of gastric mucosa is a rare pathologic finding in children [17]. However, one study reported relatively high prevalence of gastric atrophy and low-grade intestinal metaplasia in *H. pylori*-infected children compared to uninfected children [18].

13.3 Noninvasive Diagnosis of *H. pylori* Infection in Children

Because endoscopic diagnosis of *H. pylori* infection is relatively costly and invasive to apply to children, there are some limitations, especially in posttreatment settings. Therefore, noninvasive diagnostic tests with high diagnostic accuracy are preferred in childhood to detect *H. pylori* infection. There are several noninvasive diagnostic tests available in children such as UBT, HpSA test, and *H. pylori* antibody tests on serum, urine, or saliva [3, 19].

13.3.1 Urea Breath Test in Children

UBT is one of the most widely used noninvasive diagnostic methods in children. It is very safe, economic and easy to perform. In addition, UBT is a very accurate noninvasive test to identify *H. pylori* infection even in children and adolescents.

Previously noted diagnostic accuracy of the UBT is high enough to diagnose *H. pylori* infection, with sensitivity and specificity of about 95%, even in children [19, 20]. A recent

meta-analysis on ^{13}C-UBT for the diagnosis of *H. pylori* infection in children revealed that UBT has excellent diagnostic accuracy in all ages with a sensitivity of 95.7 %, specificity of 95.7 %, positive likelihood ratio (LR+) of 42.6, and negative likelihood ratio (LR-) of 0.06 [20]. In children aged 6 years or more, high accuracy was observed, with sensitivity 96.6 %, specificity 97.7 %, LR+ of 42.6, and LR- of 0.04.

Meanwhile, children less than 6 years of age have relatively high false positives and low specificity compared to children older than 6 years of age and the adults [21, 22]. According to a previous study, false positives were about 8.3 % in children younger than 6 years of age compared to 0.85 % in children older than 6 years [22]. A recent meta-analysis on ^{13}C-UBT in children also confirmed that UBT has lower accuracy, especially lower specificity, in children aged 6 years or less, with sensitivity of 95 %, specificity of 93.5 %, LR+ of 11.7, and LR- of 0.12 [20]. Hence, clinical application of UBT can be somewhat limited in this age group.

There are several hypotheses to explain high false-positive results in young children aged 6 years or less. The first hypothesis is oral urease-producing microorganisms such as *Streptococcus salivarius*, *Proteus mirabilis*, and *Klebsiella pneumonia* [23]. These bacteria colonize in the oral cavity of young children, and young children tend to hold the urea in the mouth [23]. This hypothesis was supported by another study which administered urea through nasogastric tube or a gastrostomy site to avoid the degradation of urea by oral flora [24]. Thus, young children are encouraged to rinse their mouth with clean water after ingesting urea to improve the accuracy of UBT [3].

The second hypothesis is endogenous CO_2 production that differs according to age, sex, and body weight and height [25]. Since UBT measures the isotopic ratio of $^{13}CO_2/^{12}CO_2$, the ingestion of an identical amount of ^{13}C-urea regardless of age, body weight and height may increase the isotopic ratio of $^{13}CO_2/^{12}CO_2$ in a small child. In previous studies, application of urea hydrolysis rate adjusted for age, weight, and height, instead of the conventional cutoffs using a delta over baseline, revealed better outcomes [25, 26]. Therefore, although the ^{13}C-UBT test is less accurate in assessing *H. pylori* infection status in young children younger than 6 years, this limitation may be improved by adjusting cutoff values, pretest meals, or the dosage of administered urea [20]. In a previous study, applying a cutoff value of 7.0 ‰ to children aged 6 years or less, instead of the conventional cutoff value of 2.4–4.0 ‰, yielded better accuracy [22].

The sensitivity and the specificity of UBT reach almost 100 % after eradication therapy in children. According to the recent guideline from ESPGHAN and NASPGHAN for *H. pylori* infection in children, ^{13}C-UBT is recommended as the most reliable noninvasive method that can replace invasive endoscopy to determine whether *H. pylori* has been eradicated [3, 19].

13.3.2 *H. pylori* Stool Antigen Test in Children

The HpSA test using enzyme-linked immunosorbent assay (ELISA) with polyclonal or monoclonal antibodies is an effective noninvasive test with high diagnostic accuracy to identify *H. pylori* infection in children of all ages [27–29]. According to a recent meta-analysis study on HpSA testing in children, diagnostic accuracy of ELISA monoclonal antibody revealed the best performance with pooled sensitivity of 97 %, specificity 97 %, LR+ of 29.9, and LR- of 0.03 [28]. On the other hand, HpSA test using ELISA polyclonal antibody had relatively lower accuracy with a sensitivity of 92 %, specificity of 93 %, LR+ of 16.2, and LR- of 0.09, with high heterogeneity [28]. In another meta-analysis in children, the sensitivity and the specificity of monoclonal HpSA test was 96.2 % and 94.7 %, respectively, whereas those of polyclonal HpSA test were 88.0 % and 93.0 %, respectively, also suggesting better accuracy with monoclonal techniques [29]. According to the guidelines from ESPGHAN and NASPGHAN for *H. pylori* infection in children, HpSA test using ELISA with monoclonal antibody is a more convenient noninvasive diagnostic method than UBT in

children aged 6 years or less because its accuracy is not influenced by age, and it is easy to perform even in infancy, simply requiring collection of stool samples [3].

The ELISA HpSA test is also a useful noninvasive method after eradication therapy [30]. The guidelines from ESPGHAN and NASPGHAN for *H. pylori* infection in children recommended HpSA test using a validated ELISA test as a reliable method to determine *H. pylori* status after eradication therapy [3].

One-step rapid stool antigen test using immunochromatography is also available in children, albeit with relatively low accuracy compared to the HpSA ELISA test [31–33]. Diagnostic accuracy of the one-step HpSA test using monoclonal antibody revealed a sensitivity of 88 %, specificity of 93 %, LR+ of 10.6, and LR- of 0.11, according to a meta-analysis study on pediatric population [28]. Another recent meta-analysis showed a sensitivity of 88.1 % and specificity of 94.2 % for the one-step rapid monoclonal HpSA test in children [29].

After eradication therapy, the weighted mean sensitivity and specificity were 80.9 % (71.4–88.2 %) and 97.2 % (94.8–98.7 %), respectively, with LR+ of 19.71 and LR- of 0.171 in children. Meanwhile weighted mean sensitivity and specificity were 92.6 % (91.4–93.6 %) and 93.8 % (93.0–94.6 %), respectively, with LR+ of 16.33 and LR- of 0.078 before therapy [29]. After the eradication therapy of *H. pylori* infection in children, diagnostic accuracy of the conventional HpSA test using ELISA monoclonal antibody was reported to be relatively higher than the rapid HpSA test using monoclonal antibody in children [31–33].

13.3.3 *H. pylori* Antibody Tests

Many serologic tests to detect IgA and IgG antibodies against *H. pylori* are commercially available and have been applied in children for epidemiological studies and in clinical practice [19]. The serologic test may be useful in screening *H. pylori* infection, but its application has been limited in young children owing to low sensitivity. According to a meta-analysis on antibody-based diagnostic tests for *H. pylori* infection in children reported in 2008, serologic test using ELISA IgG antibody had a low sensitivity 79.2 % (95 % confidence interval [CI], 77.3–81.0) and high specificity of 92.4 % (95 % CI, 91.6–93.3), with heterogeneity [34]. Antibody titers are reported to be relatively lower in young children because of short infection period and immature immune response to *H. pylori* [35, 36]. On the other hand, a meta-analysis on children reported that antibody-based test using Western blots had relatively good performance even in children, with a sensitivity of 91.3 % (95 % CI, 88.9–93.3), specificity of 89 % (95 % CI, 85.7–91.9), LR+ of 8.2 (95 % CI, 5.1–13.3), and LR- of 0.06 (95 % CI, 0.02–0.16) [34].

The detection of serum *H. pylori* IgG antibody is not appropriate for monitoring *H. pylori* status after treatment because serum IgG antibody titers can remain elevated for a long time even after *H. pylori* is eradicated [37].

H. pylori antibodies are also detectable in urine and saliva, as well as in blood, but these antibody tests are not recommended because of its low accuracy in the pediatric population [19]. The guidelines from ESPGHAN and NASPGHAN for *H. pylori* infection in children state that antibody tests against *H. pylori* in serum, urine, or saliva are not reliable for use in children [3].

Conclusions

Diagnosis of *H. pylori* infection in children has some unique features and different diagnostic accuracy compared to that of adults. Antral nodularity on upper endoscopy may be a pathognomonic finding of *H. pylori* infection in childhood. Histopathological findings of gastric mucosa show predominant lymphocytes and plasma cell infiltration, forming gastric MALT. Among noninvasive diagnostic tests, UBT and HpSA test are both preferred in childhood as they have good accuracy on testing before and after eradication therapy. UBT has high false positives in children aged 6 years or less and thus the HpSA test with ELISA monoclonal antibody may be more beneficial in young children.

Finally, antibody-based tests using ELISA IgG antibody technique are not recommended in children because of low sensitivity.

Therefore, when determining the diagnostic test to identify *H. pylori* status in children, whether it is invasive or noninvasive, special consideration regarding these unique aspects is required in children.

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14 Specific Conditions: Diagnosis of *H. pylori* Infection in Case of Upper Gastrointestinal Bleeding

Yoon Jin Choi

Abstract

The main causes for upper gastrointestinal (GI) bleeding are peptic ulcer disease such as benign gastric ulcer (BGU) or duodenal ulcer (DU). *Helicobacter pylori* (*H. pylori*) infection was detected up to 70 % in patients with BGU and 90 % in patients with DU. Since many studies demonstrated the benefit of *H. pylori* eradication in decreasing peptic ulcer recurrences, as well as in bleeding cases, it is very important to diagnose the infection accurately among those with peptic ulcer diseases. There are several tests for detection of *H. pylori* with variable levels of sensitivity and specificity, and upper GI bleeding would reduce sensitivity. In case of upper GI bleeding, ^{13}C-UBT urea breath test is more sensitive than tests with biopsied mucosa such as rapid urease test, histological test or culture study.

Keywords

Bleeding • *Helicobacter pylori* • Rapid urease test • Urea breath test

14.1 Introduction

Upper gastrointestinal (UGI) bleeding is the most serious complications of peptic ulcer diseases, which can lead to the mortality. The well-known risk factors for peptic ulcer diseases are *Helicobacter pylori* (*H. pylori*) infection and the administration of non-steroidal anti-inflammatory drug (NSAID). When the subjects with UGI bleeding or NSAID users were excluded, *H. pylori* infection was detected up to 70 % in patients with benign gastric ulcer (BGU) and 90 % in patients with duodenal ulcer (DU) [1]. Since *H. pylori* infection is a single predictive factor for re-bleeding in patients with DU [2] and eradication of the organism can reduce the risk of re-bleeding significantly [3, 4], international guideline has recommended the tests for diagnosis of *H. pylori* infection in case of upper GI bleeding with peptic ulcer disease [5].

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It has been reported that patients with UGI bleeding showed lower *H. pylori* positivity than those without the bleeding [6, 7]. Although this result suggested that peptic ulcer disease with the bleeding might have a different pathophysiology rather than *H. pylori*, it is more reasonable that the bleeding may decrease in sensitivity of detection of *H. pylori* leading to the underestimation of its importance. Therefore, sensitivity and specificity of various *H. pylori* tests in case of UGI bleeding were reviewed.

14.2 Accuracy of Diagnostic Methods for *H. pylori* in Upper Gastrointestinal Bleeding

14.2.1 Invasive Tests

14.2.1.1 Rapid Urease Test (RUT)

Since most patients with upper gastrointestinal bleeding undergo UGI endoscopy, rapid urease test (RUT) is the most common examination in this setting. It has been reported that RUT using gastric antral mucosa showed a high probability of false negativity according in Hong Kong [8] in accordance with several studies [9–11]. Our laboratory also has reported a consistent result that the sensitivity of RUT was lower than histology in patients with peptic ulcer bleeding [12].

The precise reason for this is unclear, but there are several mechanisms proposed for the false-negative result of RUT during peptic ulcer bleeding. The bactericidal effect of serum inducing a transient decrease in bacterial density, presence of anti-*H. pylori* antibodies inhibiting urease production, suppressed urease activity by serum enzymes or electrolytes, various buffering systems (e.g., albumin, bicarbonate, and phosphate) interfering with the pH level of the RUT reagent and concomitant administration of proton pump inhibitors (PPIs) have been suggested [13]. In one in vitro study [14], a false-negative RUT result was caused by the buffering effects of serum albumin on the pH indicator but not on urease activity. Another in vitro study concluded that large amount of gastric lavage before endoscopy could result in a false-negative RUT result [15]. However, Tu TC et al. [16] found no influence on the likelihood of a false-negative result if the gastric antral biopsy specimen was cleansed by normal saline before inoculating the wells for the CLO test. Similarly, another study concluded that an artificial blood-soaked antral specimen did not influence the results of two RUTs [17]. The bactericidal effect of human plasma [18, 19] and the reduction in bacterial load by PPIs [20] have been demonstrated. Another possible consideration is that the patchy distribution of *H. pylori*, thus using samples from the gastric antrum only may be inadequate. Blood in the stomach could induce the *H. pylori* migration to the gastric body or fundus and the decrease in bacterial density of the antrum. Fewer amounts of specimens could be obtained during emergent endoscopy procedure are other explanations of false-negative results of RUT in patients with UGI bleeding [13].

Simultaneous gastric antral and body specimens or multiple biopsies have been found to produce more positive RUTs [21, 22]. Although there is a consensus on how many biopsies is appropriate for this situation, it was reported that RUT using four antral biopsies was more sensitive than that with one piece of biopsy sample from gastric antrum (61 % vs. 74 %); the former yielded the sensitivity as the same as RUT using one piece of biopsy sample from the gastric body (73 % vs. 74 %) [23].

Most authors concluded that the RUT cannot be the only diagnostic test in such circumstances [12, 24]. If the initial diagnostic test is negative, a delayed test 4–8 weeks later can have up to an 80 % positive rate in previously negative patients [13, 25].

14.2.1.2 Histology

Low sensitivity with histologic methods in patients with bleeding peptic ulcers was reported, which is consistent with RUT sensitivity [9]. However, other studies have reported that histology is more sensitive than RUT [9, 12, 26, 27]. Patchy distribution of bacterial density, staining methods and pathologist's interpretations also influence the results [13]. The sensitivity of histology also relies on the experience of the endoscopist to take the

biopsy from the appropriate site. Therefore, combination tests should be performed to achieve a more precise diagnosis [13].

14.2.1.3 Culture

Culturing *H. pylori* gives additional information such as the susceptibility to certain antibiotics but produced a low yield of sensitivity, 70–90 % [28]. The reasons for the relatively high false negativity include the inadequate amount of obtained tissue, time to the inoculation on the culture medium, conditions of the broth, and the microanaerobic nature of *H. pylori* [29]. In particular, the author reported that culturing *H. pylori* in patients with bleeding peptic ulcers showed a decrease in the sensitivity [12]. Therefore, the culture study should not be considered as the first diagnostic test for *H. pylori* in UGI setting due to the time-consuming nature of the process and the lack of time to perform the procedure during endoscopy. However, this method can be used when the patients have resistance to anti-*H. pylori* therapy together with other diagnostic methods.

14.2.1.4 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) using either gastric or buccal mucosa has been used as an invasive test to diagnose *H. pylori* infection [30]. In one study, this test was less sensitive in patients with bleeding peptic ulcers than for those with non-bleeding peptic ulcers and chronic gastritis [31]. However, another study reported that PCR had higher sensitivity than other biopsy-based tests and similar sensitivity to noninvasive tests [32]. It has been also reported that Real-time PCR can improve *H. pylori* detection rate in a histology-negative, formalin-fixed, and paraffin-embedded tissue [33]. Since the newly developed quantitative real-time PCR is around ten times more sensitive than the conventional PCR method [34], real-time PCR in diagnosis of *H. pylori* infection in patients with UGI bleeding could be useful diagnostic methods with increase in sensitivity and additional gain of bacterial load [35]. However, further study about the cut-off value which can distinguish the real infection from asymptomatic colonization is warranted.

14.2.2 Noninvasive Tests

14.2.2.1 Urea Breath Test

Many studies have confirmed that the ^{13}C-UBT can accurately diagnose *H. pylori* infection in patients with UGI bleeding [16, 26, 27, 36]. The sensitivity of this study is not affected by blood in the stomach and is higher than those of biopsy-based methods and other noninvasive tests [37]. Nonetheless, since patients should drink a urea-containing solution with a test meal or citric acid, this method is not suitable for bleeding patients. Therefore, most UBTs are done when patients start eating, or the UBT is reserved as a delayed test if the initial invasive methods are negative. However, using low-dose encapsulated ^{13}C-urea has proven to be feasible in fasting patients or even before an endoscopy because it only takes a small amount of water to swallow the pill [38].

14.2.2.2 *H. pylori* Stool Antigen Test

The *H. pylori* stool antigen test is a relatively accurate noninvasive test, which can be performed by enzyme-linked immunosorbent assay (ELISA) with monoclonal or polyclonal antibodies or by immunochromatographic assay with monoclonal antibodies [13]. The sensitivity of this method is reduced by UGI bleeding when polyclonal ELISA or immunochromatographic stool antigens are used [36, 39, 40]. Several studies reported a high number of false-positive results in patients with UGI bleeding due to a cross-reaction with the blood [40, 41]. Therefore, the *H. pylori* stool antigen diagnostic test is not recommended for use in patients with UGI.

14.2.2.3 Serology

The author [12] and others [9] have demonstrated that serology is more sensitive than other invasive tests in cases of bleeding peptic ulcer. Although serologic test can be used as the initial invasive test, it is mostly performed when other test is negative. However, commercial serological tests must be confirmed by a local laboratory before they are used in an individual hospital [42]. More importantly, if patients have been treated for *H. pylori* infection, serological tests have revealed that serum antibodies may last for up to a year

[42]. This fact must not be overlooked when interpreting the results.

14.3 Appropriate Methods for Detection of *H. pylori* in Upper Gastrointestinal Bleeding

Although guidelines recommend diagnostic tests for *H. pylori* in UGI bleeding, there has been no consensus about which methods should be used in this setting. In spite of the reduced accuracies of *H. pylori* diagnostic tests in patients with bleeding peptic ulcers, RUT is conducted most frequently for patients with UGI bleeding because of its convenience and rapidity. Multiple biopsies on both the antrum and body for UBT and combination with other tests raise the detection rate of *H. pylori* [23]. How many pieces of biopsy need to be further studied, but this can apply to the patients differently according to the conditions. If only a single biopsy is permitted, gastric body is more suitable for the high yield [23]. Repeated biopsy in non-bleeding focus or additional ^{13}C-UBT can increase accuracy of detecting *H. pylori* [43]. On the contrary to this, stool antigen tests were less accurate, and serology, though not influenced by UGI bleeding, was not recommended as the first test.

Conclusions

Although *H. pylori* infection has been decreasing due to the prevalent anti-*H. pylori* therapies, *H. pylori* is still an important risk factor for the diseases. Moreover, aging and increasing prevalence of degenerative muscular or cardiovascular diseases increased NSAID users leading to peptic ulcer. NSAID and *H. pylori* infection may have a synergistic effect for UGI bleeding in patients with peptic ulcer disease [44], but eradication of *H. pylori* can decrease the likelihood of peptic ulcer rebleeding and associated complications. This suggests that more accurate diagnosis of *H. pylori* is important in patients with UGI bleeding. Knowledge for the characteristic and limitation of each *H. pylori* test and selection of appropriate diagnostic methods in patients with peptic ulcer bleeding could be a basis for the management of peptic ulcer patients.

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Part IV

Symptom

Symptoms of Acute and Chronic *H. pylori* Infection

15

Nayoung Kim

Abstract

Helicobacter pylori (*H. pylori*) has urease and *ureI* gene to overcome gastric acid to survive on the acidic human stomach mucous membrane. Once *H. pylori* enters the stomach, acute gastroenteritis symptoms, such as nausea, vomiting, epigastric pain, and heartburn, occur. These symptoms emerge within the first week of infection, and the symptoms reach their maximum severity on the ninth to 12th day of the infection. Then, the symptoms get better, and most of those are gone after 2 weeks of the infection. Based on a gastroscopic view of *H. pylori* infection, erythema can be seen on the gastric mucosa after the fifth day of infection, and gastric juice pH is reported to be 7.0, which is neutral. Then, the erythema findings get better after 2 weeks of infection, while the gastric juice pH is 7.5, which is indifferent. Later, the gastroscopic view is back to normal after 74 days of *H. pylori* infection, while lymphocyte and plasma cell infiltrations increase, and gastric juice pH becomes 2. Thus, continued *H. pylori* infection after 2 months can be considered as chronic infection. Once chronic infection is maintained, *H. pylori* influences on symptoms that might be related to the brain-gut axis through various immunologic mechanisms and is expected to cause symptoms on organs other than stomach. However, unequivocal data concerning the direct and immediate effect of *H. pylori* infection on the brain-gut axis are still lacking. Therefore, further studies evaluating the clinical importance of these host-bacteria interactions will improve our understanding of *H. pylori* infection pathophysiology.

Keywords

Symptom • Acute • Chronic • Infection • *Helicobacter pylori* • Brain-gut axis

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15.1 Introduction

Helicobacter pylori (*H. pylori*) is thought to have evolved continuously inside the human stomach when mankind migrated from Africa to America and Oceania [1]. *H. pylori* is a gram-negative bacteria that has urease, which takes up 10% of the bacteria's entire protein, and *ureI* gene to overcome gastric acid and to survive on the human gastric mucosa that is covered by gastric acid. In other words, *H. pylori* has gone through successful complex mutations [2, 3]. *H. pylori* is assumed to be helpful for the mankind, since it has existed for a long time with the human [4]. However, these bacteria infect 50% of global population and induce severe gastric disorders on 10% of those infected people [5–7]. Once *H. pylori* enters the stomach, acute gastroenteritis symptoms, such as nausea, vomiting, epigastric pain, and heartburn, occur, and stomach pH increases abruptly, but most of these symptoms are gone when the bacteria succeed to survive and become a chronic infection. Nonetheless, there have been reports recently that *H. pylori* infection variously affects organs other than the stomach. Thus, researches on the brain-gut axis have been conducted in full scale. This chapter is going to introduce initial symptoms when *H. pylori* enters the stomach, overcome processes of *H. pylori* and symptoms of chronic *H. pylori* infection.

15.2 The Induction Mechanisms of Symptoms After *H. pylori* Infection

When *H. pylori* enters the stomach of a human host and colonizes itself on gastric mucosa, heavy neutrophils infiltrate into the mucosa. Neutrophil infiltration and the severity of gastric mucosa damage are closely related, and it seems like gastric epithelial cell damage is caused by infiltrated leukocytes due to *H. pylori* infection [8]. A protein on *H. pylori* surface called *H. pylori* neutrophil-activating protein (HP-NAP) attracts neutrophils from blood to infected gastric mucosa and activates them [9–11]. Other than HP-NAP, urease that helps *H. pylori* to survive in acidic condition of stomach attracts neutrophils and monocytes and affects on inflammatory cytokine secretion [12] (Fig. 15.1). Also, interleukin (IL)-1β, IL-2, IL-6, and IL-8 and tumor necrosis factor (TNF)-α increase under *H. pylori* infection; IL-8 is the most powerful neutrophil-activating protein, and it is more active when *H. pylori* has *cag*-pathogenicity island (PAI) [11]. *H. pylori* infection induces active immunologic reactions on overall body or on local area, but these reactions cannot eradicate *H. pylori* and rather contribute to stomach cell damage. Th2 reaction is expected because *H. pylori* does not infiltrate into stomach cell by itself, but *H. pylori* infection induces IL-18 production, which leads to Th1 reaction, and Th1 reaction helps *H. pylori* for its sustained survival in stomach [11]. Meanwhile, there is an argument that sialic acid-binding adhesion (SabA) factor of *H. pylori* attaches to sialylated glycoconjugates on the surface of neutrophil, instead of HP-NAP, to activate neutrophil and induces acute gastroenteritis [13]. If *H. pylori* infection continues, T cell, B cell, plasma cell, and macrophage as well as neutrophil infiltrate and induce gastric epithelial cell damage [11]. Thus, these reactions contribute to acute and chronic inflammations due to *H. pylori*, and a recent research suggests that the relationship between *H. pylori* and the brain-gut axis induces the symptoms [14]. In other words, cytotoxins that are similar to VacA of *H. pylori* and IL-8, neuroinflammation due to HP-NAP, and free radical that is derived by *H. pylori* are assumed to damage the blood-brain barrier and cause alteration of neurotransmitter secretion of the gastric mucosa and spinal cord, and the changes of stomach microbiota could be the causes of gastroenterological symptoms after *H. pylori* infection [14] (Fig.15.2).

15.3 Brain-Gut Axis, a Possible Mechanism of Symptoms of *H. pylori* Infection

The fact that only 10–15% of *H. pylori*-infected patients presenting dyspeptic symptoms [15] suggest a role for other individual factors, including nervous system imbalance, as an indispensable cofactor in gastritis or ulcer disease pathogenesis

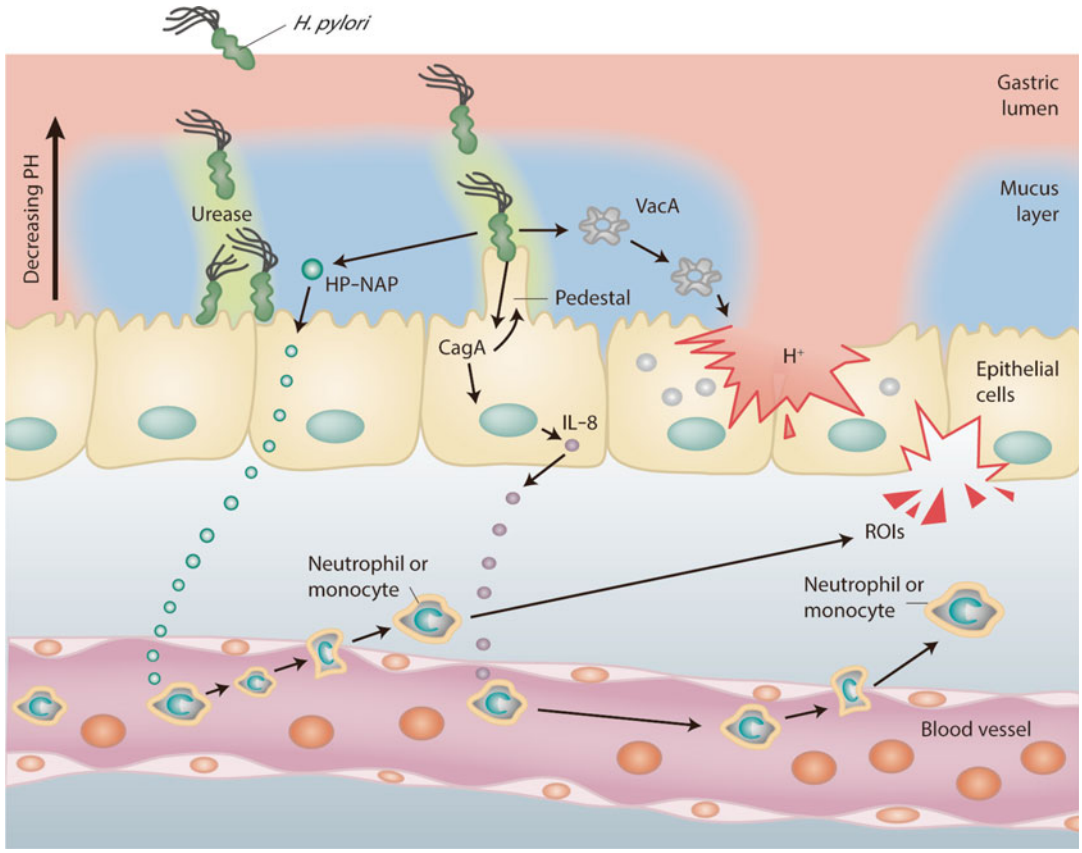

Fig. 15.1 Main virulence factors that induce diseases and help *H. pylori* surviving on the stomach mucous membrane. *HP-NAP H. pylori* neutrophil-activating protein, *ROIs* reactive oxygen intermediates (Adapted from Montecucco and Rappuoli [12])

[14]. These arguments imply that *H. pylori* infection may induce changes in the function and morphology of the digestive tract both directly through cytotoxin (CagA, VacA) release and inflammatory process activation and, indirectly, via the brain-gut axis [16]. The brain-gut axis integrates the central, peripheral, enteric, and autonomic nervous systems, as well as the endocrine and immunological systems, with gastrointestinal functions and environmental stimuli, including gastric and intestinal microbiota [14]. As *H. pylori* infection causes very active inflammation in the stomach, this process of acute infection may also induce abnormalities indirectly by affecting the brain-gut axis, similar to other microorganisms present in the alimentary tract [14]. The bidirectional relationship between *H. pylori* infection and the brain-gut axis influences both the contagion process and the host's neuroendocrine-immunological reaction to it, resulting in alterations in cognitive functions, food intake and appetite, immunological response, and modification of symptom sensitivity thresholds [14]. Furthermore, disturbances in the digestive tract permeability, motility, and secretion can occur, similar to a form of irritable bowel syndrome. Actually *H. pylori* has been an important candidate for etiological factor of functional dyspepsia (FD) [17]. *H. pylori* infection could contribute to the generation of dyspepsia or gastric symptoms by affecting the brain-gut axis which is involved in bacterial toxins, *H. pylori*-induced cytokines, or gastric neurotransmitter [14]. For instance, *H. pylori*-related gastritis may impede the production of ghrelin by causing destruction of ghrelin-producing cells [18, 19]. Choi et al. suggested that acyl ghrelin may be associated with appearance

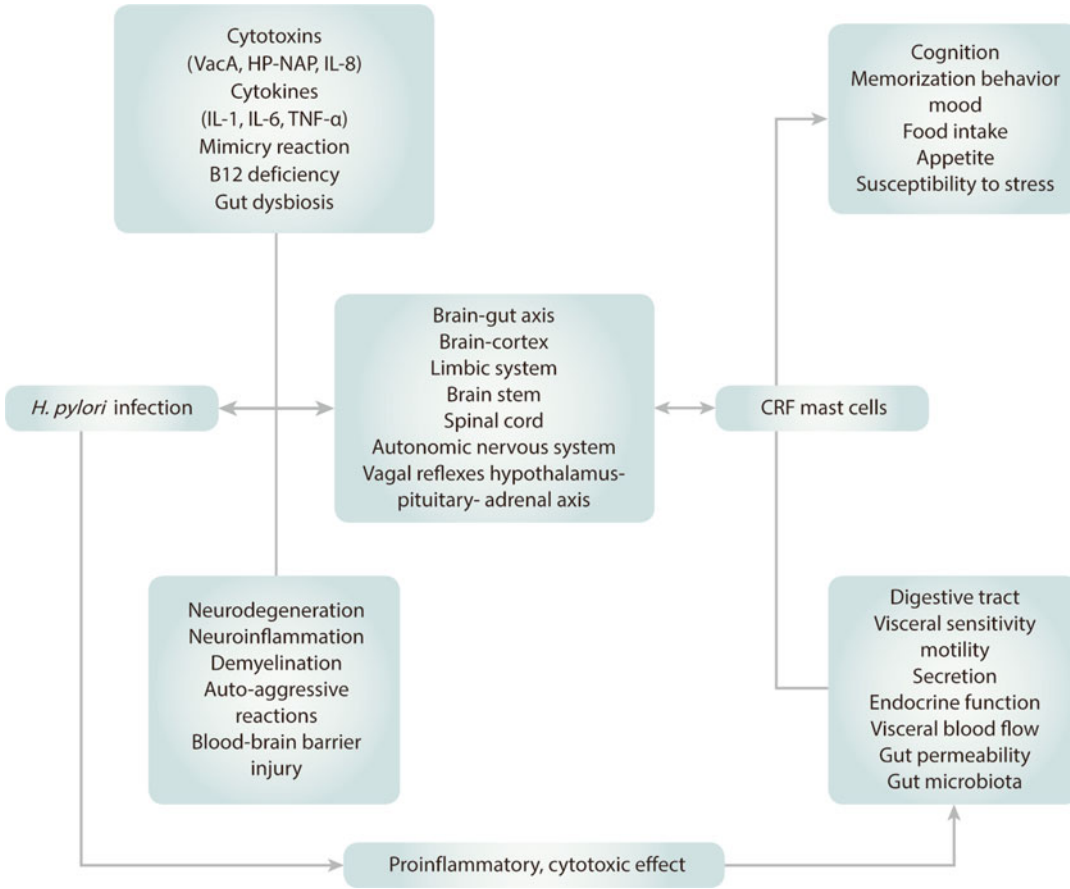

Fig. 15.2 Induction of gastroenterological symptoms that are related to the brain-gut axis due to *H. pylori* infection. *CRF* corticotropin-releasing factor, *TNF-α* tumor necrosis factor-alpha, *IL* interleukin, *HP-NAP H. pylori* neutrophil-activating protein, *HP-NAC H. pylori*-activating capacity (Adapted from Budzynski and Klopocka [14])

and exacerbation of postprandial fullness or early satiation probably related with the impaired gastric motility [20]. This is supported by the results of Shindo et al. in which postprandial discomfort syndrome (PDS)-subtype FD patients showed reduced acyl ghrelin levels correlated with delayed gastric emptying [21]. Furthermore, a recent study demonstrated that ghrelin agonist accelerated gastric emptying [22]. Actually anti-*H. pylori* therapy enhanced acyl ghrelin levels following an improvement of atrophy and correlated with improvement of PDS symptoms [20]. Similarly FD symptoms disappeared following *H. pylori* eradication in part of FD patients [23–25]. Finally, *H. pylori*-associated FD has been now accepted as one entity which is different from idiopathic FD [26]. From this background, *H. pylori* may have some relationship with the brain-gut axis through activation of neurogenic inflammatory processes or by microelement deficiency secondary to functional and morphological changes in the digestive tract. In addition, *H. pylori* can alter signaling in the brain-gut axis by mast cells, the main brain-gut axis effector, as *H. pylori* infection is associated with change of mast cell infiltration in the digestive tract [14].

15.4 Symptoms and Endoscopic and Histological Findings of Acute *H. pylori* Infection

Symptoms of acute *H. pylori* infection are thought to appear on 60% of entire infected patients [27]. Once the bacteria enter the stomach and induce infection, subjects developed variable gastrointestinal and constitutional symptoms. Dyspeptic symptoms were mild to moderate in severity and typically appeared during the first

week after challenge and peaked between days 9 and 12 [28]. Other recorded symptoms were headache ($n=1$), anorexia ($n=1$), dyspepsia ($n=7$), vomiting ($n=1$), abdominal pain ($n=6$), belching ($n=5$), and halitosis ($n=3$). These symptoms start within the first week of infection, and these symptoms reach their maximum degrees on 9–12 days after the infection [28]. Then, the symptoms are attenuated by themselves, and most of the symptoms are gone after 2 weeks of the infection [28–31]. In no instance did symptoms interfere with regular activities of daily living [28]. In the different group, symptoms typically first occurred sporadically after day 3 after challenge with 10^4–10^{10} colony-forming unit (CFU) of a *cag* PAI negative, outer membrane inflammatory protein (OipA)-positive strain of *H. pylori*, peaked in the second week, and remained relatively consistent for 1 week and then resolved [28]. Again, early symptoms included nausea and abdominal cramps, followed by feelings of abdominal fullness, belching, and mild decreased appetite after 2 weeks [28]. Other reports also described similar symptoms [29–31]. Detachment of gastric epithelial cells and multiple neutrophils on the surface of the lamina propria was observed through histological examination after the fifth day of infection in the antrum and the corpus of the stomach [28]. Erythema can be seen on gastric mucosa after the fifth day of infection via gastroscopy, and the pH of gastric juice is reported to be 7.0, which is neutral [27]. However, the erythema gets better after 2 weeks of infection, but the gastric juice pH was still 7.5 [27]. Histological examination after 2 weeks of infection showed similar observations to that of the fifth day of the infection, but the number of chronic inflammatory cells increased and showed the characteristics of both acute and chronic gastritis [28]. Also, gastroscopic observation after the 74th day of infection showed normal condition, and gastric juice pH was 2, which is acidic [28]. A histological examination at this time showed that the number of lymphocytes and plasma cells increased on both the antrum and corpus of the stomach, and lymphoid follicle formations were confirmed, which indicate chronic gastritis [28]. Graham et al. also measured mucosal cytokine levels [28]. That is, IL-1β, IL-8, and IL-6 levels increased significantly 2 weeks after inoculation (IL-1β 9.2–49.1 pg/mg protein ($p=0.05$); IL-6 0.0–0.14 pg/mg protein ($p=0.005$); IL-8 4.1–179.4 pg/mg protein ($p=0.005$)) [28]. IL-8 levels increased more than 20-fold in specimens obtained 2 weeks after inoculation. In corporal biopsy specimens, there was only a significant increase in IL-8 levels (4.6–96.9 pg/mg protein) ($p=0.05$) [14]. After 12 weeks, IL-8 levels tended to decrease compared with 2 weeks, especially in the antral specimens, but IL-1β levels were not significantly different between 2 and 12 weeks [28].

15.5 Symptoms of Chronic *H. pylori* Infection

H. pylori constantly gets used to its host environment and continuously affects on the host to induce symptoms as long as it survives. Chronic inflammation induces atrophic gastritis and intestinal metaplasia on gastric epithelial cells and causes abnormal physiologic activities along with its relationship with the brain-gut axis, so functional dyspepsia could be induced as a result [14] (Fig. 15.2). The brain-gut axis relationship with chronic *H. pylori* infection has been suspected to induce various clinical symptoms due to (1) changes of dietary pattern [32, 33]; (2) amnestic and cognitive disorder [32–34]; depression and anxiety [33]; (3) hormone alterations, such as substance P, somatostatin, vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP) [35], gastrin, and neuropeptides leptin and ghrelin [36, 37]; (4) effects on autonomic nervous system balance and vagus nerve reflection [38]; (5) abnormal gastrointestinal tract movement [39]; (6) visceral nerve hypersensitivity to chemo- and mechanostimulants [40, 41]; (7) abnormal gastric secretion [39, 42]; (8) increase of intestinal permeability [43, 44]; and (9) effects on intestinal microbiota [45, 46] (Fig. 15.2). Moreover, *H. pylori* infection is associated with the upregulation of toll-like receptors and cytokine overproduction, especially TNF-α, IL-1, IL-6, and IL-8 [47–49], thereby indirectly influencing the brain-gut axis. These immune mediators may stimulate mast cells in the gastric mucosa, as well as the hypothalamus and brain stem (via neuroendocrine-immune crosstalk) [50, 51], thereby activating the sympathetic autonomic

nervous system (ANS) and pituitary-suprarenal axis, resulting in increased cortisol and adrenalin secretion [44, 50–52]. These associations could be quite exaggerated, but it is also true that the normalization of some of the symptoms has been observed after *H. pylori* eradication [20, 23–25, 38, 53–58], so chronic *H .pylori* infection can explain the symptoms of both inside and outside of digestive system. Therefore, *H. pylori* eradication gets more important in terms of gastric cancer prevention and treating symptoms of the inside and the outside of the digestive system. However, clear explanations are still lacking, and more researches about various mechanisms of symptoms due to chronic *H. pylori* infection are needed.

Conclusions

H. pylori is the only bacteria that colonize and survive on the human stomach, although the host produces various immunologic reactions. Acute *H. pylori* infection can be seen from 60 % of infected patients and induces symptoms of headache, nausea, epigastric pain, and body aches. Then, the infection becomes a chronic state after 2 months of *H. pylori* infection. As *H. pylori* keeps getting used to its host environment, this bacteria constantly affect on the host to induce symptoms. In other words, the chronic infection causes not only atrophic gastritis and intestinal metaplasia but also visceral hypersensitivity, abnormal gastric secretions, and abnormal gastrointestinal movement. Other than that, *H. pylori* could influence on symptoms that are related to the brain-gut axis through various immunologic mechanisms, but their pathogeneses are still unclear.

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Part V

Disease

Synopsis of *H. pylori*-Associated Diseases

16

Nayoung Kim

Abstract

Helicobacter pylori (*H. pylori*) has urease and *ureI* gene to overcome gastric acid to survive on the acidic gastric mucosa. Colonization with *H. pylori* is a condition that affects the relative risk of developing various clinical disorders of the upper gastrointestinal tract or extragastroduodenal disorders. That is, long-term inflammation due to *H. pylori* infection causes progressive damage to the gastric mucosa and plays a causative role in a number of important diseases, including duodenal ulcer, gastric ulcer, gastric cancer, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma in addition to atrophic gastritis and intestinal metaplasia. Two decades of intense research into *H. pylori* virulence factors such as the VacA and CagA proteins have revealed many aspects of the relationships between this bacterium, the gastric mucosal surface, and the induction of disease. Disease outcome is the result of the intricate, ongoing interplay between environmental, bacterial, and host factors. In the continuous interactions with the host, the bacteria are able to adapt by mutations and DNA rearrangements, rendering novel genotypes, and overcome the host immune mechanism. On the host side, variations in the host immune response to the chronic presence of *H. pylori* and genetic polymorphism of host directly impact *H. pylori*-associated gastric disease, resulting in the disease outcome. However, *H. pylori* colonization pattern such as antrum-predominant gastritis and corpus-predominant pangastritis is known to determine the disease outcome, and this gastritis pattern is mainly decided by the acid secretion when *H. pylori* starts to colonize in the gastric mucosa.

Keywords

Synopsis • Disease • Outcome • *Helicobacter pylori*

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16.1 Introduction

Colonization with *Helicobacter pylori* (*H. pylori*) is not a disease in itself but a condition that affects the relative risk of developing various clinical disorders of the upper gastrointestinal tract or extragastroduodenal disorders [1]. *H. pylori* causes progressive damage to the gastric mucosa and is now accepted as playing a causative role in a number of important diseases, including duodenal ulcer, gastric ulcer, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [2–4]. Indeed, *H. pylori*-induced gastritis is considered as the most important risk factor for peptic ulcer and its complications as well as for gastric cancer [4]. Testing for *H. pylori* therefore has no relevance by itself but should be performed to find the cause of an underlying condition, such as peptic ulcer disease, or for the purpose of disease prevention, such as in gastric cancer relatives [5, 6]. In these cases, a positive test result justifies treatment, and a negative test result may indicate the need to search for other etiologic factors or preventive measures [1]. For these reasons, a correct understanding of the clinical course of *H. pylori*-associated disorders and the effect of *H. pylori* eradication is needed. Two decades of intense research into *H. pylori* virulence factors such as the VacA and CagA proteins have revealed many aspects of the relationships between this bacterium, the gastric mucosal surface, and the induction of disease [1]. The reason for the increment of gastric cancer possibility can be explained by intestinal metaplasia (IM), dysplasia, and gastric cancer cascade due to atrophic gastritis (AG) from *H. pylori* infection. Disease outcome is the result of the intricate, ongoing interplay between environmental, bacterial, and host factors [1]. Strain-to-strain genetic variability in bacterial virulence factors such as *vacA* and *cagA* not only affects the ability of the organism to colonize and cause disease but also affects inflammation and gastric acid output [1]. The decrease gastric output due to *H. pylori*-associated gastritis can explain why the prevalence of reflux gastritis decreases in the *H. pylori*-infected host. Long-term interaction between *H. pylori* and host causes fundamental change to both of *H. pylori* and host. That is, in the continuous interactions with the host, the bacteria are able to adapt to the host condition by mutations and DNA rearrangements. On the host side, variations in the host immune response to the chronic presence of *H. pylori* directly impact *H. pylori*-associated gastric disease and affect gastric acid output and thereby the density and location of *H. pylori* cells [1]. In this chapter synopsis of *H. pylori*-associated diseases such as gastritis, peptic ulcer, non-ulcer dyspepsia, MALT lymphoma, gastric cancer, and extragastroduodenal disorders will be briefly described.

16.2 *H. pylori*-Associated Diseases

Although gastric colonization with *H. pylori* inevitably induces histologic gastritis in the infected individuals, 10–20 % of *H. pylori*-positive patients have risk of developing ulcer disease and a 1–2 % risk of developing noncardiac gastric cancer [7–9]. The risk of development of these disorders in the presence of *H. pylori* infection depends on a variety of bacterial, host, and environmental factors that mostly relate to the pattern and severity of gastritis [1] (Fig. 16.1).

16.2.1 Acute and Chronic Gastritis

Chronic active gastritis, which occurs after colonization with *H. pylori*, can be observed in all *H. pylori*-positive subjects. The intragastric distribution and severity of this chronic inflammatory process depend on a variety of factors, such as characteristics of the colonizing strain, host genetics and immune response, diet, and the level of acid production [1]. *H. pylori*-induced ulcer disease, gastric cancer, and MALT lymphoma are all complications of this chronic inflammation; ulcer disease and gastric cancer in particular occur in those individuals and at those sites with the most severe inflammation [1]. Colonization with *H. pylori* virtually always leads to infiltration of the gastric mucosa in both the antrum and

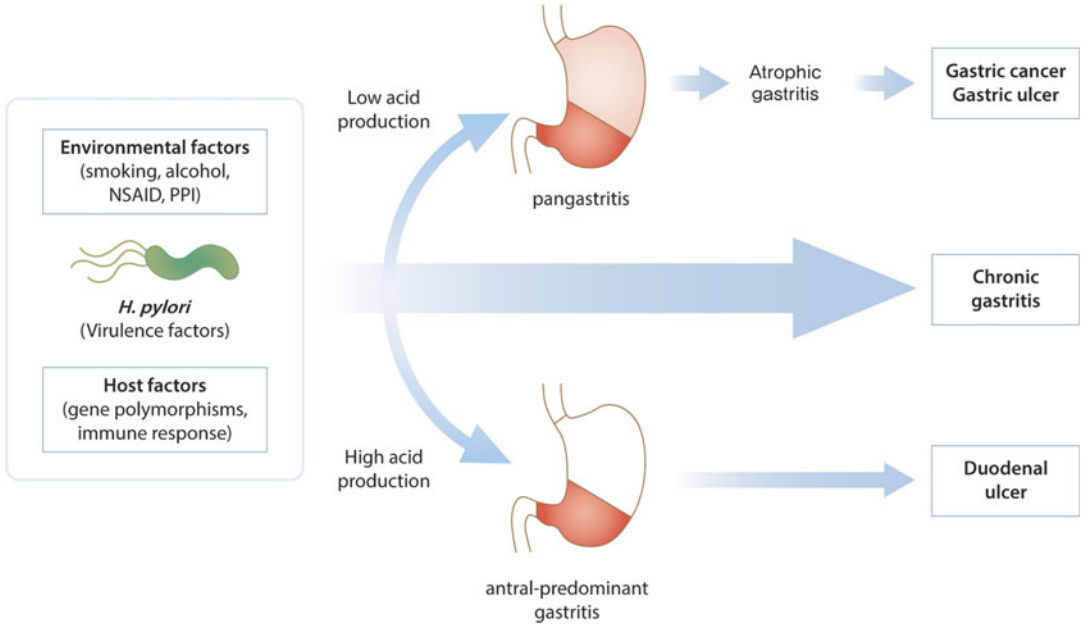

Fig. 16.1 Schematic representation of the factors contributing to gastric pathology and disease outcome in *H. pylori* infection. *NSAID* nonsteroidal anti-inflammatory drug, *PPI* proton pump inhibitor (Modified from Kusters et al. [1])

corpus with neutrophils and mononuclear cells, which consist of chronic inflammatory process.

16.2.1.1 Acute Gastritis

When *H. pylori* enters the stomach of a human host and colonizes itself on gastric mucosa, heavy neutrophils infiltrate into the mucosa. Neutrophil infiltration and the severity of gastric mucosa damage are closely related, and it seems like gastric epithelial cell damage is caused by infiltrated leukocytes due to *H. pylori* infection [10]. Once the bacteria enter the stomach and induce infection, subjects developed variable gastrointestinal and constitutional symptoms. Dyspeptic symptoms were mild to moderate in severity and typically appeared during the first week after challenge and peaked between days 9 and 12 [11]. These symptoms start within the first week of infection, and these symptoms reach their maximum degrees on 9–12 days after the infection [11]. Then, the symptoms are attenuated by themselves, and most of the symptoms are gone after 2 weeks of the infection [11–14]. It is unclear whether this initial colonization can be followed by spontaneous clearance and resolution of gastritis and, if so, how often this occurs [1]. Follow-up studies of young children with serology or breath tests suggested that infection may spontaneously disappear in some patients in this age group [15–17]. However, it does not occur in adults except disappearance of *H. pylori* in case of AG and IM [18]. In addition, studies of homozygotic twins showed a concordance in their *H. pylori* status irrespective of whether they had grown up together or apart, but this concordance was not observed among heterozygotic twins [19]. These results suggest that some individuals are prone to *H. pylori* colonization, while others may be able to prevent colonization or clear an established infection [1].

16.2.1.2 Chronic Gastritis

When colonization does become persistent, a close correlation exists between the level of acid secretion and the distribution of gastritis, which determines the gastric hormonal responses with dynamic changes of gastric mucosa. Finally, they determine the outcomes of *H. pylori* infection. In subjects with intact acid secretion, *H. pylori* in particular colonizes the gastric antrum, where

few acid secretory parietal cells are present because they like weak acid environment such as pH 5.0 instead of strong acid. This colonization pattern is associated with an antrum-predominant gastritis [1] (Fig. 16.1). Histological evaluation of gastric corpus specimens in these cases reveals limited chronic inactive inflammation and low numbers of superficially colonizing *H. pylori* bacteria [1]. In this state duodenal ulcer (DU) can easily develop [1] (Fig. 16.1). Subjects in whom acid secretion is impaired have a more even distribution of bacteria in the antrum and corpus, and bacteria in the corpus are in closer contact with the mucosa, leading to a corpus-predominant pangastritis [1, 20] (Fig. 16.1). In this state the acid secretion will decrease and could be a condition for gastric ulcer or gastric cancer development when the bacteria continuously interact with the host. As predisposition condition of DU and gastric cancer is contradictory, the chance of development of gastric cancer is known to be half in case of DU history. Similarly, there was a report that gastric cancers occurred only in patients with previous benign gastric ulcer (BGU) and not in patients with former DU [21]. When this chronic inflammation continues, AG defined as the loss of glands [22] occurs and slowly progresses to IM, which is defined as the replacement of the surface, foveolar, and glandular epithelium in the gastric mucosa by intestinal epithelium with the presence of Paneth cells, goblet cells, and absorptive cells [23]. *H. pylori* infection was the most important risk factor of AG and IM, and AG is considered to be an antecedent to IM [24]. Actually the time difference of AG and IM could be different, but Korean report suggested 10 years [24]. According to the Correa model, the pathogenesis of intestinal-type gastric cancer can be explained by a multistep process from chronic gastritis through AG, intestinal metaplasia IM, and dysplasia to cancer [22]. The risk of gastric cancer increases with greater extent and higher degree of gastric mucosal atrophy [23] and more than tenfold by IM [25]. Several studies suggest that AG and IM are not related with gastroduodenal symptoms [26], but they are major precursor lesions of gastric cancer [25, 27, 28].

16.2.2 Non-ulcer Dyspepsia

Non-ulcer or functional dyspepsia (FD) is defined as the presence of symptoms of upper gastrointestinal distress without any identifiable structural abnormality during diagnostic workup, in particular including upper gastrointestinal endoscopy [29]. Uninvestigated dyspepsia is defined as the presence of dyspeptic symptoms for which no further diagnostic evaluation has been performed. These symptoms are frequently experienced by 20–30 % of the adult population of the Western world [29]. FD was divided into four symptoms based on the Rome III criteria as follows: epigastric pain, epigastric burning, bothersome postprandial fullness, and early satiation (prevents finishing regular-sized meals). Rome III criteria classified FD into two subcategories of FD based on cohort and population-based studies: postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS) [29]. A recent meta-analysis reported that the odds ratio (OR) for the occurrence of post-infectious FD was 2.54 (95 % confidence interval [CI], 1.76–3.65) more than 6 months after acute gastroenteritis compared with the control [30]. Another meta-analysis found an OR of 2.18 (95 % CI, 1.70–2.81) for FD risk following acute gastroenteritis [31]. These results suggest that gastrointestinal infection is associated with an increased risk of FD, supporting an inflammatory and immunological mechanism in the pathogenesis of FD [32]. *H. pylori* infection is the main cause of gastroduodenal inflammation [33] and provokes activation of a complex cytokine and chemokine response in the gastric mucosa [34], which may induce dyspepsia [35]. However, 30–60 % of patients with functional dyspepsia carry *H. pylori*, but this prevalence is not much different from that in the unaffected population [28, 36]. This result could be explained by that FD is a multifactorial disease rather than *H. pylori*-dependent disease. Thus, the effect of eradication on FD could be the only method whether *H. pylori* infection is related with FD or not. A Cochrane meta-analysis was performed on 17 randomized controlled trials and identified an association between *H. pylori* eradication and improvement in FD

symptoms [37]. A small but significant benefit of *H. pylori* eradication therapy was observed with a number needed to treat of 14 (95 % CI, 10–25) [37]. In another recent study, the number needed to treat was 8 [38]. In addition, a systemic review and meta-analysis from China reported that the summary OR for improvement in FD patients after *H. pylori* eradication was 3.61 (95 % CI, 2.62–4.98) [39]. Similarly, a recent meta-analysis of randomized controlled trials with 12 months follow-up revealed that dyspeptic symptoms were significantly improved in the eradication group (OR 1.38; 95 % CI, 1.18–1.62) [40]. Based on these considerations, the Kyoto global consensus proposed that patients who remain symptomatic after successful *H. pylori* eradication should be regarded as having *H. pylori*-associated dyspepsia separated from FD [41]. In addition, *H. pylori* eradication has been recommended as first-line treatment for dyspeptic patients with *H. pylori* infection, because eradication therapy for dyspeptic symptoms is better than placebo in *H. pylori*-infected dyspeptic patients [41]. However, as symptom resolution takes months after completion of eradication therapy, this time delay makes some scholars to not agree with this proposal.

16.2.3 Gastric or Duodenal Ulcers

Gastric or duodenal ulcers are defined as mucosal defects with a diameter of at least 0.5 cm penetrating through the muscularis mucosa [1], but the guideline could be different to 0.3 cm. BGU mostly occurs along the lesser curvature of the stomach, in particular, at the transition from the corpus to antrum mucosa [42]. DU usually occurs in the duodenal bulb, which is the area most exposed to gastric acid. In Western countries, DU is approximately fourfold more common than BGU; elsewhere, BGU is more common, especially, when aged population increases [1]. For instance, the ratio of BGU ($n=265$) to DU ($n=210$) was 1.26:1 in Korea [43]. In terms of age, DU frequently occurs between 20 and 50 years of age, while BGU predominantly arises in subjects over 40 years old. Recently *H. pylori* prevalence rapidly decreases, the mean age of DU also increases, but still there is age difference between DU and BGU [43]. Both gastric and duodenal ulcer diseases are strongly related to *H. pylori*. In the first decade after the discovery of *H. pylori*, approximately 95 % of DU and 85 % of BGU occurred in the presence of *H. pylori* infection [9]. Several cohort studies estimated that the lifetime risk for ulcer disease in *H. pylori*-positive subjects is three to ten times higher than in *H. pylori*-negative subjects [44]. Furthermore, *H. pylori* eradication provided strong evidence for a causal relationship between *H. pylori* and ulcer disease by showing that eradication of this bacterium strongly reduced the risk of recurrent ulcer disease [45–50]. Ulcer development in the presence of *H. pylori* is influenced by a variety of host and bacterial factors [51]. If acid output is decreased, the gastric transitional zone between the corpus and antrum is the place where gastric ulcer can occur [1]. If acid production is normal to high, the most severe inflammation usually is found in the distal stomach and proximal duodenum, giving rise to juxtapyloric and duodenal ulcer disease [1]. Recently the incidence and prevalence of peptic ulcer have been decreasing [52]. Hygiene improvement and low prevalence of *H. pylori* infection are suspected as main reasons, but the increase of aging population and the use of nonsteroidal anti-inflammatory drug (NSAID) become the major cause of peptic ulcer diseases instead of *H. pylori* infection. In addition, idiopathic peptic ulcer rapidly increases [53].

16.2.4 Gastric MALT Lymphoma

The gastric mucosa does not normally contain lymphoid tissue, but MALT nearly always appears in response to colonization with *H. pylori*. In rare cases, a monoclonal population of B cells may arise from this tissue and slowly proliferate to form a MALT lymphoma [1]. The histological criteria for the diagnosis of gastric MALT lymphoma and the differentiation from polyclonal reactive infiltrates remain

controversial. In particular diagnosis is based on histological appearance during routine microscopy and on demonstration of clonality by immunohistochemistry or molecular techniques, such as PCR. Nearly all MALT lymphoma patients are *H. pylori* positive [54], and *H. pylori*-positive subjects have a significantly increased risk for the development of gastric MALT lymphoma [55]. Because of the diagnostic controversies and the relative rarity of this disorder, the exact incidence in *H. pylori*-positive subjects is unknown, but MALT lymphomas occur in less than 1 % of *H. pylori*-positive subjects [56]. *H. pylori* eradication can lead to complete remission in approximately 60–80 % of patients with stage IE MALT lymphoma confined to the stomach, but some 10 % continue to have signs of minimal residual disease, and the remainder shows no response or disease progression [57–61]. Ten to 35 % of those who initially reach complete remission after *H. pylori* eradication show recurrent disease during further follow-up. A major predictor for response appears to be the presence of a t(11;18)(q21;q21) translocation. This translocation is associated with *API2-MALT1* fusion, the former being involved in regulation of apoptosis, the latter resembling a caspase-like protein, but with as-yet-unknown biological function. Together, the fusion leads to suppression of apoptosis. Several studies have reported that MALT lymphomas with this translocation do not at all or only rarely respond to *H. pylori* eradication [61–63].

16.2.5 Gastric Cancer

Despite the global decrease, gastric cancer is still a burdensome disease internationally. It is the fifth most common cancer and the third leading cause of cancer mortality worldwide [64]. The prognosis of gastric cancer patients depends on the tumor stage at the time of initial diagnosis that the 5-year survival rates of early gastric cancer exceeding 90 % make a striking contrast to those of advanced gastric cancer reaching below 50 % [65–68]. Therefore, the effort to detect gastric cancer when the tumor is in early stage can reduce the overall socioeconomic burden of gastric cancer. Traditionally, gastric cancer was thought to develop due to dietary carcinogens produced by old ways of food preservation such as smoking and salting. However, in recent decades, *H. pylori* has been marked as the main culprit of gastric cancer development [69]. According to the Lauren's classification system [70], gastric cancer can be classified into two major histological variants: an intestinal type and a diffuse type. It is generally accepted that intestinal-type gastric adenocarcinoma arises through a multistep process from chronic gastritis that progresses through stages of atrophy, IM, and dysplasia and finally intestinal-type cancer [22]. On the other hand, diffuse-type gastric cancer is thought to be primarily genetically determined and to be less associated with environmental factors than the intestinal type and not to progress through severe AG [71]. In a meta-analysis of 19 cohort or case-control studies, a summary odds ratio for gastric cancer was estimated to be 1.92 (95 % CI, 1.32–2.78) in *H. pylori*-infected subjects compared to uninfected subjects regardless of histological type of gastric cancer such as intestinal type or diffuse type [72]. Furthermore, several randomized control studies have shown that *H. pylori* eradication reduces the risk of gastric cancer [73]. Among those, the most recent study reported that *H. pylori* eradication reduced gastric cancer incidence by 25 % [74], confirming the importance of *H. pylori* eradication for the prevention of gastric cancer. In addition to *H. pylori* infection, low socioeconomic status, smoking, and family history of gastric cancer were established as risk factors of gastric cancer [5, 6, 75–78]. Factors such as alcohol, fruits, vegetables, and salty and spicy food intake have been subjects of controversy [79–82]. Regarding ABO blood type's association with gastric cancer, blood group A was associated with increased risk of gastric cancer [83, 84], and B allele was associated with decreased risk of gastric cancer in a Japanese study [85]. These findings suggest that gastric cancer is a multifactorial disease with *H. pylori* being the primary cause, and *H. pylori*'s effect

on carcinogenesis is modulated by microbial, environmental, and host factors [86].

16.2.6 Gastroesophageal Reflux Disease

Many studies suggested that *H. pylori* might protect against the development of gastroesophageal reflux disease (GERD) and as such also be of benefit to their hosts [1]. This slowly emerging concept came from repeated observations of a low prevalence of *H. pylori* among GERD patients, particularly of more virulent strains [87], opposing time and geographical trends for *H. pylori* prevalence compared with the incidence of GERD and its complications, a potentially increased incidence of GERD after *H. pylori* eradication [88], and the recognition that *H. pylori*-induced corpus gastritis reduced acid secretion. The hypothesis was that *H. pylori*-induced inflammation of the gastric corpus had an acid-suppressive effect, thus preventing patients from contracting GERD. However, there was no evidence that *H. pylori* eradication has a considerable impact on either the new development of GERD [89, 90]. These data show that although epidemiologic data suggest that there may be an inverse relation between *H. pylori* and GERD, the risk for new development or worsening of preexistent GERD is not an issue in the decision of whether or not to treat *H. pylori*.

16.2.7 Extraintestinal Manifestations of *H. pylori* Infection

H. pylori has been linked to a variety of extraintestinal disorders. These include coronary heart disease, asthma, dermatological disorders such as rosacea and idiopathic urticaria, autoimmune thyroid disease and thrombocytopenic purpura, iron deficiency anemia, Raynaud's phenomenon, scleroderma, migraine, and Guillain-Barré syndrome [1]. The underlying hypothetical mechanisms include chronic low-grade activation of the coagulation cascade, accelerating atherosclerosis, and antigenic mimicry between *H. pylori* and host epitopes leading to autoimmune disorders [91]. This has led to large numbers of case studies of patients with these disorders. Several groups in particular have studied patients with idiopathic thrombocytopenic purpura and showed that when these patients are colonized with *H. pylori*, eradication therapy has a significant effect over placebo for improvement of thrombocyte counts [92–94] and iron deficiency anemia [95, 96]. Currently, according to American College of Gastroenterology Guideline and the Maastricht IV/Florence Consensus Report, *H. pylori* eradication is recommended in unexplained iron deficiency anemia and idiopathic thrombocytopenic purpura [4, 97]. In patients with other conditions mentioned above, there is as yet no role for *H. pylori* eradication, and further adequate, randomized trials are needed.

Conclusions

H. pylori causes progressive damage to the gastric mucosa and is now accepted as playing a causative role in a number of important diseases, including gastritis, duodenal ulcer, gastric ulcer, gastric cancer, gastric MALT lymphoma, and extraintestinal disorders. This disease outcome is the result of the intricate, ongoing interplay between environmental, bacterial, and host factors. Long-term interaction between *H. pylori* and host causes fundamental change to both *H. pylori* and host. That is, in the continuous interactions with the host, the bacteria are able to adapt to the host condition by mutations and DNA rearrangements. On the host side, variations in the host immune response to the chronic presence of *H. pylori* directly impact *H. pylori*-associated gastric disease and affect gastric acid output and thereby the density and location of *H. pylori* cells. It is well known that *H. pylori* colonization pattern such as antrum-predominant gastritis and corpus-predominant pangastritis is very important for the disease outcome, and gastritis pattern is mainly decided by the acid secretion when *H. pylori* starts to colonize in the gastric mucosa because *H. pylori* likes the weak acid condition instead of strong acid.

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Atrophic Gastritis and Intestinal Metaplasia

17

Nayoung Kim and Yo Han Park

Abstract

Helicobacter pylori (*H. pylori*) infection causes atrophic gastritis (AG) and intestinal metaplasia (IM), which are known as the main precursor lesions of gastric cancer. The prevalence of AG and IM varies depending on countries, even it represents diverse results in the same nation. Usually AG is antecedent of IM, but the risk factors of AG and IM are not always the same. Furthermore, the management strategy of AG and IM has not been established, yet. However, eradication of *H. pylori* is very important to prevent the progression of AG and IM to gastric cancer. Therefore, early diagnosis of AG and IM is very important, especially in high incidence area of gastric cancer. However, as the endoscopic, histological, and serological diagnostic tool had a limitation, a multifactorial assessment is needed to ameliorate the diagnostic accuracy of AG and IM.

Keywords

Atrophic gastritis • Intestinal metaplasia • *Helicobacter pylori* • Premalignant lesion

17.1 Introduction

Helicobacter pylori (*H. pylori*) causes progressive gastric damage that is initially most prominent in the antrum and advances into the corpus [1, 2]. Gastric cancer risk is associated with the extent and severity of atrophic injury, which is recognized by loss of normal glandular elements (atrophy), and the development of metaplastic epithelia (pseudopyloric or spasmolytic polypeptide-expressing type and intestinal type) [1, 3, 4]. The risk of developing gastric cancer increases with the rate of development of these atrophic changers [1]. In Western countries

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such as the United States, the incidence of gastric cancer was noted to decline rapidly such that it fell from being the most common cancer in the first quarter of the twentieth century to an uncommon disease by the beginning of the twentieth century [5]. The different *H. pylori*-related diseases are associated with different patterns of gastritis (i.e., atrophic pangastritis or corpus-predominant gastritis in gastric ulcer and gastric cancer and antral-predominant with duodenal ulcer) suggesting that the rapid changes in disease manifestation were accompanied by similar changes in the rate of development of atrophic gastritis (AG) [1, 3–7]. When the normal gastric mucosa is replaced by mucosa which resembles that of the intestine, this condition is called as intestinal metaplasia (IM). As the gastric cancer risk increases very rapidly, AG and IM are considered to be premalignant lesions of gastric cancer. For this reason, effective diagnosis and management of AG and IM is a very important research topic to prevent gastric cancer [8, 9]. According to a recent meta-analysis, the incidence rates of AG are in a wide range from 0% to 10.9% per year [10]. This wide range of incidence of AG could be explained by the different settings in which the diagnoses of AG were made [10]. *H. pylori* infection is the most important risk factor of AG and IM. As AG is considered to be an antecedent to IM [11]; thus, the risk factors for AG are expected to be similar to those for IM. However, bacterial factors of *H. pylori* have been found to play an important role for AG, while environmental and host factors are more important for IM [11]. From this background, the aim of this chapter is to provide comprehensive information regarding the epidemiology, etiology, diagnosis, and management of AG and IM, especially in regard to *H. pylori*, which will lay a foundation to establish strategies to prevent gastric cancer.

17.2 Atrophic Gastritis and Intestinal Metaplasia as Precursor Lesions of Gastric Cancer

Chronic inflammation can damage inflamed cells and trigger a multistep process of carcinogenesis. In premalignant tissues associated with chronic inflammation, tumor cells and leukocytes of various kinds such as neutrophils, macrophages, monocytes, mast cells, eosinophils, dendritic cells, and lymphocytes are present [12, 13]. These inflammatory cells contribute to cancer initiation, promotion, and metastasis by producing cytokines, reactive oxygen species, and reactive nitrogen species. Various oxidant products can damage cellular DNA, RNA, and proteins by chemical reactions such as oxidation, nitration, nitrosation, and halogenation. Damages of cellular components result in increased mutations and altered functions of important enzymes and proteins in premalignant tissues, so contributing to the multistage carcinogenesis process [14]. According to the Correa model, chronic inflammation of gastric mucosa triggers a pathway of chronic active gastritis, multifocal atrophy, IM, gastric dysplasia, and finally invasive gastric adenocarcinoma [15] (Fig. 17.1). The pathogenesis of intestinal-type gastric cancer can be explained by a multistep process from chronic gastritis through AG, IM, and dysplasia to cancer. The presence of AG, which has been traditionally defined as the loss of glands [16], is well known as a risk factor of gastric cancer. The risk of gastric cancer increases with greater extent and higher degree of gastric mucosal atrophy [17]. Gastric IM is defined as the replacement of the surface, foveolar, and glandular epithelium in the gastric mucosa by intestinal epithelium with the presence of Paneth cells, goblet cells, and absorptive cells [17]. Several studies suggest that AG and IM are major precursor lesions of gastric cancer [3, 18, 19]. These relationships were observed before the identification of *H. pylori* infection in 1982 [20]. Gastric cancer is divided into two major histological types, intestinal type and diffuse type, by Lauren classification [21]. Intestinal-type cancers are believed to arise secondary to AG and IM [22]. On the contrary, diffuse-type gastric cancer usually arises independently of IM. This raised doubts about the association between IM and gastric cancer development [23]. It is thought that diffuse type is more likely to have a primary genetic etiology, and the involvement of *H. pylori* is probably limited to a subset of sporadic cases [22, 23].

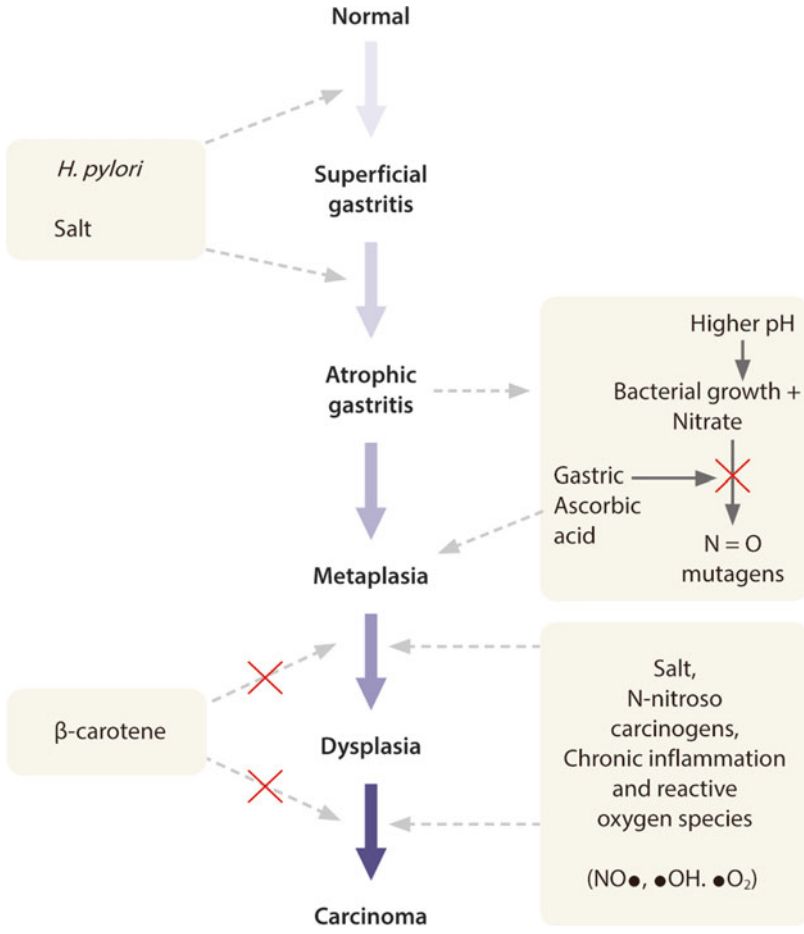


Fig. 17.1 Correa's theory regarding human gastric carcinogenesis: a multistep and multifactorial process. Gastritis begins from superficial gastritis and progresses into atrophic gastritis, metaplasia, dysplasia, and intestinal-type gastric cancer (Modified from Correa [15])

However, many studies suggest that both intestinal and diffuse types of gastric carcinoma are associated with *H. pylori* infection [24, 25]. Compared with other risk factors of gastric cancer, AG and IM increase the risk of intestinal-type gastric cancer exponentially. That is, the risk of gastric cancer in subjects with severe fundal AG was 5.76 times higher than that in those having little or no fundal AG [26]. In case of IM, it increased risk of developing gastric cancer more than tenfold [27]. In a prospective study of 1,526 subjects, 1,246 patients had *H. pylori* infection and 280 did not [3]. During mean follow-up of 7.8 years, gastric cancer developed in the individuals (36 patients, 2.9 %) infected with *H. pylori* but not in the uninfected persons [3]. Histological severe gastric atrophy, corpus-predominant gastritis, or IM were increasing risk factors of gastric cancer [3]. In addition, in a cohort of 4,655 healthy asymptomatic subjects, the risk of gastric cancer increased stepwise from chronic atrophic gastritis (CAG)-free gastritis [*H. pylori*(+)/CAG(−) group] (hazard ratio [HR] 7.13; 95 % confidential interval [CI], 0.95–53.33) to CAG [*H. pylori*(−)/CAG(+) group] (HR 14.85; 95 % CI, 1.96–107.7) and finally to severe CAG with extensive IM [*H. pylori*(−)/CAG(+)group] (HR 61.85; 95 % CI, 5.6–682.64) in which *H. pylori* was lost [18]. Probably *H. pylori* alone is not directly associated with

gastric carcinogenesis. Instead, *H. pylori*-induced chronic inflammation can provide the seed of cascade leading to gastric cancer, which can progress continuously even in the absence of *H. pylori* [28]. The patients with *H. pylori* infection and IM had more than 6.4-fold increased risk of gastric cancer than the subjects of infected with *H. pylori* infection but without IM [3]. In a cohort study of 2,224 subjects conducted in South Korea, the group with IM had 10.9-fold increased risk of gastric cancer [19]. *H. pylori* infection triggers a multistep progression from chronic gastritis, gastric atrophy, IM, and finally into gastric cancer [29]. *H. pylori*, a gram-negative, flagellated bacterium about 3 μm long with a diameter of about 0.5 μm [30], became the first bacterium to be classified as a type I carcinogen by IARC working group in 1994 [31]. In a meta-analysis of 19 cohort or case-control studies, the summary odds ratio for gastric cancer in *H. pylori*-infected subjects was 1.92 (95 % CI, 1.32–2.78) [32]. In another meta-analysis of 42 cohort or case-control studies, the summary odds ratio for *H. pylori* infection in relation to gastric carcinoma was 2.04 (95 % CI, 1.69–2.45) [33]. So, these studies show the clear association between *H. pylori* and gastric adenocarcinomas [32, 33]. However, among *H. pylori*-positive patients, only 1–2 % subjects develop gastric cancer [17], suggesting that the final effects of *H. pylori* infection could be determined by prevalence, environmental factors, bacterial factors, and host factors [34]. Despite the clear relationship between *H. pylori* infection and gastric adenocarcinomas through AG and IM, especially in the intestinal type of gastric cancer, the mechanisms of process about chronic inflammation and developing gastric cancer are under investigation. The enhanced production of free radicals by chronic *H. pylori* infection causes mutations in target cells so the neoplastic clones are established [35]. In addition, tumor necrosis factor (TNF)-α plays major roles in the growth, invasion, and metastasis of neoplasm, and this mechanism is called as a "perigenetic pathway" [35]. Furthermore, TNF-α-inducing protein (Tipα) from *H. pylori* binds to and enters the nucleus through a specific binding molecule, which might act as a carcinogen of gastric cancer [36].

17.3 Prevalence of Atrophic Gastritis and Intestinal Metaplasia

A meta-analysis of 14 studies on incidence of AG was reported in 2010 [10], which showed the incidence rates of AG ranged from 0 % to 10.9 % per year [10]. This wide range of incidence could be explained by the particular settings in which the diagnosis of AG was made [10]. The lowest incidence rates (0 %) were found in patients with reflux esophagitis and in patients successfully treated for *H. pylori* infection [37, 38]. The highest incidence rate (10.9 %) was observed in a study conducted in patients who underwent vagotomy for ulcer disease [39]. Regarding the *H. pylori* infection, the AG incidence rate was higher in the *H. pylori*-positive patients than in the *H. pylori*-negative ones [10]. In a meta-analysis, rate ratios comparing the incidence of AG in *H. pylori*-positive patients to that in *H. pylori*-negative ones ranged from 2.4 to 7.6 with a summary estimate of 5.0 (95 % CI, 3.1–8.3) [10]. In contrast, reports on the incidence of IM are rare in asymptomatic general population, because upper gastrointestinal endoscopy with histological examination is needed to estimate the prevalence of IM. In a study about the relationship between IM and *H. pylori* infection in the Netherlands, IM was found more often in *H. pylori*-positive patients than in *H. pylori*-negative ones (33.9 % vs. 15.2 %, $p<0.001$) [40]. The mean age of IM-positive patients was 64 years and that of IM-negative ones was 72 years ($p<0.005$) [40]. In a Japanese prospective study with a follow-up of 7.8 years, IM was detected in as many as 37 % of 1,426 *H. pylori*-positive patients (mean age: 52.3 years) while in only 2 % of 280 uninfected patients (mean age: 52.7 years) [3]. There were several studies regarding the prevalence of AG and IM in South Korea, where the incidence of gastric cancer is very high. In a cohort study consisted of 389 subjects (≥16 years), the prevalence of AG in the antrum and corpus was 42.5 % and 20.1 %, and the prevalence of IM was 28.6 % and 21.2 %, respectively [11]. In another study reporting age-adjusted prevalence in South Korea, the prevalence of AG was 42.7 % for men and 38.1 % for women ($p=0.194$) and that of IM was 42.5 % for men and 32.7 % for women

Table 17.1 The prevalence of atrophic gastritis and intestinal metaplasia in the world

Author, year	Country	Diagnostic methods	Study population (n)	AG (%) antrum/body	IM (%) antrum/body
Kim et al. (2008) [11]	Korea	Histology	389	42.5/20.1	28.6/21.2
Kim et al. (2008) [41]	Korea	Histology	713	42.7/38.1	42.5/32.7
Park et al. (2012) [42]	Korea	Endoscopy	25,536	27.1[b]	7.1[b]
Joo et al. (2013) [43]	Korea	Endoscopy	4,023	40.7[b]	12.5[b]
Weck et al. (2007) [44]	Germany	Serology[a]	9,444	6.0[b]	
Borch et al. (2000) [45]	Sweden	Histology	501	9.4[b]	
Asaka et al. (2008) [46]	Japan	Histology	2,455	55.5[b]	24.2[b]
Kamada et al. (2014) [4]	Japan	Histology	216 (1970s)	98/82[b]	63.9/32.4[b]
			417 (1990s)	80/67[b]	37.4/21.3[b]
			107 (2010s)	33/19[b]	15.0/4.7[b]
Zou et al. (2011) [47]	China	Histology	1,022	63.8[b]	
Eriksson et al. (2008) [48]	Finland	Histology	505		18.8/7.1
Almouradi et al. (2013) [49]	USA	Histology	437		15.0[b]

Modified from Park and Kim [50]
AG atrophic gastritis, *IM* intestinal metaplasia, *USA* United States of America
[a]Serology by *H. pylori* IgG antibodies
[b]Prevalence of atrophic gastritis or intestinal metaplasia in the antrum and/or body

($p=0.005$) [41]. The prevalence of AG and IM increased significantly with age for both men and women [41]. When a multicenter study of South Korea in 2006, with 25,536 asymptomatic subjects, evaluated the prevalence of endoscopic AG and IM, they were 27.1 % and 7.1 %, respectively, which were lower than those estimated with histological diagnoses [42]. The proportions of endoscopic AG and IM in the patients aged 40 years or less were 14.9 % and 2.7 %, respectively, and in those aged 60 years or more were 43.5 % and 12.3 %, respectively [42]. This study showed that the prevalence of AG and IM increased significantly with age and more frequent in male than in female [42]. Interestingly, the prevalence of endoscopic AG and IM were 40.7 % and 12.5 % in a multicenter study of 4,023 subjects in 2011, significantly higher than those of a multicenter study in 2006 [43]. This increase in prevalence of AG and IM may be attributed to aggressive diagnosis made by physicians in 2011 relative to 2006 instead of real increase of prevalence rate of AG and IM [43]. In case of Japan the prevalence of histological atrophy in the antrum and in the corpus was significantly lower in the 2010s (33 % and 19 %, respectively) compared to those evaluated in either the 1970s (98 % and 82 %, respectively) ($p<0.001$) or 1990s (80 % and 67 %, respectively) ($p<0.001$) [44]. The severity of atrophy and IM also declined remarkably among those with *H. pylori* infection [44]. Actually, the prevalence of AG and IM varied (9.4–63.8 % in case of AG; 7.1–42.5 % in case of IM) depending on diagnostic methods and countries [4, 11, 41–50] (Table 17.1).

17.4 Risk Factors of Atrophic Gastritis and Intestinal Metaplasia

Several studies have suggested that dietary causes such as excessive salt intake, deficient ascorbic acid, and insufficient carotene could be risk factors of AG, IM, and gastric cancer [15, 51]. However, among the many risk factors of AG and IM, *H. pylori* infection was found as the most important [3, 18, 19, 31]. Especially, bacterial virulence genes such as *cagA* and *vacA* were found to be the important risk factors of AG, IM, and gastric cancer [52–56]. In a study of 58 subjects of *H. pylori* infection, with a mean follow-up period of 11.5 years, *cagA* was associated with a significant risk of AG and IM (odds ratio [OR] 3.48; 95 % CI, 1.02–12.18) [53]. Infection with *cagA*-positive strains further increased the risk for gastric cancer by 1.64 (95 % CI, 1.21–2.24) overall in a meta-analysis [54].

Table 17.2 Risk factors of histological atrophic gastritis by multivariate analysis

Antrum	AG grade 0[a]	AG grade 1–3[a]	Adjusted OR	95 % CI	*p* value
H. pylori (%)					
Negative	56 (83.6)	11 (16.4)			
Positive	55 (43.7)	71 (56.3)	5.69	1.87–17.3	<0.001
Age (years) (%)					
≤47	58 (69.9)	25 (30.1)			
48–60	32 (48.5)	34 (51.5)	2.23	0.87–5.70	0.094
≥61	21 (47.7)	23 (52.3)	3.75	1.31–10.9	0.014
cagA (%)					
Negative	52 (64.2)	29 (35.8)			
Positive	22 (40.7)	32 (59.3)	2.67	1.05–6.84	0.040
Corpus	AG grade 0[a]	AG grade 1–3[a]	Adjusted OR	95 % CI	*p* value
H. pylori (%)					
Negative	84 (91.3)	8 (8.7)			
Positive	115 (73.2)	42 (26.8)	3.63	1.58–8.34	0.003
Age (years) (%)					
≤47	91 (90.1)	19 (9.9)			
48–60	64 (74.4)	22 (25.6)	3.90	1.36–11.2	0.011
≥61	44 (71.0)	18 (29.0)	4.94	1.61–15.2	0.005
vagA m (%)					
Negative	80 (84.2)	15 (15.8)			
m1	37 (62.7)	22 (37.3)	3.48	1.54–7.87	0.003
m2	9 (83.3)	1 (16.7)	1.04	0.10–11.0	0.973

Adapted from Kim et al. [11]
AG atrophic gastritis, *OR* odds ratio, *CI* confidence interval, *H. pylori Helicobacter pylori*
[a]Updated Sydney system scores, 0 = none, 1 = slight, 2 = moderate, 3 = marked

CagA protein secreted by *H. pylori* translocates into cytoplasm of gastric mucosa cells via its type IV secretion system after *H. pylori* attachment. CagA interrupts signaling pathways by phosphorylation-dependent and phosphorylation-independent mechanisms, leading to cytoskeletal change, motility, and abnormal proliferation in normal gastric epithelial cells [34]. *vacA* gene is another well-known virulence factor, s1 and m1 forms of *vacA* are more common in disease-associated *H. pylori* strains, and s1m1 genotype was related to gastric epithelial damage, AG, and IM [52, 55, 56]. From a study of a total of 370 *H. pylori*-infected patients, a high association was observed between the presence of AG or IM and the prevalence of *vacA* s1, m1, and *cagA* strains of *H. pylori* genotypes [56]. That is, the presence of atrophy was associated with *vacA* s1 (OR 46.9; 95 % CI, 8.6–256.4), *vacA* m1 (OR 10.1; 95 % CI, 4.3–23.7), and *cagA* (OR 11.2; 95 % CI, 4.5–27.8) [56]. Similarly, presence of IM was associated with *vacA* s1 (OR 14.9; 95 % CI, 4.2–53.2), *vacA* m1 (OR 6.8; 95 % CI, 2.9–15.9), and *cagA* (OR 4.8; 95 % CI, 2.1–10.6) [56]. Furthermore, other virulence factors such as SabA (outer membrane protein) were significantly associated with AG, IM, and gastric cancer [57]. Many studies showed that the *vacA* and *cagA* genotypes of *H. pylori* are not equally distributed all over the world [58–60]. There are significant differences in *H. pylori* genotypes among populations from Asia, different parts of Europe, and North and South America. Given the geographic distribution of specific *H. pylori* genotypes, it could be speculated that there might be differences in incidence of gastric cancer [58–60].

In a single-center prospective study in 389 subjects without significant gastroduodenal diseases in South Korea, risk factors of histological AG in the antrum and in the corpus were *H. pylori* infection, *cagA*, and *vacA* m1 positivity and age ≥61 years [11] (Table 15.2). The risk factors of histological IM in the antrum and in

the corpus were age ≥61 years, *H. pylori* infection, strong spicy food, a smoking history, and the presence of *IL10-592* C/A as against A/A [11] (Table 15.3). Finally, virulence factor of *H. pylori* was the most important risk factor of AG, while environmental or host factors were more important risk factors for IM, which suggests a kind of difference of risk factors between AG and IM [11]. In another study on the risk factors of endoscopic AG and IM in the 4,023 health

Table 17.3 Risk factors of histological intestinal metaplasia by multivariate analysis

Antrum	IM grade 0[a]	IM grade 1–3[a]	Adjusted OR	95 % CI	*p* value
H. pylori (%)					
Negative	119 (89.5)	14 (10.5)			
Positive	156 (61.9)	96 (38.1)	8.22	3.27–20.7	<0.001
Age (years) (%)					
≤47	133 (88.1)	18 (11.9)			
48–60	102 (78.5)	28 (21.5)	2.28	1.04–4.98	0.039
≥61	69 (65.7)	36 (34.3)	3.02	1.34–6.78	0.008
Smoking (%)					
Never	145 (81.0)	34 (19.0)			
Current	33 (91.7)	3 (8.3)	0.39	0.05–4.58	0.372
Past	46 (67.6)	22 (32.4)	3.49	1.3–8.75	0.008
Spicy food (%)					
Low	51 (79.7)	13 (20.3)			
Moderate	90 (70.3)	38 (29.7)	0.78	0.66–1.34	0.788
Strong	37 (58.7)	26 (41.3)	2.38	1.16–4.88	0.017
IL6 -572 genotype (%)					
C/C	92 (64.8)	50 (14.3)			
G carrier	88 (74.6)	30 (25.4)	0.50	0.26–0.94	0.033
Corpus	IM grade 0[a]	IM grade 1–3[a]	Adjusted OR	95 % CI	*p* value
H. pylori (%)					
Negative	122 (91.7)	11 (8.3)			
Positive	182 (71.9)	71 (28.1)	3.65	1.51–8.87	0.004
Age (years) (%)					
≤47	133 (88.1)	18 (11.9)			
48–60	102 (78.5)	28 (21.5)	2.47	0.96–6.35	0.061
≥61	69 (65.7)	36 (34.3)	9.98	3.78–26.3	0.005
Smoking (%)					
Never	235 (78.8)	51 (21.2)			
Current	39 (92.9)	3 (7.1)	0.18	0.02–1.34	0.093
Past	21 (46.7)	24 (53.3)	7.10	2.48–20.3	<0.001
Occupation (%)					
Professional	103 (81.1)	24 (18.9)			
Non-professional	43 (72.9)	16 (27.1)	3.53	1.17–10.7	0.025
Unemployed	20 (64.5)	11 (35.5)	3.79	1.28–11.2	0.017
IL10-592 genotype (%)					
A/A	91 (82.0)	20 (18.0)			
C/A	83 (68.6)	38 (31.4)	3.69	1.65–8.21	0.001
C/C	24 (75.0)	8 (25.0)	1.32	0.41–4.21	0.639

Adapted from Kim et al. [11]
IM intestinal metaplasia, *OR* odds ratio, *CI* confidence interval, *H. pylori Helicobacter pylori*
[a]Updated Sydney system scores, 0 = none, 1 = slight, 2 = moderate, 3 = marked

Table 17.4 Risk factors of endoscopic atrophic gastritis by multivariate analysis

Variables	Estimate	SE	*p* value	OR	95 % CI
Age (years)					
<40				1.00	
40–59	1.039	0.165	<0.001	2.55	2.05–3.18
≥60	1.980	0.203	<0.001	5.00	3.71–6.74
Male	0.603	0.155	<0.001	1.38	1.17–1.64
H. pylori IgG positivity	0.377	0.112	<0.001	1.41	1.19–1.66
Intestinal metaplasia	1.309	0.147	<0.001	4.29	3.35–5.50
Education below college	−0.131	0.216	0.046	1.35	1.01–1.79

Adapted from Joo et al. [43]
SE standard error, *OR* odds ratio, *H. pylori Helicobacter pylori*, *CI* confidence interval

Table 17.5 Risk factors of endoscopic intestinal metaplasia by multivariate analysis

Variables	Estimate	SE	*p* value	OR	95 % CI
Age (years)					
<40				1.00	
40–59	1.150	0.205	<0.001	3.16	2.11–472
≥60	1.178	0.236	<0.001	3.25	2.05–5.15
Male	0.631	0.154	<0.001	1.88	1.39–2.54
H. pylori IgG positivity	0.775	0.119	<0.001	2.17	1.72–2.74
Atrophic gastritis	1.303	0.113	<0.001	3.68	2.95–4.60
Relatives of gastric cancer	0.395	0.143	0.006	1.48	1.12–1.96
Smoking	0.170	0.138	NS	1.19	0.91–1.55
Alcohol	0.112	0.131	NS	1.20	0.87–1.45
Education below college	-0.384	0.162	0.018	1.47	1.06–2.00
Consumption of dairy product	0.338	0.116	0.004	1.40	1.12–1.76

Adapted from Joo et al. [43]
SE standard error, *OR* odds ratio, *H. pylori Helicobacter pylori*, *NS* not significant, *CI* confidence interval

check-up subjects, the risk factors for AG were male, age groups of 40–59 years and >60 years, positive *H. pylori* serology, IM, and education below the college level (OR; 5.00, 2.55, 1.38, 1.41, 4.29, and 1.35, respectively) [43] (Table 15.4). The risk factors for IM were male, AG, age groups of 40–59 years and >60 years, positive *H. pylori* serology, having relatives with gastric cancer, consumption of dairy products, and education below the college level (OR; 3.25, 2.17, 3.16, 1.88, 3.68, 1.48, 1.40, and 1.47, respectively) [43] (Table 15.5).

17.5 Classification of Atrophic Gastritis and Intestinal Metaplasia

Although several classifications of gastritis have been proposed, there is no universally accepted classification of gastritis. The first classification based on histopathological examination of gastric mucosa collected by so-called blind biopsies and samples collected during surgical procedures was created by Schindler [61]. Schindler described that gastritis was divided into a superficial gastritis

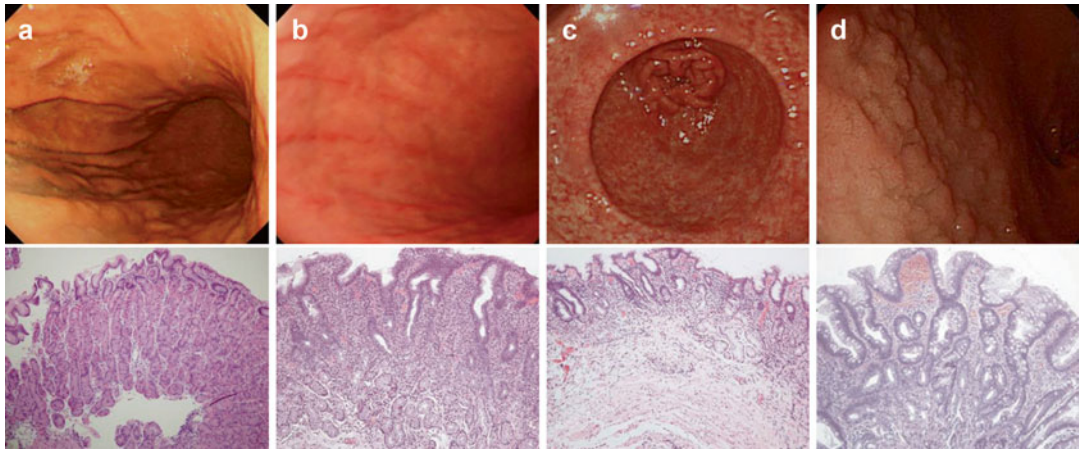


Fig. 17.2 Classification of gastritis (**a**) normal, (**b**) superficial gastritis, (**c**) atrophic gastritis, and (**d**) intestinal metaplasia. Upper panel is endoscopic and lower panel is hitologic finding (Adapted from Park and Kim [50])

(Fig. 17.2b) that may progress to AG (Fig. 17.2c) and IM (Fig. 17.2d) with time, which is different from normal (Fig. 17.2a). Additionally, Schindler proposed that there were different courses and prognoses of disease by the type of the gastritis [61]. And a novel classification and grading of gastritis were devised by a group of experts at the 9th World Congress of Gastroenterology in Sydney, Australia, in 1990 [62]. The histological division of Sydney system is a practical guideline upon which the morphological features of gastritis in endoscopic biopsy samples should be documented. Type, severity, and extent of gastric inflammation composed to possible etiology should be detailed depending on the chart. The Sydney system asserted the routine gastric biopsy sampling protocol (two from the antrum and two from the corpus, both from anterior and posterior walls) and sample fixation in adequately labeled separate containers [62]. Now the updated Sydney system in 1996 is most widely used as the classification of gastritis [63]. The original Sydney classification of gastritis dividing into gastritis acute, chronic, and special forms and grading of chronic inflammation, neutrophil activity, atrophy, IM, and *H. pylori* density into mild, moderate, and marked categories were kept [62]. The updated Sydney system introduced a visual analogue scale for evaluating the severity of histological grading [63] (Fig. 17.3). The histological features of the gastric mucosa were recorded using updated Sydney system scores, that is, 0 = none, 1 = mild, 2 = moderate, and 3 = marked [63]. It changed the routine of endoscopic biopsy sampling by the introduction of biopsy sampling from the incisura angularis and modified the corpus and antrum biopsy locations from the two opposite walls in lesser and greater curvature of both parts [63] (Fig. 17.4). Endoscopic description of acute gastritis by Sydney system is divided into edema, exudates, erosions, and hemorrhage. In contrast, endoscopic diagnosis and classification of chronic gastritis are reliable depending on the interobserver [63]. In addition, recently a global consensus for gastritis was developed based on etiology-based classification of gastritis and duodenitis, for international classification system as well as further research [64].

However, all of these classifications do not reflect prognosis of gastric cancer risk in subjects with CAG. Therefore, the Operative Link on Gastritis Assessment (OLGA) was developed to improve the histological staging system for gastric atrophy [65]. OLGA system uses gastric biopsy sampling protocol defined by Sydney system and the histological grading system recommended by the updated Sydney system [65] (Fig. 17.5). Long-term follow-up studies with follow-up ranges from 144 to 204 months proved that the OLGA staging reflects relevant information on clinicopathological outcome of gastritis, and therefore *H. pylori*-negative patients with

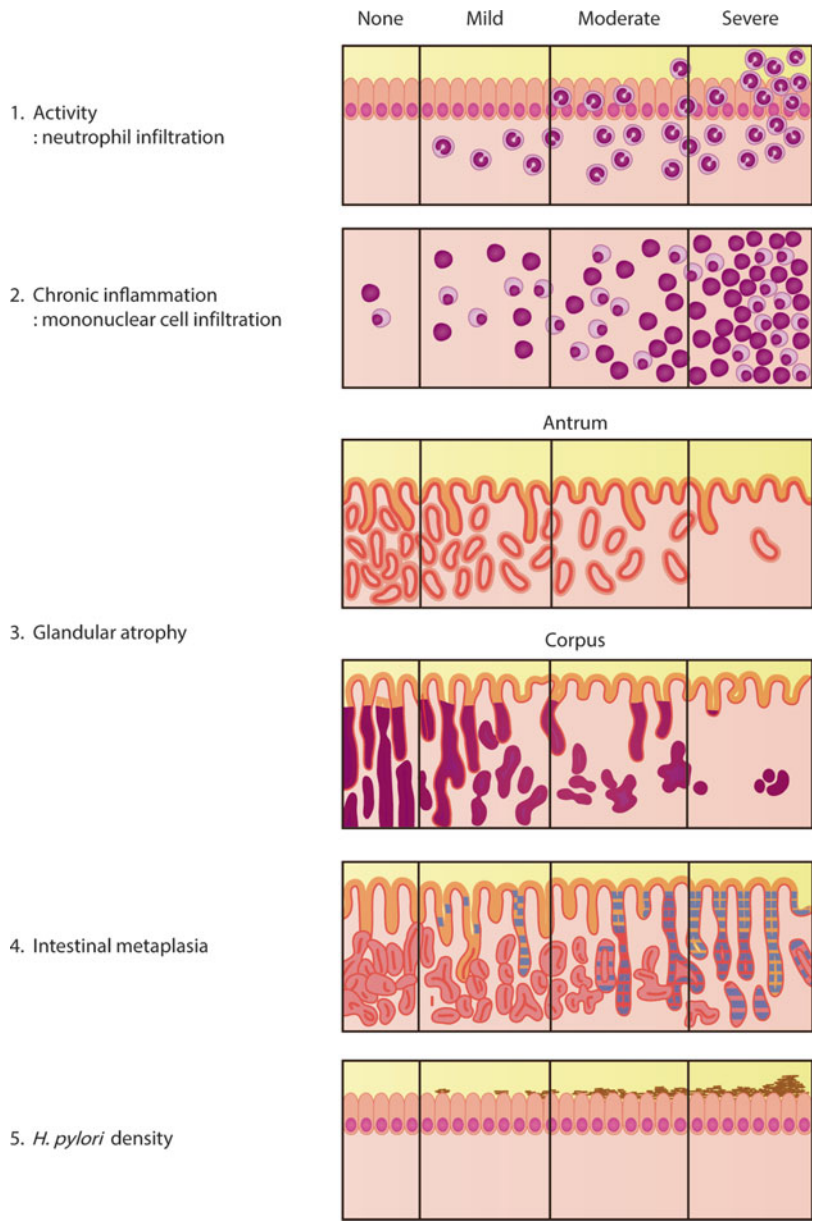


Fig. 17.3 Grading of gastritis by Sydney System: acute inflammation, chronic inflammation, atrophic gastritis, intestinal metaplasia, and *H. pylori* density (Adapted from Dixon et al. [63])

low OLGA stages could be excluded from secondary preventive surveillance of gastric cancer [66], whereas patients with higher OLGA stages (stages III and IV) should be considered definitely candidates for endoscopic examination [66]. However, there are no universally acceptable classification methods for gastritis, yet. For the optimization of the methods to interpret gastritis, repetitive communication between the endoscopists and pathologists should be required.

IM could be classified according to the phenotype of mucin, and it will be touched more in

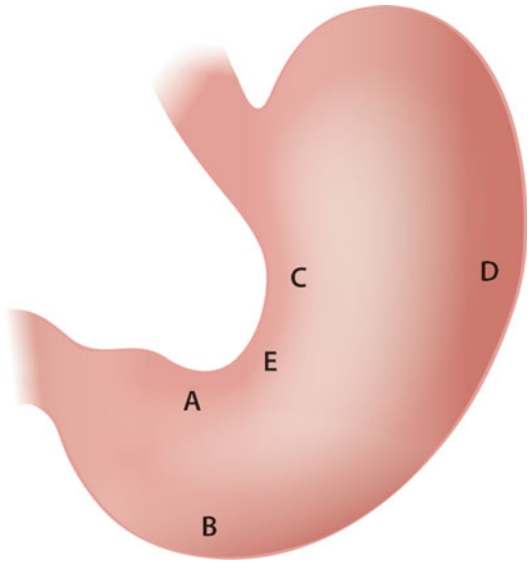


Fig. 17.4 The optimal gastric biopsy sites recommend by updated Sydney system. Biopsy specimens are taken at five different sites: (*A*) lesser curvature of the antrum; (*B*) greater curvature of the antrum; (*C*) lesser curvature of the corpus; (*D*) greater curvature of the corpus; and (*E*) incisura angularis (Adapted from Dixon et al. [63] and Park and Kim [50])

detail in the histological diagnosis part of this chapter. Type I IM expresses only sialomucins, type III expresses sulfomucins, and type II expresses a mixture of gastric and intestinal mucins [67]. Several studies reported that type III or incomplete IM increases the risk of gastric cancer [68, 69]. However, a contrary result has been published [70]. Furthermore, IM subtyping was not found to play a major role in the prediction of gastric cancer development in South Korea [71]. International guideline did not recommend subtyping of IM for clinical practice in 2012 [72]. However, a recent systemic review concluded that the incomplete IM was significantly related to the prevalence of gastric cancer [73]. Moreover, it reported that the relative risks of gastric cancer were from 4- to 11-fold higher for the presence of incomplete type in comparison to complete type or in comparison to the absence of incomplete type [73]. This systemic report concluded that subtyping of IM has the scientific evidence on the evaluation of gastric cancer risk [73].

		CORPUS			
		No atrophy (grade 0)	Mild atrophy (grade 1)	Moderate atrophy (grade 2)	Severe atrophy (grade 3)
ANTRUM	No atrophy (grade 0)	STAGE 0	STAGE I	STAGE II	STAGE II
	Mild atrophy (grade 1)	STAGE I	STAGE I	STAGE II	STAGE III
	Moderate atrophy (grade 2)	STAGE II	STAGE II	STAGE III	STAGE IV
	Severe atrophy (grade 3)	STAGE III	STAGE III	STAGE IV	STAGE IV

Fig. 17.5 Gastritis staging: the Operative Link on Gastritis Assessment (OLGA) system. Atrophy is defined as loss of appropriate glands (with or without metaplasia). In each compartment, atrophy is scored in a four-tiered scale (0–3) according to the visual analogue scale of the updated Sydney system (Adapted from Rugge et al. [65])

17.6 Diagnosis of Atrophic Gastritis and Intestinal Metaplasia

In most of previous studies about the clinical relevance of endoscopic and histological diagnosis of the gastritis, the diagnosis of gastritis should have been based on histological examination of the gastric mucosa [74, 75]. Especially in the young age group, a high index of suspicion of gastric atrophy is important, and confirmation of the diagnosis by histology is necessary [76]. However, in another study, endoscopic and histological diagnosis displayed high correlation [77]. Particularly, the benefits of upper endoscopy to conform atrophic change of gastric mucosa are generally accepted [78, 79]. Endoscopic feature of AG is the visibility of a vascular pattern of gastric mucosa (Fig. 17.2c) than normal gastric mucosa (Fig. 17.2a). Endoscopic finding of gastric IM (Fig. 17.2d) is defined as the replacement of the surface, foveolar, and glandular epithelium in the oxyntic or antral mucosa by intestinal epithelium. Actually, endoscopic finding of IM is observed as a mucosal nodular pattern, usually occurring after the occurrence of the AG. It is not difficult to diagnose severe cases of AG and IM properly by endoscopic findings, but it is difficult to make the diagnoses of mild AG and IM [76]. Sometimes endoscopic diagnosis is not correlated to histological diagnosis. Therefore, it is proper to biopsy in suspected cases of AG and IM. However, as atrophic mucosal changes are not distributed similarly in the whole gastric mucosa, multiple endoscopic biopsy of gastric mucosa does not always represent AG. Furthermore, it is difficult to take multiple biopsies of all subjects with simple gastritis [80, 81]. To resolve this clinical difficulty, noninvasive tests for precursor lesions such as serum level of pepsinogen (PG), gastrin-17, and *H. pylori* immunoglobulin G antibodies could be used as biomarkers of AG and IM to replace endoscopic biopsy [82]. Actually, the endoscopic, histological, and serological AG showed a relatively good correlation in the 2,558 Korean subjects (Fig. 17.6) [83]. However, as these three methods had a limitation, a multifactorial assessment might be needed to ameliorate the diagnostic accuracy of AG [83]. From this background, we will discuss about three diagnostic methods for AG and IM: endoscopic, histological, and PG I/II ratio in this chapter.

17.6.1 Endoscopic Diagnosis

Endoscopic diagnosis of AG and IM is the basic method. However, there is the possibility of low sensitivity, specificity, and interobserver variation. Endoscopic diagnosis of AG is made when the visibility of a vascular pattern followed by loss of gastric mucosal gland is present (Fig. 17.2c). There are various criteria to endoscopic classification of AG. AG due to *H. pylori* infection is more common in the East, and atrophic mucosal change progresses from the antrum to the corpus along with the lesser curvature (Fig. 17.7c); these endoscopic features reflect Kimura-Takemoto classification well [84]. The boundaries of the mucosal atrophy are called the F line (Fig. 17.7b), and AG is classified into six types. The closed-type AG indicates that the atrophic border remains on the lesser curvature, while the open-type AG means that the atrophic border no longer exists on the lesser curvature but extends along the anterior and posterior walls of the stomach (Fig. 17.7a). However, Western physicians lack a good understanding of Kimura-Takemoto classification because the ratio of autoimmune gastritis is greater in the West than in the East and *H. pylori* infection rate is rather low [85]. In addition, the interobserver reliability is rather low in Kimura-Takemoto classification from endoscopic features. Other limitation of the endoscopic diagnosis of AG and IM is the lower sensitivity and specificity than the pathological diagnosis. In the study of 1,333 subjects in South Korea, the sensitivity of the prevalence of endoscopic diagnosis of atrophy was significantly low in the age below 50 years old [76]. The sensitivity and specificity of endoscopy for the diagnosis of gastric atrophy based on histological findings were 61.5 % and 57.7 % in the antrum and were 46.8 % and 76.4 % in the corpus of the stomach [76]. Especially, endoscopic diagnosis of AG was

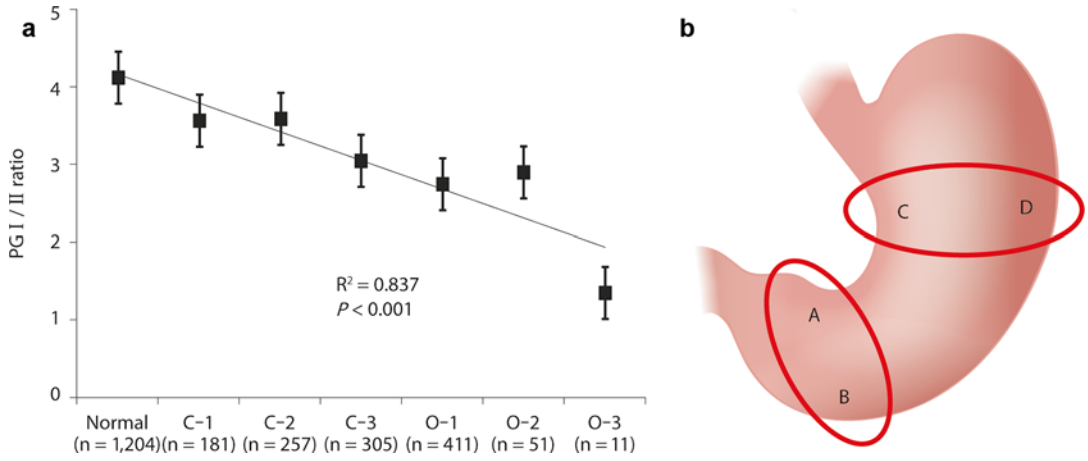


Fig. 17.6 Correlation between serum pepsinogen (PG) I/II ratio and endoscopic atrophic gastritis and gastric biopsy sites in the antrum and body. (**a**) The serum PG I/II ratio decreased significantly as gastric mucosal atrophy progressed. Data are presented as mean ± SE. (**b**) The gastric biopsy was taken from the lesser curvature (*A*) and greater curvature in the antrum (*B*) and from the lesser curvature (*C*) and greater curvature in the corpus (*D*) (Adapted from Lee et al. [83])

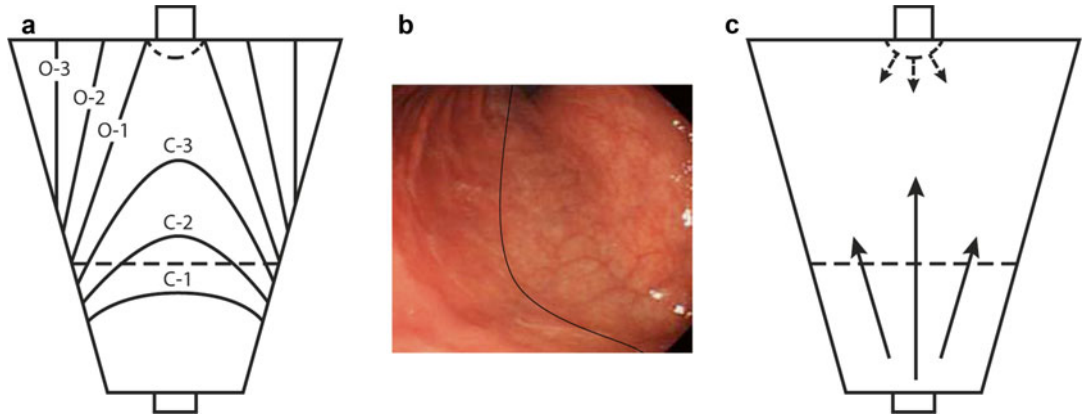


Fig. 17.7 Kimura-Takemoto classification of chronic atrophic gastritis: (**a**) atrophic gastritis (AG) is classified into six types. The closed-type AG indicates that the atrophic border remains on the lesser curvature of the stomach, while the open-type AG means that the atrophic border no longer exist on the lesser curvature but extends along the anterior and posterior walls of the stomach, (**b**) the boundaries of the mucosal atrophy is called as the F line, (**c**) progression of atrophic mucosal change from antrum to the corpus along with lesser curvature (Adapted from Kimura and Takemoto [78])

inaccurate below 50 years of age because mild atrophic mucosal change may look normal in endoscopy [76]. In addition, the sensitivity and specificity of endoscopic IM diagnosis were also low in study by Lim et al. [86]. The sensitivity and specificity of endoscopic diagnosis of IM based on histology were 24.0 % and 91.9 % for the antrum and were 24.2 % and 88.0 % for the corpus. As indicated by a multivariate analysis, the activity of mucosal inflammation and the presence of endoscopic AG were associated with the sensitivity of endoscopic diagnosis of IM in the antrum, while benign gastric ulcers, dysplasia, and the presence of endoscopic AG were associated with the sensitivity of endoscopic diagnosis of IM in the corpus [86]. Thus, a high

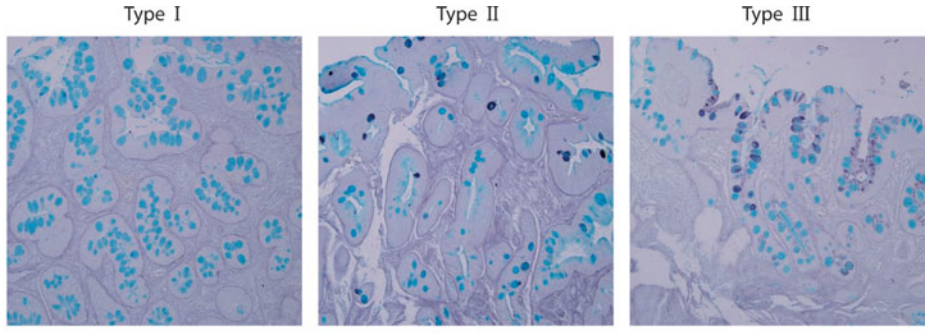
level of suspicion is important to increase the sensitivity of endoscopic diagnoses of IM, especially when adenoma, endoscopic AG, and ulcer are present, and confirmation of the doubt by histological diagnosis is necessary.

Other methods for endoscopic diagnosis of AG or IM are magnification chromoendoscopy and narrow band imaging (NBI). Several studies have suggested that chromoendoscopy with magnification could help to identify lesions of IM and dysplasia [87]. However, high-resolution magnifying endoscopy without chromoendoscopy also appears superior to standard endoscopy, allowing great accuracy for the diagnosis of *H. pylori* gastritis, IM, and dysplasia [88, 89]. The recent technology of NBI has been found to have good sensitivity and specificity for the diagnosis of gastric precancerous lesions [90, 91]. However, there is no agreement on which NBI patterns are associated with gastric precancerous lesions. So, further study is needed to apply NBI in diagnosis of AG and IM.

17.6.2 Histological Diagnosis

The second method of diagnosis of AG is histopathology. The updated Sydney system is the most widely accepted for classification and grading of gastritis. The updated Sydney system recommended five biopsies, two from the antrum, one from the incisura, and two from the corpus, because atrophic mucosal change and IM of gastric mucosa progress from antrum to corpus [63] (Fig. 17.3). If it is difficult to obtain at these three points, biopsies from the antrum and corpus are recommended. It is desirable to examine specimens from the antrum and corpus of the lesser and greater curvature, yet there are practical difficulties. Clinically, one biopsy specimen from the lesser curvature and the other from greater curvature are used to evaluate AG and IM [74, 83, 86].

Histological diagnosis is difficult when the specimens are inadequate. Deep portion of normal gastric mucosa are composed of different types of cells in the antrum and in the corpus. Normal gastric mucosa of the antrum is composed of mucous glands which secrete gastric mucus, and that of the corpus is composed of parietal cell and chief cell which secrete gastric acid and digestive enzymes. Sometimes severe inflammation obscures the gland's population, making it impossible to assess mucosal atrophy reliably. Such cases can be labeled as "indefinite for atrophy," and the final judgment can be deferred until the inflammation has regressed [92]. In addition, the diagnosis of "indefinite for atrophy" may be controversial among pathologists due to the difficulty in histological diagnosis [92]. IM is defined as the replacement of the surface, foveolar, and glandular epithelium in the oxyntic or antral mucosa by intestinal epithelium. These IM has been subtyped by classification of mucin expression. There are several classification systems for IM. The most widely used and useful classification was made by Jass and Filipe in 1981 [93]. Type I IM (complete) is positive for the sialomucin, and type II IM (incomplete) is positive for sialomucin in goblet and columnar cell, while type III IM (incomplete) is positive for sulfomucin [93]. Complete-type IM is similar to the small intestine in pathologic features and tests positive for the MUC2 secreted by goblet cells, while incomplete-type IM is similar to the large intestine in pathologic features and positive for MUC5AC and MUC6 secreted by the gastric mucosa and MUC2 [71, 94]. In addition, high iron diamine-alcian blue (HID-AB) stain can help classify the types of IM. Type I IM expresses only sialomucins (bright blue) (Fig. 17.8, type I), and types II and III express sialomucins (bright blue) and sulfomucins (black) (Fig. 17.8, type II and type III) [71]. Jass and Filipe reported that type III IM was associated with intestinal-type gastric cancer [93]. Other researchers have suggested that incomplete-type IM is associated with gastric cancer. As such, it is still controversial discussing which type of IM is related with gastric carcinogenesis [68, 69, 71]. In a cohort study of 861 subjects conducted in South Korea, type III IM was associated with aging ($p = 0.036$), and type II IM was associated with gastric carcinogenesis in the presence of *H. pylori* infection ($p < 0.05$) [71].



	Type I		Type II		Type III	
	Sialomucin	Sulfomucin	Sialomucin	Sulfomucin	Sialomucin	Sulfomucin
Goblet cell	+	−	+	±	+	+
Columnar cell	−	−	+	−	±	+

Fig. 17.8 Phenotype of intestinal metaplasia (IM) classified by mucin: type I IM expresses only sialomucins (*bright blue*), and types II and III express sialomucins (*bright blue*) and sulfomucins (*black*) (Adapted from Kang et al. [71])

17.6.3 Diagnosis by Serum Pepsinogen I/II Ratio

The third diagnostic method of AG or IM is a measurement of serum PG I, II, and I/II ratio. The radioimmunoassay method was used to measure serum PG levels. Recently, a new method has emerged to measure PG level [95, 96]. The present methods to measure PG level are latex-enhanced turbidimetric immunoassay (HBi Corp, Seoul, Korea, imported from Shima Laboratories, Tokyo, Japan) [91, 95, 96] and enzyme-linked immunosorbent assays (Biohit ELISA kit; Biohit, Helsinki, Finland) [97, 98]. PG has been used to screen AG, IM, and gastric adenocarcinoma for more than 20 years because of its noninvasiveness and cost-effectiveness. In Japan, PG screening for gastric cancer has been used to improve population compliance [99]. However, PG screening is not accepted as a generalized screening method of AG or IM worldwide because of low positive predictive value in other countries [100].

PG I is produced by chief cells in the antrum and corpus, while PG II is produced by the chief cells and mucous neck cells of the whole gastric mucosa. When gastric atrophy develops, chief cells are replaced by pyloric glands, leading to a decrease in PG I level, while PG II levels is relatively unaffected, so a low PG I/II ratio reflects the severity of AG [101, 102]. In a prospective study of 5,113 subjects in Japan, screening for gastric cancer with PG I <70 ng/mL and PG I/II ratio <3 as the cut-off points had the sensitivity and specificity of 84.6 % and 73.5 %, respectively [103]. Generally PG I/II ratio 3.0 or less has been widely accepted as a cut-off value in several studies [103, 104]. However, in a recent Korean study which evaluated the relationship between PG level and AG, a new cut-off value of PG I/II ratio, 3.2, was suggested to diagnose AG [83]. This study suggested that serum PG I/II ratio remarkably decreased in correlation with the extent of atrophy by the Kimura-Takemoto classification [83]. In another Korean research, PG I and PG II were negatively correlated with AG, and only PG I/II ratio was positively correlated with AG [96]. Serum PG I and PG II were higher in *H. pylori*-positive than in *H. pylori*-negative subjects, because a growth rate of PG II was higher than that of PG I in *H. pylori*-positive individuals [83, 95, 96]. Moreover, a significantly positive correlation was found between age and the PG II ($p < 0.001$) and a negative correlation between age and the PG I/II ratio ($p < 0.001$) but no correlation between age and the PG I [96]. In addition, men had significantly higher PG I levels compared with women ($p < 0.001$) and had a slightly higher PG II levels without statistical significance, suggesting that the difference in the PG I levels observed between sex could be related to

hormonal effects [96]. Consequently these results show that multiple factors could change the serum PG level. Thus, the proper cut-off value of PG level should be established in each country in order to increase sensitivity and specificity [83, 96].

17.7 Management for Atrophic Gastritis and Intestinal Metaplasia

Up to the present time, there are no unified clinical guidelines for prevention of gastric cancer regarding the classification of high-risk groups progressing to gastric cancer [105]. However, through many studies, AG and IM are considered as precancerous lesions. Prevention and treatment of AG and IG could decrease the prevalence of gastric cancer, requiring strategies to manage AG and IM [106]. As *H. pylori* infection triggers a multistep progression from chronic gastritis, AG, IM, and finally to invasive gastric cancer [29], *H. pylori* eradication is a key step in management strategy for AG and IM even there are still arguments regarding the efficacy of *H. pylori* eradication, especially in case IM [9]. Another strategy is the surveillance to detect early gastric cancer (EGC) in the subjects with AG or IM, with additional managements of several risk factors of AG and IM [106]. Adequate consumption of vegetables and fruits seems to reduce the risk of cancer and decrease the incidence of gastric cancer in the West. Although some studies for premalignant gastric lesions have shown positive results of vitamin C, folic acid, and beta-carotene supplementation, these results were not confirmed in a large meta-analysis [107–110]. In summary *H. pylori* eradication could be the main strategy for prevention of gastric cancer in individuals with AG and IM, but it may be helpful to change diet and supply vitamin C, folic acid, and β-carotene. Consequently, further large cohort study is needed to establish the optimal strategy of gastric cancer prevention in the presence of AG and IM.

Conclusions

To reduce the prevalence of gastric cancer, it is very important to classify and manage the high-risk groups of gastric cancer. Furthermore, *H. pylori* infection triggers a multistep inflammation from chronic gastritis, AG, IM, and finally to gastric cancer. Many studies have proved that AG and IM are precancerous lesions of gastric cancer and *H. pylori* infection is the most important cause of AG and IM. Diagnostic methods of AG and IM are endoscopic, histological findings, and PG I/II ratio. Each method has advantages and disadvantages, and it is necessary to select the proper test according to the individual cases. As the infection of *H. pylori* is the most important risk factor of AG and IM, it is important to perform *H. pylori* eradication to prevent the progression to gastric cancer. The appropriate time to prevent gastric cancer by *H. pylori* eradication is still under discussion. Controlling other risk factors such as diet, vitamin C, beta-carotene, and folic acid may be useful to decrease the prevalence of gastric cancer.

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Functional Dyspepsia

18

Sung Eun Kim

Abstract

Functional dyspepsia (FD) is defined as a clinical condition in which pain arises from the gastroduodenal area in the absence of any organic, systemic, or metabolic disease that could explain the symptoms. Dyspeptic symptoms must be present for the previous 3 months with symptom onset at least 6 months before diagnosis according to the Rome III criteria. *Helicobacter pylori* (*H. pylori*) infection is considered a predisposing factor for FD, because *H. pylori* is known not only to induce pathologic change of the gastric mucosa but also to affect several gastrointestinal hormones. Furthermore, recent studies showed that *H. pylori* could influence molecular biology causing motility disorders. Unfortunately, the results are still controversial, and further studies are needed to evaluate the role of *H. pylori. H. pylori* eradication is suggested as one of the treatment options for FD. In countries with a high prevalence of *H. pylori* infection, such as Korea, *H. pylori* eradication might improve the symptoms of FD patients. Several meta-analyses reported that *H. pylori* eradication could improve symptoms in patients with FD compared to placebo; however, there are limitations, including heterogeneity of enrolled studies. Therefore, well-designed, prospective studies are required to confirm the effect of *H. pylori* eradication in FD.

Keywords

Disease eradication • Dyspepsia • *Helicobacter pylori*

18.1 Introduction

Dyspepsia is one of the most prevalent gastrointestinal diseases, and its prevalence is presumed to be about 5 % of all patients who visit primary healthcare clinics. Patients with dyspeptic symptoms usually suffer from heartburn, epigastric

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pain, postprandial discomfort, bloating, and a heavy feeling in the upper abdominal area. These symptoms are generally chronic and can decrease quality of life. Some patients with dyspeptic symptoms are diagnosed with functional dyspepsia (FD), which is defined as chronic and recurrent gastroduodenal discomfort without evidence of organic or systemic disease, such as peptic ulcer, gastrointestinal malignancy, gastroesophageal reflux disease, or pancreatobiliary disease. Of patients who were referred to the department of gastroenterology at a tertiary hospital in Korea, about 8–20 % had organic diseases, and about 70–92 % had functional gastrointestinal disorders (FGIDs). FD was the most common disorder among the patients with FGIDs [1]. According to the National Health Insurance Corporation database in Korea, FGIDs lead to a significant socioeconomic burden [2].

Several factors have been suggested to induce the symptoms of FD, including disturbed gastroduodenal motility, visceral hypersensitivity, psychological factors, and diet. Among them, the direct connection between *Helicobacter pylori* (*H. pylori*) infection and FD remains controversial. Given this background, the aims of this chapter are to evaluate the relationship between FD and *H. pylori* infection, to assess the role of *H. pylori* in FD pathogenesis, and to assess the effect of *H. pylori* eradiation on treatment of FD.

18.2 Definition of Functional Dyspepsia

Prior to investigating the relationship between *H. pylori* infection and FD, we reviewed the definition of FD in detail. According to the Rome III criteria, FD is defined as the existence of symptoms seeming to originate in the gastroduodenal area but with a negative diagnostic workup for organic or metabolic diseases that would explain the pain, including upper endoscopy, abdomen ultrasonography, laboratory findings, computed tomography, or other modalities [3]. FD was divided into four symptoms based on the Rome III criteria as follows: epigastric pain, epigastric burning, bothersome postprandial fullness, and early satiation (prevents finishing regular-sized meals). These symptoms must have been present for the previous 3 months with symptom onset at least 6 months before diagnosis. In addition, they presented two novel subcategories of FD based on cohort and population-based studies: postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS) [4–6]. PDS must include one or both of the following symptoms: bothersome postprandial fullness after regular-sized meals at least several times per week and early satiety preventing finishing a regular-sized meal at least several times per week. In addition, patients with PDS can experience bloating in the upper abdominal area, postprandial nausea, or belching. Furthermore, PDS may coexist with EPS. EPS includes epigastric pain or burning of at least moderate severity at least once per week. This epigastric pain or burning should be intermittent, should not be generalized or localized to other abdominal or chest areas, should not be alleviated by defecation or passage of flatus, and should not be associated with gallbladder or sphincter of Oddi disorders. Additionally, the pain is usually induced or resolved by intake of a meal but may also happen during a fasting state [3, 7] (Table 18.1).

18.3 Pathophysiology of Functional Dyspepsia

FD is a complex disease that can arise from a variety of causes. Unfortunately, as stated above, the association between FD and *H. pylori* infection has not been clearly established. From the perspective of the Rome committee, *H. pylori* infection is not included as an organic cause of FD. Thus, *H. pylori*-infected patients with dyspeptic symptoms can be considered to have FD. Recently, several studies have suggested that the relationship between FD and *H. pylori* infection may play a role in FD pathophysiology.

Several studies have reported that some patients acquired irritable bowel syndrome (IBS) symptoms after a gastrointestinal infection. Similarly, several studies showed that a subset of patients developed FD following an episode of

Table 18.1 Rome III criteria for functional dyspepsia

Diagnostic criteria[a] for functional dyspepsia must include one or more of the following symptoms:
(a) Bothersome postprandial fullness
(b) Early satiety
(c) Epigastric pain
(d) Epigastric burning
With no evidence of structural disease that is likely to explain symptoms (including upper endoscopy)
1. Postprandial distress syndrome
Diagnostic criteria[a] must include one or both of the following symptoms:
(a) Bothersome postprandial fullness, occurring after ordinary-sized meals, at least several times per week
(b) Early satiety that prevents finishing a regular meal, at least several times per week
Other supportive criteria:
(a) Upper abdominal bloating or postprandial nausea or excessive belching can be present
(b) Epigastric pain syndrome may coexist
2. Epigastric pain syndrome
Diagnostic criteria[a] must include all of the following symptoms:
(a) Pain or burning localized to the epigastrium of at least moderate severity at least once per week
(b) The pain is intermittent
(c) Not generalized or localized to other abdominal or chest regions
(d) Not relieved by defecation or passage of flatus
(e) Not fulfilling criteria for gallbladder and sphincter of Oddi disorders
Other supportive criteria:
(a) The pain may be of a burning quality, but without a retrosternal component
(b) The pain is commonly induced or relieved by ingestion of a meal but may occur while fasting
(c) Postprandial distress syndrome may coexist

Modified from Lacy et al. [7] and Tally and Vakil [8]
[a]Criteria must be fulfilled for the previous 3 months with symptom onset at least 6 months before diagnosis

gastrointestinal infection [9, 10]. A recent meta-analysis reported that the odds ratio (OR) for the occurrence of post-infectious FD was 2.54 (95 % confidence interval [CI], 1.76–3.65) more than 6 months after acute gastroenteritis compared with the control [11]. Another meta-analysis found an OR of 2.18 (95 % CI, 1.70–2.81) for FD risk following acute gastroenteritis [12]. Taken together, gastrointestinal infection is associated with an increased risk of FD, supporting an inflammatory and immunological mechanism in the pathogenesis of FD [13]. *H. pylori* infection is the main cause of gastroduodenal inflammation [14] and provokes activation of a complex cytokine and chemokine response in the gastric mucosa [15], which may induce dyspepsia [16].

H. pylori infection induces gastric acid hypersecretion in the gastric antral mucosa [17]. Antral-predominant gastritis occurs in about 10–15 % of patients with *H. pylori* infection, likely due to gastric acid hypersecretion [18]. Patients with antral-predominant gastritis due to *H. pylori* infection showed decreased somatostatin release in the antral gland area, which leads to increased gastrin release with subsequent rise in acid secretion. This mechanism may explain dyspepsia.

In addition to clinical symptoms, *H. pylori* infection induces macroscopic and microscopic changes in the gastric mucosa that, along with lifestyle factors, may increase the risk of atrophic gastritis and intestinal metaplasia, leading to gastric cancer [19–21]. However, the results of studies that investigated the relationship between severity of histological gastritis and dyspepsia symptoms were varied. Czinn et al. [22] revealed an association between the severity of inflammation and epigastric pain. Likewise, another study group also revealed an indirect association between the severity of inflammation on the gastric corpus and the severity of symptoms [23]. However, there was no significant difference between the severity of dyspepsia symptoms and the severity of histological gastritis in other studies [24, 25]. Consequently, it is difficult to draw firm conclusions about the relationship between the severity of gastritis and the severity of dyspeptic symptoms, and further studies are needed to investigate this association.

Among the gastrointestinal hormones studied so far, ghrelin and leptin are representative hormones controlling appetite. Ghrelin promotes appetite and food intake and stimulates gastric emptying and gastric acid secretion [26]. These functions are partially achieved via vagal nerve pathways [27]. Plasma ghrelin concentrations are

elevated in gastroduodenal mucosal injury for the purpose of gastroduodenal cytoprotection [28, 29]. However, plasma ghrelin levels decrease in the severe gastric mucosal atrophic state induced by *H. pylori* infection [30, 31]. Thus, *H. pylori* infection may be related to induction of gastric motor dysfunction and reduction of appetite by suppression of ghrelin secretion [16]. Ghrelin may therefore play a role in the occurrence of FD, especially that related to *H. pylori*. Studies have revealed that patients with FD experience changing plasma ghrelin levels, which are frequently related to FD symptom scores [32, 33]. A few studies reported that plasma ghrelin levels were significantly decreased in dysmotility-like FD patients with postprandial fullness and/or early satiety [34, 35]. A recent study revealed that the Leu72Met (408C > A) single nucleotide polymorphism of the ghrelin gene was significantly related to early-phase gastric emptying in patients with FD [36]. Akamizu et al. [37] revealed that repeated ghrelin administration increased food intake in patients with FD; however, another study showed that there was no significant difference between patients with dysmotility-like FD and healthy controls [32]. Increased plasma ghrelin levels in patients with FD were also reported [38]. Leptin also activates vagal nerve terminals, decreases appetite, and enhances gastric mucin secretion [39]. Similar to ghrelin, leptin may induce the occurrence of FD. A study in Iran reported that serum leptin levels were higher in patients with dysmotility-like dyspepsia [40]. Moreover, *H. pylori*-infected patients had increased serum leptin concentrations and leptin mRNA expression in the gastric mucosa [40, 41], suggesting that *H. pylori* infection could cause decreased appetite by stimulating serum leptin secretion. Unfortunately, there are only a few studies investigating the correlation of *H. pylori*-positive FD patients with ghrelin or leptin. Thus, the association between gastrointestinal hormones and *H. pylori*-associated dyspepsia remains speculative.

A recent study evaluated the role of microRNAs (miRNAs) in gastric motility disorders related to *H. pylori* infection [42]. Expression levels of muscle-specific miRNAs in the stomach of *H. pylori*-infected mice, such as miR-1, miR-133a, and miR-133b, were downregulated. On the other hand, the expression profiles of histone deacetylase 4 and serum response factor, which are target genes of miR-1 and miR-133 to reinforce muscular hyperproliferation, were accelerated. Furthermore, notable thickening of the muscular layer in the gastric corpus of *H. pylori*-infected mice was identified on histologic examination. Additionally, gastric emptying was significantly enhanced in *H. pylori*-infected mice. Taken together, chronic *H. pylori* infection influences the expression of muscle-specific miRNAs, histone deacetylase 4, and serum response factor, which might induce hyperplasia of the gastric muscular layer and acceleration of gastric emptying in *H. pylori*-infected mice. Though no study has tested this proposed mechanism in humans, these results suggest new molecular biology perspectives and provide a theoretical underpinning for *H. pylori*-associated dyspepsia to be an organic disease rather than a functional disorder.

Current literature suggests increased duodenal acid secretion in patients with dyspeptic symptoms [43] and a relationship between duodenal eosinophilia and FD. When eosinophil counts in patients with FD and asymptomatic controls were investigated, the OR for FD patients with high eosinophil counts in the duodenal bulb and duodenal second portion were 11.7 (95 % CI, 3.9–34.9) and 7.3 (95 % CI, 2.9–18.1), respectively, compared with asymptomatic controls [44]. *H. pylori* infection induces eosinophil aggregation in the gastric mucosa [45]. Another recent study also reported that eosinophilia in the duodenal second portion was significantly increased in FD patients with postprandial fullness, early satiety, and abdominal pain [46]. Therefore, *H. pylori* infection might be related to duodenal eosinophilia alongside the occurrence of FD. Intraepithelial lymphocytes in patients with FD and healthy controls were also evaluated by a study in France [47]. The number of intraepithelial lymphocytes was significantly higher in *H. pylori*-positive FD patients compared to healthy controls, but there was no significant difference between *H. pylori*-negative FD patients and healthy controls. The expression of

CD95/Fas and HLA-DR expressing CD3+ intraepithelial lymphocytes was significantly lower in *H. pylori*-negative FD patients than in healthy controls. These results suggest that the phenotypic characteristics of intraepithelial lymphocytes might explain the difference between *H. pylori*-positive FD and *H. pylori*-negative FD.

Taken together, *H. pylori*-positive dyspepsia may represent a new area of study for FD. Recent studies suggested that *H. pylori*-positive dyspepsia, or *H. pylori*-associated dyspepsia, is not a functional disorder but an organic disease with many and various mechanisms of pathophysiology. Therefore, some study groups proposed that *H. pylori*-associated dyspepsia is different from FD and should be separated [16, 19]. A recent report from Kyoto global consensus clarified that *H. pylori* infection is the cause of dyspepsia in a subset of patients, and *H. pylori*-associated dyspepsia is a different entity compared to FD [48]. Further studies are needed to evaluate the true association between *H. pylori* infection and symptoms of FD.

18.4 Diagnostic Approach to Functional Dyspepsia

The committee of the American College of Gastroenterology issued guidance on the evaluation of FD patients [8]. Dyspeptic patients more than 55 years old or those with alarm features (Table 18.2) should undergo prompt endoscopy to rule out peptic ulcer disease, esophagogastric malignancy, and other rare upper gastrointestinal tract diseases. In patients aged 55 years or younger with no alarm features, the clinician may consider two approximately equivalent management options: (i) test and treat for *H. pylori* using a validated noninvasive test and a trial of acid suppression if eradication in successful but dyspeptic symptoms do not resolve, or (ii) an empiric trial of acid suppression with a proton pump inhibitor (PPI) for 4–8 weeks. The test-and-treat option is preferable in populations with a moderate-to-high prevalence of *H. pylori* infection (≥10%), whereas the empirical PPI strategy is preferable in low prevalence situations (Fig. 18.1).

Table 18.2 Alarm features

(a) Bleeding
(b) Anemia
(c) Early satiety
(e) Progressive dysphagia
(d) Unexplained weight loss (>10% body weight)
(f) Odynophagia
(g) Persistent recurrent vomiting
(h) A family history of gastrointestinal cancer
(i) Previous esophagogastric malignancy
(j) Previous documented peptic ulcer
(k) Lymphadenopathy
(l) Abdominal mass
(m) Fever
(n) Jaundice
(o) New onset dyspepsia[a]

Modified from Talley and Vakil [8] and Miwa et al.[49]
[a]Miwa et al. [49] defined age of new onset dyspepsia as over 40 years of age in populations with a high prevalence of upper gastrointestinal malignancy and over 45 and 50 years in populations with intermediate and low prevalence, respectively

In 2012, an Asian consensus report on FD was developed [49]. All of the consensus members agreed that if patients have any alarm features including new onset dyspepsia in a patient over 40 years of age in a population with high prevalence of upper gastrointestinal malignancy, such as China, Korea, or Japan, they should undergo further testing (Fig. 18.2). Compared to the guidelines of the West, those in Asia recommend a younger patient age at which new onset of dyspepsia should prompt endoscopic examination.

According to Korean guidelines for the treatment of FD in 2012, which compared endoscopy with "test and treat" for *H. pylori*, endoscopy may be a more effective initial strategy for managing patients with FD in Korea given the high incidence of gastric cancer and low cost of endoscopy [50]. The usefulness of *H. pylori* serology testing before endoscopy in patients with dyspepsia was investigated in Korea [51]. The sensitivity and negative predictive value of anti-*H. pylori* IgG for organic disease were 76.6% and 85.5%, respectively, in patients with dyspepsia under 40 years old. In patients with dyspepsia over 40 years old, the sensitivity and negative predictive value of anti-*H. pylori* IgG for organic dis-

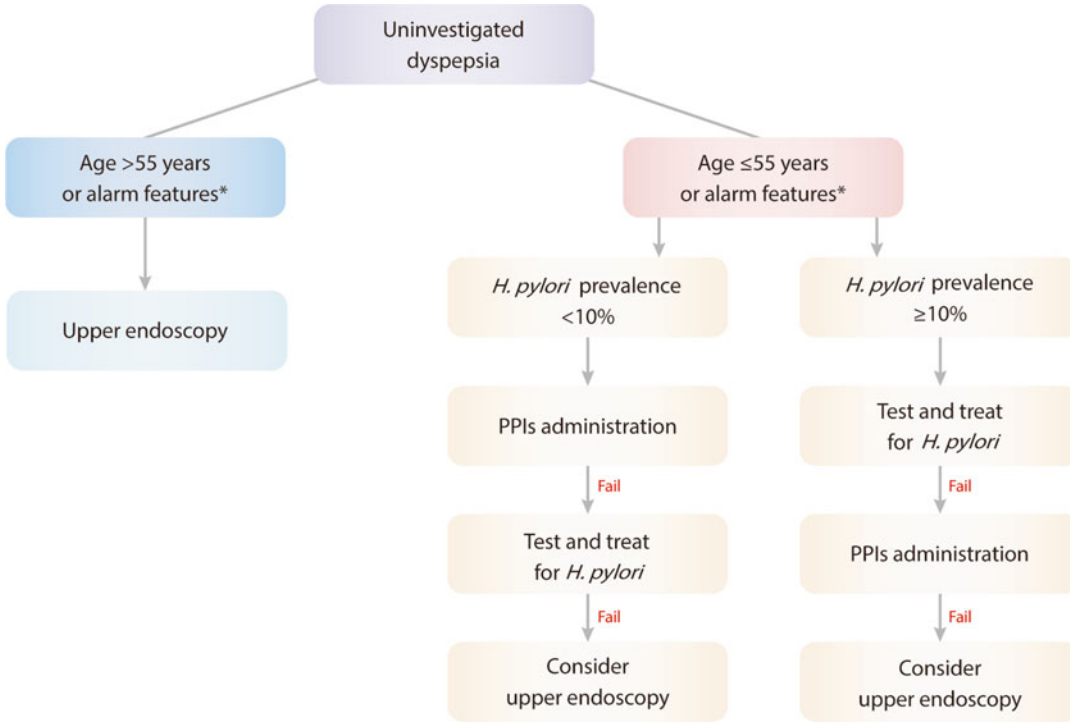
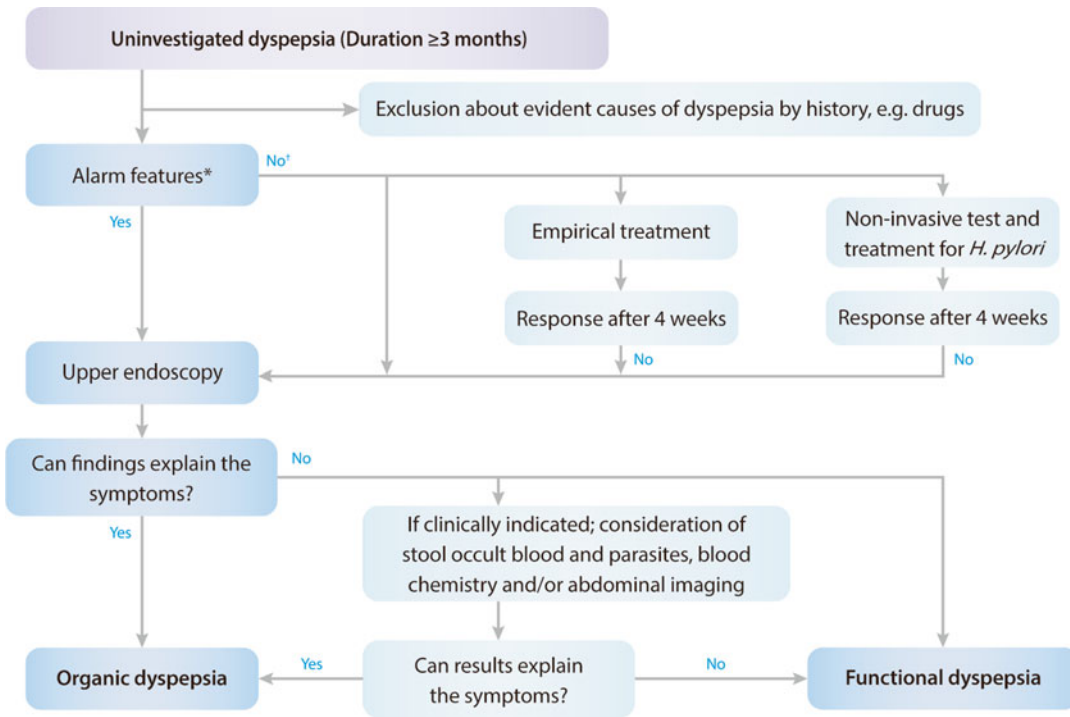


Fig. 18.1 Management algorithm of uninvestigated dyspepsia. *Alarm features can be found in Table 18.2. *H. pylori Helicobacter pylori*, *PPIs* proton pump inhibitors (Adapted from Tally and Vakil [8], with permission from nature publishing group)



ease were 61.9 % and 64.0 %, respectively. Although further study will be needed, the "test-and-treat" *H. pylori* approach might not the preferred initial step in treating patients with FD in Korea. Rather, as mentioned earlier, endoscopy may be the more effective initial strategy for managing patients with FD in Korea [50, 52].

Other diagnostic tests, such as serum testing for anemia, liver disease, and pancreatitis (amylase and lipase) and upper abdominal ultrasound or computed tomography, can be helpful in diagnosing FD [49].

18.5 Treatment of Functional Dyspepsia

There are several options for treating FD, including PPIs, histamine-2 receptor antagonists, prokinetic agents, and antidepressant and anxiolytic agents. Among them, we focus on *H. pylori* eradication.

Several epidemiologic studies have revealed that the *H. pylori* infection rate in patients with FD is higher than in matched control populations. A meta-analysis expressed a summary OR of 1.6 (95 % CI, 1.4–1.8) for *H. pylori* infection in FD [53]. Although this has not yet been confirmed, this result suggests that eradication of *H. pylori* could improve FD symptoms [54].

A Cochrane meta-analysis was performed on 17 randomized controlled trials and identified an association between *H. pylori* eradication and improvement in FD symptoms. A small but significant benefit of *H. pylori* eradication therapy was observed with a number needed to treat of 14 (95 % CI, 10–25) [55]. In another recent study, the number needed to treat was eight [43]. The cumulative long-term benefit of *H. pylori* eradication in patients with FD was also performed in UK. Dyspeptic symptoms in the *H. pylori* eradication group were significantly decreased compared to the placebo group, and OR was 0.84 (95 % CI, 0.71–1.00) [56]. The effect of *H. pylori* eradication on Asian FD patients might be different from FD patients in the West due to the varying prevalence of *H. pylori* strains including polymorphisms of *cagA* gene, gastric acid levels, and the severity of gastritis [57]. A systemic review and meta-analysis from China reported that the summary OR for improvement in FD patients after *H. pylori* eradication was 3.61 (95 % CI, 2.62–4.98) [58]. A study in Singapore demonstrated a 13-fold increased chance of FD symptom resolution in successfully eradicated *H. pylori* FD patients compared to those with persistent *H. pylori* infection (95 % CI, 1.1–17.7) [59]. In addition, a recent meta-analysis of randomized controlled trials with 12 months follow-up revealed that dyspeptic symptoms were significantly improved in eradication group (OR 1.38; 95 % CI, 1.18–1.62) [60]. Unfortunately, there are no randomized controlled studies evaluating the effect of *H. pylori* eradication on FD in Korea. Our study group showed that *H. pylori* eradication (OR 5.81; 95 % CI, 1.07–31.59) was related to improvement of FD at one year [61]. Studies in meta-analysis evaluated the effect of *H. pylori* eradication in patients with functional dyspepsia since 2000 and were summarized in Table 18.3.

However, Zullo et al. [63] pointed out several problems related to meta-analyses studying the effect of *H. pylori* eradication in FD patients. The type and number of dyspeptic symptoms of patients in enrolled studies were different, and the definitions of symptom improvement or regression varied widely. Many studies were performed to verify the effectiveness of *H. pylori* eradication in FD patients; however, only 12 studies were well-designed, double-blind, placebo-controlled studies with at least 50 patients followed for more than 6–12 months with final confirmation of *H. pylori* status by endoscopy with biopsy or a ^{13}C-urea breath test. Unfortunately, the type and

Fig. 18.2 Diagnostic algorithm of functional dyspepsia. *Alarm features can be found in Table 18.2. †The appropriate choice from the three options according to patient symptoms, patient wishes, risk of *H. pylori* infection, and gastric cancer in each country as well as primary healthcare settings. *H. pylori Helicobacter pylori* (Adapted from Miwa et al. [49], with permission from The Korean Society of Neurogastroenterology and Motility)

Table 18.3 Meta-analysis studies about *Helicobacter pylori* eradication in patients with functional dyspepsia since 2000

Authors	Year	Enrolled studies	Follow-up	Number	Symptom improvement	OR (95 % CI)	NNT
Gisbert et al. [62]	2002	9 RCT	At least 6 months	Patients; 953 Control; 958	Eradication group; 43 % (95 % CI, 40–46 %) Control group; 39 % (95 % CI, 36–42 %)	1.20 (0.91–1.58)	25
Moayyedi et al. [55]	2006	17 RCT	At least 3 months	Patients; 1,934 Control; 1,632	Eradication group; 36 % (range 15–75 %) Control group; 29 % (range 7–51 %)	0.90 (0.86–0.94), 10 % relative risk reduction (95 % CI, 6–15 %) in eradication group	14
Jin et al. [58]	2007	7 RCT	At least 1 month	Patients; 395 Control; 366	Eradication group; 74.4 % (294/395) Control group; 47.3 % (173/366)	3.61 (2.62–4.98)	NM
Zhao et al. [60]	2014	14 RCT	At least 12 months	Patients; 1,490 Control; 1,503	Eradication group; 40.6 % (605/1,490) Control group; 34.0 % (511/1,503)	1.38 (1.18–1.62)	15

OR odds ratio, *CI* confidence interval, *NNT* number needed to treat, *RCT* randomized controlled trials, *NM* not mentioned

number of symptoms and the definition of symptom improvement were different even in these 12 well-designed studies. Considering these findings, we could assume that the studies evaluating the effectiveness of *H. pylori* eradication in patients with FD are difficult to interpret. There is thus no definitive conclusion about the usefulness of *H. pylori* eradication in FD patients.

Despite these limitations, the American Gastroenterological Association recommends *H. pylori* eradication as the initial management strategy for uncomplicated dyspepsia in patients younger than 55 years, especially in countries with high prevalence (>10 %) of *H. pylori* infection, like Korea [7]. The Korean College of *Helicobacter* and Upper Gastrointestinal Research also reported that *H. pylori* eradication helps long-term dyspeptic symptom improvement in some patients with FD [64].

On another note, *H. pylori* eradication is useful in only some patients with FD. A recent study reported that FD patients with microscopic duodenitis showed greater symptom improvement after *H. pylori* eradication than those without microscopic duodenitis [65]. Sugano et al. [19] who proposed that *H. pylori*-associated FD should be separated from FD also suggested that FD patients with *H. pylori* infection should be treated before meeting criteria for a diagnosis of FD. Because *H. pylori* infection clearly influences pathological and physiological changes, residual dyspepsia symptoms after complete eradication of *H. pylori* infection might be regarded as real FD coexisting with *H. pylori* infection. Based on these considerations, the Kyoto global consensus also agreed that patients who remain symptomatic after successful *H. pylori* eradication should be regarded as having FD [48] (Fig. 18.3). In addition, *H. pylori* eradication is first-line treatment for dyspeptic patients with *H. pylori* infection, because eradication therapy for dyspeptic symptoms is better than placebo in *H. pylori*-infected dyspeptic patients.

All things considered, *H. pylori* eradication is effective in some patients with dyspepsia. Considering the short duration and acceptable

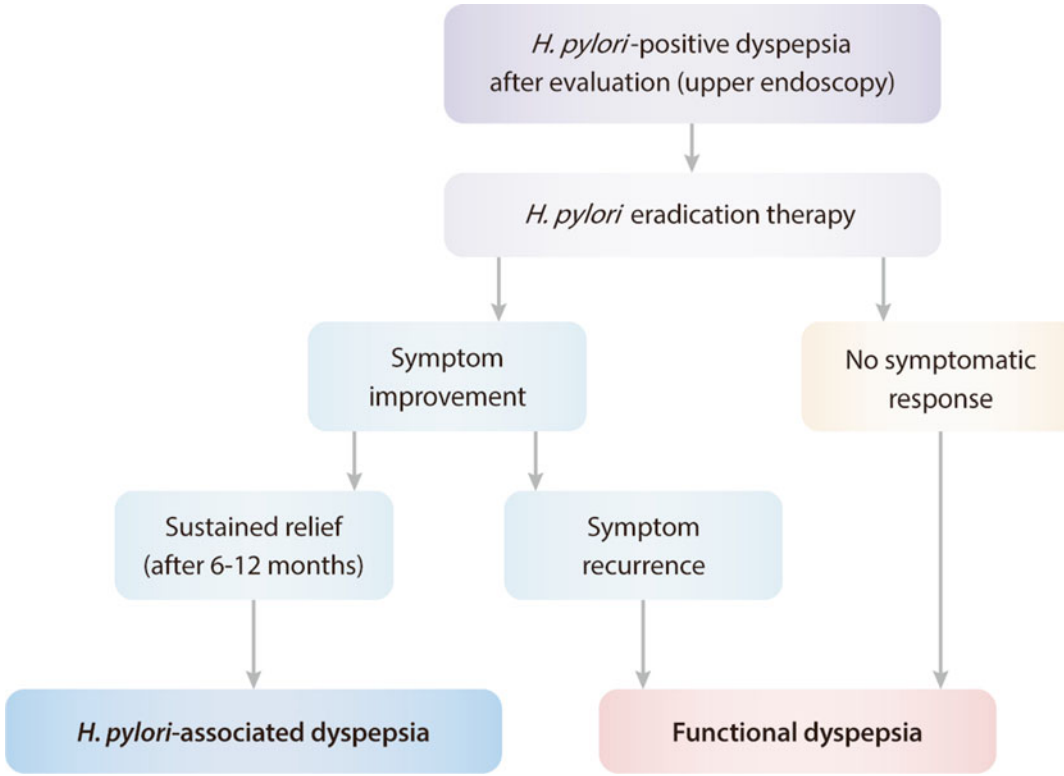


Fig. 18.3 Diagnostic algorithm of *H. pylori*-associated dyspepsia. *H. pylori Helicobacter pylori* (Adapted from Sugano et al. [48], with permission from BMJ publishing group Ltd.)

cost-benefit analysis for improving dyspeptic symptoms, *H. pylori* eradication could be proposed as the treatment for those with *H. pylori* infection.

Conclusions

FD is one of the most prevalent gastrointestinal disorders; however, many physicians are hard to diagnose FD due to the varied pathophysiology and symptoms. Among the many causes of FD, *H. pylori* infection is considered to have some effect on the onset of FD worldwide. *H. pylori* eradication is suggested as one of the therapeutic options for FD patients with *H. pylori* infection, and several meta-analyses reported that *H. pylori* eradication improved dyspeptic symptoms in FD patients compared to placebo. Thus, *H. pylori* eradication could be attempted for treatment of FD patients. Some limitations still exist, such as lack of well-designed, double-blinded, placebo-controlled studies to establish the effectiveness of *H. pylori* eradication in FD patients. Therefore, additional well-designed, prospective studies are needed to demonstrate the association between FD and *H. pylori* infection.

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Peptic Ulcer

19

Chang Seok Bang and Gwang Ho Baik

Abstract

The incidence and prevalence of peptic ulcer have been decreasing with the advancement of medical management and low prevalence of *Helicobacter pylori* (*H. pylori*) infection. However, complications of peptic ulcer are still common, and admission rate has not been decreasing with the wide use of nonsteroidal anti-inflammatory drugs (NSAIDs) and antiplatelet agents. *H. pylori* and NSAIDs are the most common and important etiologies of peptic ulcer. Early diagnosis with endoscopic and radiologic examinations is important for the prevention of complications. Similar endoscopic features with peptic ulcer can be seen with malignancy and should be excluded for the differential diagnosis. Elimination of causative factors and proton pump inhibitor -based medications are main treatment strategies. The clinical characteristics of idiopathic or non-*H. pylori*, non-NSAID ulcer need more investigation.

Keywords

Helicobacter pylori • Nonsteroidal anti-inflammatory agents • Peptic ulcer

19.1 Introduction

Gastrointestinal ulcers can be found anywhere in the gastrointestinal tract from the esophagus to colon, and various causative factors are related to the development of ulceration. Peptic ulcer usually refers to mucosal injury more than 5 mm diameter confined to the mucosa and submucosa in the stomach and duodenum [1]. Since digestive enzyme pepsin involved in gastric acid secretion had been suspected as the main responsible factor for the development of ulcers, terminology of peptic ulcer has been used. This chapter will

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review about epidemiology, etiology, clinical features, diagnosis, treatments, and complications of peptic ulcer.

19.2 Epidemiology

The incidence and prevalence of peptic ulcer have been decreasing [2]. Hygiene improvement and low prevalence of *Helicobacter pylori* (*H. pylori*) infection are suspected as main reasons. The annual global incidence is reported as 0.1–1.5 %, and prevalence is reported as 0.03–0.19 % [2, 3]. However, there are also reports indicating that complications of peptic ulcer are still common and admission rate has not been decreasing [4, 5]. The reasons are supposed to be wide use of NSAIDs or aspirin, especially in elderly patients [4–6].

According to the Korean studies, the prevalence of peptic ulcer is estimated as 2–3 % [7–9], and it has not been decreasing [10, 11]. The prevalence of duodenal ulcer is reported to be constant or slightly decreasing, but that of gastric ulcer is reported to be increasing [11, 12]. The mean age of the patients showed higher in gastric ulcer than that of duodenal ulcer, and patients with gastric ulcer take more NSAIDs or aspirin [12].

19.3 Causative Factors

The pathophysiology of peptic ulcer had been suspected as an imbalance between mucosal injury factors and mucoprotective defensive factors. However, *H. pylori* infection and NSAIDs or aspirin use are known to account for more than 70 % and considered as the most important causative factors of peptic ulcer now [1]. Attention to idiopathic or non-*H. pylori*, non-NSAID peptic ulcer is increasing because of high recurrence and mortality rates [13, 14].

19.3.1 *H. pylori* Infection

Although *H. pylori* infection is still the most important causative factor of peptic ulcer, the seroprevalence of *H. pylori* in Korean population (over 16 years) decreased from 66.9 % in 1998 to 59.6 % in 2005 [15]. The detection rate of *H. pylori* showed slightly higher in duodenal ulcer as 73–100 % than that of gastric ulcer as 65–100 % [16–21]. However, another study reported increased detection rate from 66.1 to 73.1 % in gastric ulcer (from the 1990s to 2000s) and decreased detection rate from 79.3 to 68.1 % in duodenal ulcer (from the 1990s to 2000s) [12]. Several theories have been proposed for the development of duodenal ulcer. Increased acid secretion by colonized *H. pylori* in gastric metaplasia developed in duodenal mucosa and in the gastric antrum developed as antral-dominant gastritis has been proposed. Decreased bicarbonate secretion by *H. pylori* in the duodenum has been also suspected as one of the pathogeneses. Gastric ulcer is characterized by normal or decreased acid secretion. Defect in mucosal protective function and reflux of duodenal digestive enzymes by loosening of pyloric sphincter have been suspected as the pathogenesis of gastric ulcer [1]. Only about 5–10 % of patients with *H. pylori* infection has peptic ulcer. Thus, variation of bacterial characteristics itself, host immunity, and unknown factors other than *H. pylori* infection are suspected to be influential for the development of peptic ulcer [1].

19.3.2 NSAIDs

With the aging of the population, NSAIDs or aspirin is widely used in patients with chronic musculoskeletal or cardiovascular diseases. Long-term use of NSAIDs has been reported to increase the risk of peptic ulcer and complications [22]. Low-dose aspirin, which is widely used for the prevention of cardiovascular diseases, is also known to increase the risk of peptic ulcer and complications [23].

There are various pathophysiologic mechanisms for the development of peptic ulcer in patients taking NSAIDs. The main mechanism is inhibition of cyclooxygenase (COX) (mainly COX-1) by NSAIDs, followed by decreased

production of prostaglandin, which is responsible for mucosal protection [24]. Direct mucosal injury by NSAIDs and increased inflammatory response by activation of lipoxygenase (LOX) are suspected as another mechanism.

Risk factors for the development of peptic ulcer in NSAID users are past medical history of peptic ulcer or complications, higher dose, long-term use, old age, other comorbidities, and co-administration of steroid or anticoagulants [25–27]. NSAID use and *H. pylori* infection are independent risk factors for the development of peptic ulcer. Patients with both risk factors are known to have a higher risk for the development of peptic ulcer.

19.3.3 Non-*H. pylori*, Non-NSAID Ulcer

Attention to idiopathic or non-*H. pylori*, non-NSAID peptic ulcer is increasing. This is because the recurrence rate is high (35 % within 2 years, 42 % within 7 years) in non-*H. pylori* peptic ulcer patients and the definite cause is not identified in 28 % of non-NSAID ulcer patients [12–14, 28].

The prevalence is estimated as 16.2–35.7 % in Korean studies, although the geographical difference [12, 28, 29]. The presumptive causes are as follows: increased gastric acid secretion; smoking; malignancy; use of antiplatelet agents (clopidogrel, ticlopidine, prasugrel, etc.), potassium chloride, and bisphosphonate; cytomegalovirus infection; Herpes simplex virus infection; tuberculosis; syphilis; portal hypertension; Behcet's disease; stress; and other comorbidities [30–35]. Although suspected causes are listed above, the possibility of false-negative results for *H. pylori* infection should be considered, and unrecognized use of NSAIDs should be excluded through the meticulous history taking.

Among the causative factors of peptic ulcer development, the frequency is relatively low in this group of patients. However, more investigation is needed for these patients, because they usually need sustained antiulcer treatments.

19.4 Clinical Features

Patients with peptic ulcer show gastrointestinal symptoms, such as abdominal pain or dyspepsia. However, no symptom is known to be sensitive or specific for the diagnosis or prediction of complications of peptic ulcer. Especially, patients with silent ulcer who have decreased visceral sensitivity show no symptoms. Thus, diagnosis or making a decision of diagnostic procedure based on clinical symptoms has limitations [36].

The abdominal pain can be heartburning or colicky. It could accompany reflux symptom or dyspepsia. The symptom of duodenal ulcer develops in the acid secretion state without food buffer. Ingested food is expelled from the stomach within 2–3 h, and gastric acid secretion is sustained for 3–5 h after food intake. Thus, symptom of duodenal ulcer usually develops 2–5 h after food intake. The characteristic symptoms are midnight abdominal pain when the gastric acid secretion is active or pain relieved by food or antacid intake. In contrast to the duodenal ulcer, patients with gastric ulcer show abdominal pain lasting for 1–2 h after food intake and relieving by food passage from the stomach.

19.5 Diagnosis

Endoscopic examination is recommended for the patients with clinical symptoms, risk factors, or who suspected for the development of complications of peptic ulcer (Fig. 19.1). Gastric cancer is prevalent in East Asia including Korea. Early detection and treatment by endoscopic resection in the specific subset of early gastric cancer have shown similar treatment outcome with surgery. Gastric ulcer should be followed up by endoscopic examination, and malignancy should be excluded by histologic examination, because of similar morphologic life cycle of early gastric cancer [37] (Table 19.1). Advanced gastric cancer shows irregular appearance (relatively irregular edge and margin), but differential diagnosis is sometimes necessary in Borrmann type II or III categories [38]. In addition to the diagnosis of peptic ulcer by endoscopy, history taking for

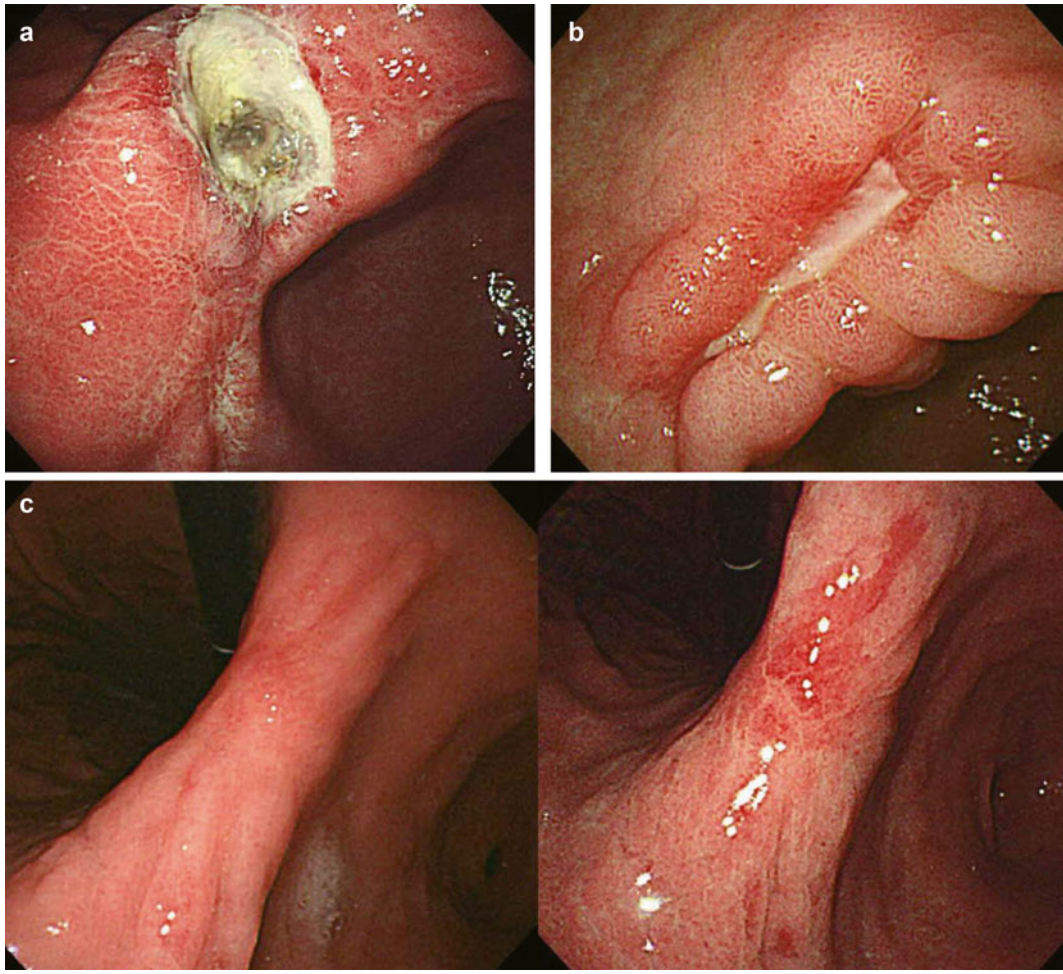

Fig. 19.1 Endoscopic feature of peptic ulcer. (**a**) Active stage gastric ulcer on angle which has regular margin and edge with hemorrhagic stigmata on the base of ulcer. (**b**) Healing stage gastric ulcer on angle with round edges and regular regenerative epithelium. (**c**) Scar stage gastric ulcer on angle (*left*, *white scar*; *right*, *red scar*)

Table 19.1 Endoscopic morphology of benign ulcer and malignancy

	Peptic ulcer	Malignancy
Folds	Regular folds that extend to the margins of the base	Prominent folds that do not extend to the margins
	Radiation of smooth, symmetric folds to the ulcer crater	Interrupted, nodular, cutting, fused, or clubbing-shaped folds
Margin	Smooth margin with round edges, regular regenerative epithelium, and normal surrounding mucosa	Overhanging, irregular, thickened, nodular, asymmetrical hyperemic, discolored or moth-eaten margin
Base	Flat and regular base	Necrotic or hemorrhagic base with some islands of regenerative epithelium

the causative factors, such as NSAID intake and tests for *H. pylori* infection, are needed.

19.6 Treatment

The aim of treatment is symptomatic relief and prevention of complications and recurrence with the healing of ulceration. Medical treatments are a mainstay, but complications usually need additional endoscopic management. Proton pump inhibitor (PPI), which is the most potent acid secretion blocker of the gastric parietal cell, is used. Antacid and mucosal protective agents are also used for the treatment of peptic ulcer. A total of 4–8 weeks of PPIs are used, including an eradication period for the treatment of peptic ulcer. Six to 8 weeks for gastric ulcer and 4–6 weeks for duodenal ulcer are generally recommended for the treatment period of peptic ulcer [39]. Duodenal ulcer without complications is not recommended for the sustained use of PPI. However, in cases of peptic ulcer with complications, sustained use of PPI is recommended [40].

Removal of causative factors is essential for the treatment and prevention of complications. *H. pylori* eradication is the most effective management for the healing of ulcer and prevention of recurrence in the *H. pylori*-associated peptic ulcer. In the NSAID-induced peptic ulcer, medical managements with stopping NSAIDs are recommended. In cases when discontinuing NSAIDs is impossible, combination of PPI or misoprostol with NSAIDs is recommended, and PPI showed better efficacy than misoprostol [41].

In cases of NSAID-induced peptic ulcer, the risk of peptic ulcer complications is increased when the *H. pylori* infection is present. On the contrary, there have been reports about the beneficial effect of *H. pylori* infection in patients with NSAID-induced peptic ulcer through increasing COX-2 in the gastric mucosa, which is responsible for the mucosal healing [42, 43]. Therefore, *H. pylori* eradication is recommended after healing of ulcers in patients with NSAID-induced gastric ulcer [39].

Stopping antiplatelet agent, including aspirin, prevents complications of peptic ulcer. However, it could induce or aggravate cardiovascular or cerebrovascular events. Therefore, balancing risk and benefit of continuing or stopping medication and personalized evaluation are important [27].

In the non-*H. pylori*, non-NSAID peptic ulcers, the possibility of *H. pylori* infection should be considered, and unrecognized use of NSAIDs should be evaluated. PPI is usually used for 4–8 weeks. The treatment response is relatively low in this group of patients. Therefore, sustained treatments are frequently required [44, 45].

Refractory ulcer is defined as nonresponse to 8–12 weeks of antisecretory treatments (12 weeks for gastric ulcer and 8 weeks for duodenal ulcer). Adherence to drug, smoking history, use of NSAIDs, Crohn's disease, Zollinger-Ellison syndrome, and other comorbidities should be evaluated. Follow-up endoscopic examination with histology to exclude malignancy is important.

19.7 Complications

The complications of peptic ulcer, including hemorrhage, perforation, penetration, and gastric outlet obstruction, develop in 25% of patients with peptic ulcer [46]. In contrast to the decreasing prevalence of peptic ulcer, the admission rate from complications of peptic ulcer is reported to be constant or increasing [4, 5]. Peptic ulcer hemorrhage is the most frequent complication, and perforation, gastric outlet obstruction, and penetration follow in the order. *H. pylori* infection and NSAID use are also risk factors, and non-*H. pylori*, non-NSAID peptic ulcer is drawing attention for the developing complications of peptic ulcer.

19.7.1 Gastroduodenal Hemorrhage

Upper gastrointestinal hemorrhage is the most frequent complication and develops in 15–20% of patients with peptic ulcer [46, 47]. In contrast to the decreasing trend of complications of organic perforation or gastric outlet obstruction, upper gastrointestinal hemorrhage from peptic ulcer is increasing, mainly due to the wide use of NSAIDs or aspirin in elderly patients [47].

Hematemesis, melena, or hematochezia is the frequent complaint. However, meticulous history taking and physical examination are important because 20 % of elderly patients taking NSAIDs showed silent peptic ulcer hemorrhage without definite symptoms [48].

Risk stratification system guides to classify the high rebleeding risk lesions and how to treat the culprit lesions. Blatchford (Table 19.2) or AIMS65 score (Table 19.3), based on laboratory results and clinical features, or Rockall score (Table 19.4) including results of endoscopic examination can be used [9–11]. Forrest classification system (Table 19.5) is the most frequently used tool for the prediction of rebleeding and determination of endoscopic treatments [15]. Gastric lavage with cold saline through nasogastric tube had been used for the diagnosis of active hemorrhage, clearance of endoscopic view, and hemostasis. However, it is no longer recommended because it could delay the endoscopic procedure and diagnostic sensitivity of gastric lavage is low [12–14]. Moreover, endoscopic view can be obtained with changing position of patients [12–14].

Stabilization of vital sign and stopping causative factors, such as NSAIDs, are the first step of treatment. Intravenous PPI is injected for the ulcer healing and prevention of rebleeding,

Endoscopic hemostasis includes local injection, mechanical hemostasis, thermal coagulation, and local infusion therapy. No single treatment is known to be more effective than the other remaining managements. Combinations of hemostatic treatments are known to be more effective than single treatment in patients with active hemorrhage [16, 17].

The hemostasis success rate is reported to be over 90 %, but rebleeding also develops in 10–25 % of patients [7]. NSAID discontinuation should be confirmed, and all patients should be tested for the *H. pylori* infection. If *H. pylori* infection was confirmed, the eradication therapy should be followed for the prevention of rebleeding [18]. If impossible to stop the aspirin, combination of PPI with aspirin is recommended [19, 20].

Second-look endoscopic examination, which is performed within 24 h from the first hemostasis, has not shown the beneficial effect in overall mortality and operation rate, although it should be performed when the hemostasis was incomplete or vital sign is unstable [7, 21].

Surgical treatment should be considered when the hemorrhage recurs despite of repeated attempts of endoscopic hemostasis or when the

Table 19.2 Blatchford scoring system

Parameter	Score value
Blood urea nitrogen (BUN)	
<18.2 mg/dL (<6.5 mmol/L)	0
18.2≤ BUN <22.4 mg/dL (6.5≤ BUN <8 mmol/L)	2
22.4≤ BUN <28 mg/dL (8≤ BUN <10 mmol/L)	3
28≤ BUN <70 mg/dL (10≤ BUN <25 mmol/L)	4
70 mg/dL ≤ (25 mmol/L ≤)	6
Hemoglobin (Hb) (male)	
13 g/dL ≤(130 g/L ≤)	0
12≤ Hb <13 g/dL (120≤ Hb <130 g/L)	1
10≤ Hb <12 g/dL (100≤ Hb <120 g/L)	3
Hemoglobin (Hb) (female)	
12 g/dL ≤(120 g/L ≤)	0
10≤ Hb <12 g/dL (100≤ Hb <120 g/L)	1
Hemoglobin (Hb) (male or female) <10 g/dL (<100 g/L)	6
Systolic blood pressure (SBP)	
110 mmHg ≤	0
100–109 mmHg	1
90–99 mmHg	2
<90 mmHg	3
Other markers	
Pulse ≥100/min	1
Melena at presentation	1
Syncope at presentation	2
Hepatic disease present	2
Cardiac failure present	2

Table 19.3 AIMS 65 scoring system

Parameter	1 point for each parameter	Alternative description
Albumin	<3.0 g/dL (30 g/L)	
INR	>1.5	
Mental status	Glasgow coma score <14	Disorientation, lethargy, stupor, or coma
Systolic blood pressure	<90 mmHg	
Age	>65	

Table 19.4 Rockall scoring system

	Score			
Variable	0	1	2	3
Pre-endoscopy				
Age	<60	60–79	≥80	
Shock	No shock with systolic BP ≥100 mmHg and pulse <100/min	Tachycardia (pulse ≥100/min) with systolic BP ≥100 mmHg	Hypotension with systolic BP <100 mmHg	
Comorbidity	No major		Cardiac failure, ischemic heart disease, any major comorbidity	Renal failure, hepatic failure, disseminated malignancy
Post-endoscopy				
Diagnosis	Mallory-Weiss tear, no major lesions, no stigmata of recent hemorrhage	All other diagnosis	Upper gastrointestinal tract malignancy	
Major stigmata of recent hemorrhage	None (or dark area only)		Blood in upper gastrointestinal tract (adherent clot, visible or spurting vessel)	

Table 19.5 Forrest classification

Classification and description
Acute hemorrhage
Class Ia – spurting hemorrhage
Class Ib – oozing hemorrhage
Signs of recent hemorrhage
Class IIa – nonbleeding visible vessel
Class IIb – adherent clot
Class IIc – flat pigmented spot
Lesions without active bleeding
Class III – clean ulcer base

vital sign is unstable [22]. Angiographic embolization showed comparable efficacy to surgery in the prevention of rebleeding and mortality, and it could be substituted for surgery when the surgical risk of patient is too high [23]. Malignancy should be considered if the ulcer healing is incomplete, and endoscopic biopsy should be performed for this lesion to exclude the malignancy, if it was not massive hemorrhage [6].

19.7.2 Intestinal Perforation

Intestinal perforation occurs in 2–10 % of peptic ulcer, and it is the most common cause of operation in patients with peptic ulcer [24]. Anterior wall of duodenal bulb is the most common site (60 %), and the antrum (20 %) and lesser curvature side of gastric body (20 %) follow [6, 12]. However, gastric ulcer perforation is reported to be increasing with the wide use of NSAIDs [6, 12]. Typical symptoms and signs of peritonitis or septic shock could be observed. However, it could be alleviated in elderly, diabetic patients or patients taking steroid, immunosuppressant, and narcotic analgesics [6, 25]. The symptom onset of retroperitoneal perforation is relatively late, and typical signs are not present [26]. History taking and physical examination are important, and detecting free air in X-ray could direct to the diagnosis. However, there is no free air in 10–20 % of duodenal ulcer perforation [27, 28]. Therefore, additional study, such as computed tomography, ultrasound, or upper gastrointestinal contrast study, is frequently needed [27, 28].

After making a diagnosis, salvage managements including nasogastric tube drainage, saline infusion, PPI, and wide-spectrum antibiotics are started [6]. Bacterial peritonitis from free perforation is the indication of emergency operation [25]. Surgical methods and resection area could be changed according to the perforation site. If clinical features are stable, nonsurgical management could be considered.

The mortality rate of intestinal perforation from peptic ulcer was reported to be 6–14 %, and

Table 19.6 American Society of Anesthesiologists (ASA) physical status classification system

Classification	Description
ASA 1	Healthy patients
ASA 2	Mild to moderate systemic disease caused by the surgical condition or by other pathological processes and medically well controlled
ASA 3	Severe disease process which limits activity but is not incapacitating
ASA 4	Severe incapacitating disease process that is a constant threat to life
ASA 5	Moribund patient not expected to survive 24 h with or without an operation
ASA 6	Declared brain-dead patient whose organs are being removed for donor purposes

delay of diagnosis and treatment, comorbidities, NSAID use, and old age (>70 years) were proved to be risk factors associated with mortality [7, 30]. Various risk stratification systems for the prediction of prognosis have been developed. Boey score and ASA score (Table 19.6) are the most frequently used scoring system, but the usefulness of each method is different from study [31].

19.7.3 Gastric Outlet Obstruction

Gastric outlet obstruction refers to the intestinal obstruction of prepyloric area, duodenal bulb, or post-bulbar area. Malignancy is the most common etiology taking 66 % of all causes, and peptic ulcer is the most common cause among the benign etiologies taking 5–8 % of all causes [6, 25, 34]. The mechanism of development is inflammation, edema, and fibrosis from repeated ulceration. Nausea, vomiting, early satiety, and weight loss are a frequent complaint.

Endoscopy or contrast study is used for the diagnosis. Malignancy should be excluded before the initiation of treatment. Computed tomography or endoscopic ultrasound can be used for the exclusion of malignancy and measuring the stenotic lesion. Balloon dilatation using the endoscopic instrument is used for the treatment, and only focal stenotic lesions could be managed. For the prevention of restenosis after balloon dilatation, steroid injection can be performed using endoscopic ultrasound [34]. Obstructive lesion should be observed before the therapeutic procedure, and active ulcer should be managed with antiulcer medication or *H. pylori* eradication. Mechanical dilatation without the management of treatable ulcer cannot achieve the satisfactory results [34]. Balloon dilatation is repeated approximately every 1–3 weeks interval. The definite endpoint of treatment is not decided. According to one study, endpoint of 41 Fr. balloon and 15 mm diameter of gastric outlet showed the 80 % of immediate symptomatic relief [35]. Graded or stepwise approach is used in every single procedure (8 → 10 mm, 10 → 12 mm, 12 → 15 mm).

Treatment outcome is reported to be various, but the overall long-term therapeutic outcome is estimated over 75 %, if *H. pylori* eradication, PPI use, and stopping NSAIDs were accomplished [34]. Cautious interpretation is also needed because some of the studies did not include *H. pylori* eradication or prescription of PPI in the treatment, and various therapeutic indicators, such as symptomatic relief or recurrence of gastric outlet obstruction, were used for the determination of therapeutic efficacy. Short-term efficacy showed satisfactory results, but repeated procedure is usually needed. The development of intestinal perforation is the most serious complication. Patients with hematologic coagulopathy, cardiovascular diseases, or acute stage ulcer that can lead to the perforation are not recommended for the procedure. Nonresponders to medication or patients with recurrent gastric outlet obstruction should be considered for surgical management.

19.7.4 Penetration and Fistula

The penetration from peptic ulcer develops when the ulcer progress to the direction of organic axis without the development of perforation. The nature, location, and duration of pain changes with the development of penetration and patients with penetration usually have a history of long-standing

peptic ulcer [32]. The penetration can be made in the near organs, such as pancreas, liver, biliary tract, colon, or vessels [33]. The penetration made between the gastric antrum to bulb of duodenum and located near the normal pylorus is called double pylorus [33]. The diagnosis is made through the endoscopic or radiologic examination [33]. Treatment includes PPI, antiulcer medications, *H. pylori* eradication, and stopping NSAIDs and surgical managements are only needed in cases with serious conditions.

Conclusions

Peptic ulcer is decreasing with the use of PPI and decreasing prevalence of *H. pylori* infection. However, complications of peptic ulcer are still common in the clinical practice. Early detection and diagnosis with endoscopic and radiologic examination leads to the high treatment success and low recurrence rate. Multidisciplinary approach of treatments including medications, endoscopic procedures, angiographic hemostasis, and surgical managements is important for the treatment of peptic ulcer and its complications. Further studies about non-*H. pylori*, non-NSAID ulcer will be useful for the prevention of complications and recurrence of peptic ulcer.

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Gastric Extranodal Marginal Zone B-Cell Lymphoma of MALT

20

Yoon Jin Choi

Abstract

Low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma occurs worldwide and is mostly found in gastric antrum and gastric body. Endoscopic features vary from shallow ulcer to simple color change of mucosa, nodularity, or mass. Low-grade gastric MALT lymphomas with such nonspecific endoscopic findings are often difficult to differentiate from benign diseases. Low-grade gastric MALT lymphoma is strongly associated with *Helicobacter pylori* (*H. pylori*). *H. pylori* infection rate has been reported to be as high as 90 % among patients with low-grade gastric MALT lymphoma. Therefore, diagnosis and treatment of *H. pylori* infection is the first step in the management of gastric MALT lymphoma independent of the stage of disease. *H. pylori* eradication has now become the treatment of choice for low-grade gastric MALT lymphoma, and the remission rate of stage I disease has reached almost 80 %. Although most remission occurs within 6–12 months after *H. pylori* eradication, it takes more to be completely healed for some cases. Therefore, the time point of diagnosing complete remission and choosing future treatment option when the eradication is not led to the tumor regression are not clearly established yet.

Keywords

Helicobacter pylori • Mucosa-associated lymphoid tissue (MALT) • MALT lymphoma

20.1 Introduction

Primary gastric lymphoma is a rare disease accounting 1–7 % of gastric malignant tumors, but accounts 60 % among gastrointestinal tract lymphoma. The prevalence of primary gastric lymphoma is increasing compared to the

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decreasing prevalence of gastric adenocarcinoma. Around 80 % of primary gastric lymphoma cases are B-cell non-Hodgkin lymphoma, which is divided by mucosa-associated lymphoid tissue (MALT) lymphoma and high-grade diffuse large cell lymphoma. In 1983, Isaacson and Wright first established the concept of mucosa-associated lymph originating from MALT, and the term MALT lymphoma became widely used since then [1]. The gastrointestinal (GI) tract is the most frequent site of MALT lymphoma, and the stomach is involved in up to two-thirds of these cases. It sometimes occurs in the ocular adnexa, lung, urinary tract, thyroids, and gall bladder other than the GI tract. Low-grade gastric MALT lymphoma was revealed to be caused by *Helicobacter pylori* (*H. pylori*) as an immune response on the gastric mucosa. Patients with low-grade gastric MALT lymphoma are reported to have very high *H. pylori* infection rate up to 90 %.

Since low-grade gastric MALT lymphomas have nonspecific endoscopic findings, it is often difficult to differentiate from benign diseases. The author, herein, reviewed the clinicopathologic characteristics, diagnosis, treatment, and prognosis.

20.2 Pathogenesis of Gastric MALT Lymphoma

20.2.1 *H. pylori*

Low-grade MALT lymphoma of the stomach is strongly associated with *H. pylori* infection. Up to 90 % of patients with gastric MALT lymphoma are *H. pylori* positive [2]. Although there is no lymphoid tissue in normal mucosa, infection with *H. pylori* triggers proliferation of T cells and B cell by antigen presenting eventually resulting in the formation of acquired lymphoid tissues [3]. The bacteria directly contacts with gastric epithelial cells, and this promotes to the production of multiple inflammatory cytokines, such as interleukin (IL)-1ß, IL-2, IL-6, IL-8, and tumor necrosis factor (TNF)-α. Among them, it is believed that IL-8 is the most important mediator for the inflammatory reaction in mucosa [4]. Cells in the MALT lymph tissue proliferate leading to lymphoid follicular hyperplasia. Activated cells are transformed into tumor cells and finally result in malignant lymphoma through cytogenetic changes, chromosomal abnormality, and induction of autoimmunological clones or dysregulation of apoptosis. Persistent infection with *H. pylori* causes chronic gastritis that, in some people, can develop into more organized gastric MALT with the histological and functional similarities to Peyer's patches of the small intestine [5]. In the earliest stage, the neoplastic cells (sometimes known as centrocyte-like cells) adopt a perifollicular distribution, but the infiltrate extends into the lamina propria away from the follicles and this may be a helpful diagnostic feature. The neoplastic cells infiltrate into gastric gland epithelium causing eosinophilic change to the epithelial cells and destruction of the architecture (lymphoepithelial lesion).

The neoplastic cells have variable morphology including mature round lymphocyte cells resembling germinal center centrocytes with irregular nuclei, cells with monocytoid/marginal zone B-cell appearance, and cells with lymphoplasmacytic appearances. All cases have a variable number of large transformed cells. However, when large neoplastic cells are present in sheets, the diagnosis of an associated diffuse large B-cell lymphoma (DLBCL) should be made. The clinical relevance of distinguishing two subgroups of diffuse large cell lymphomas of the stomach according to the presence or absence of a low-grade MALT component is still a matter of debate [6].

20.2.2 Genetic Variation

Several genetic changes are implicated in the development of gastric MALT lymphoma. Translocations t(11;18)(q21;q21), t(1;14)(p22;q32), and t(14;18)(q32;q21) are most frequently found in MALT lymphoma [7]. Importantly, nuclear factor kappa B (NF-κB) is known to mediate cell survival and antiapoptotic signals [8], it has been speculated that its upregulation may contribute to the malignant

transformation of *H. pylori*-independent growth of MALT lymphomas with refractory to anti-*H. pylori* therapy [9].

Most common genetic variation is t(11;18)(q21;q21), which can be found 15–24 % of MALT lymphoma [10]. Translocation t(11;18)(q21;q21) fuses the N-terminus of the *API2* gene to the C-terminus of the *MALT1* gene and generates a functional API2-MALT1 fusion product [9] (Fig. 20.1). The chimeric protein of t(11;18)(q21;q21) itself leads to constitutive NF-κB activity through self-oligomerization of the baculovirus IAP repeat (BIR) domain of the API2 molecule. This translocation is a specific marker for *H. pylori* independence of low-grade gastric MALT lymphoma [11].

Another important genetic aberration, t(1;14)(p22;q32), which is found in less than 5 % of MALT lymphomas, juxtaposes the *BCL10* gene of chromosome 1p to the immunoglobulin gene locus of chromosome 14q. This results in strong expression of a truncated BCL10 protein in the nuclei and cytoplasm [12]. BCL10 is an intracellular protein that positively regulates lymphocyte proliferation by linking antigen receptor stimulation to activate NF-κB signaling. Finally, translocation t(14;18)(q32;q21) is only rarely found in gastric MALT lymphoma [13]. Besides these translocations, it has been recently reported that chromosome 6q was frequently deleted in ocular marginal zone B-cell lymphoma and identified *TNFAIP3/A20*, a negative regulator of NF-κB pathways, as the primary target for 6q deletion [14]. However, this has been found in only 7 % of gastric MALT lymphoma, and further study about the association of this genetic alteration and gastric MALT lymphoma is needed.

20.3 Clinical Feature and Diagnosis

20.3.1 Clinical Characteristics and Endoscopic Features

Low-grade gastric MALT lymphoma occurs over a wide range of age, but most common from 55 to 60 years old [15]. The sex ratio incidence is essentially equal [15]. It is usually localized on the antrum and body but can occur everywhere in the stomach [16]. The symptoms are absent or vague in most patients with early-stage low-grade lymphoma. Therefore, gastric MALT lymphoma is sometimes diagnosed incidentally following an upper endoscopy performed for regular cancer-screening program. Similarly, various and nonspecific endoscopy findings have been described for low-grade gastric MALT lymphoma. It is frequently characterized simply by erosions, small nodules, and thickening of gastric folds, generally suggesting a benign condition, or even by normal gastric mucosa. Although there is no consensus for the endoscopic classification of gastric MALT lymphoma, it can be divided to exophytic type, ulcero-infiltrative type, superficial type, and others [17]. By using the classification of Yokoi et al. [18], superficial lesions could be grouped into six categories: IIc-like type, submucosal tumor type, multiple erosion type, cobblestone-mucosa type, partial-fold swelling type, and discoloration type (Fig. 20.2). In Korea, superficial type is most common. Erosions or ulcers in gastric MALT lymphoma often seem irregular, geographical, or multiple unlike the usual benign ulcers or cancers which show a centripetal finding. However, such a neoplasia can be detected

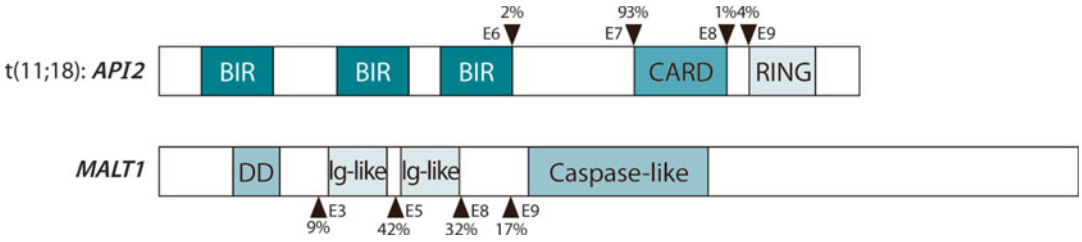

Fig. 20.1 Representation of the *API2* and *MALT1* genes involved in t(11;18). *Vertical arrows* represent the known breakpoints that give rise to the *API2-MALT1* fusion and their incidence in percentages. *B1R* baculovirus IAP repeat, *CARD* caspase recruitment domain, *Ring* really interesting new gene, *DD* death domain

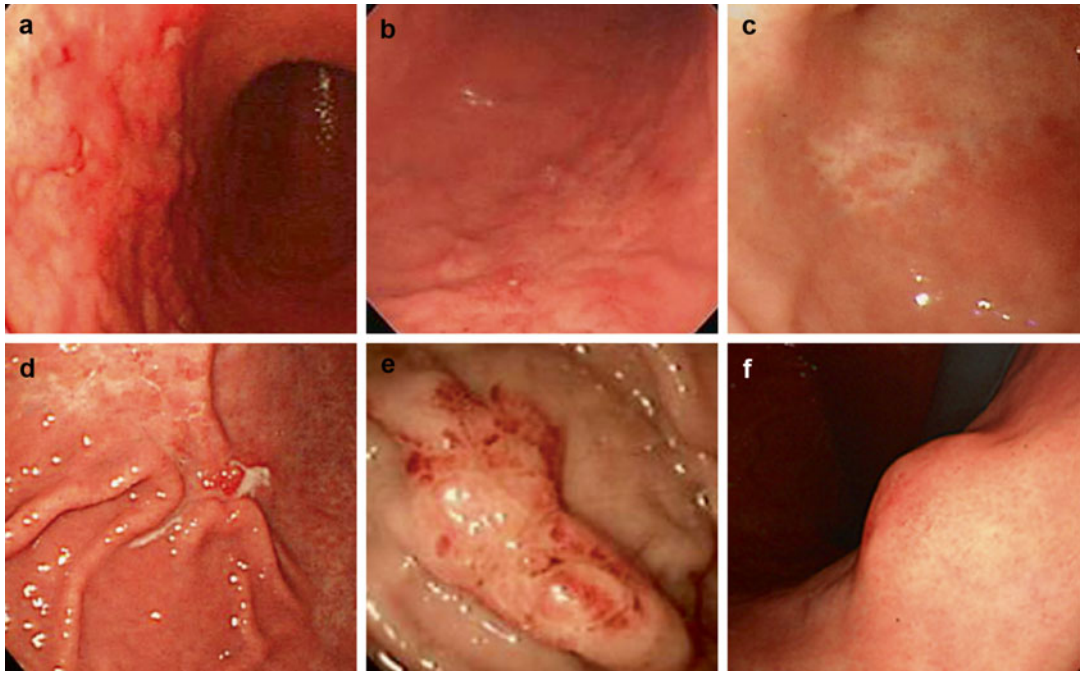

Fig. 20.2 Various endoscopic findings of low-grade gastric MALT lymphoma. (**a**–**f**) Superficial lesions: (**a**) multiple erosion type, (**b**) nodularity (or cobblestone-mucosa type), (**c**) discoloration type, (**d**) IIc-like type, (**e**) partial-fold swelling type, (**f**) submucosal tumor type (Adapted from Choi et al. [16])

on normal-appearing mucosa with solely patch discoloration or mild nodularity. The concordance of the first endoscopic impression and histological diagnosis is less than 80 % [19]; thus, multiple biopsies of any suspicious lesions including surrounding normal mucosa should be done for the diagnosis of the disease. In cases where gastric MALT lymphoma is suspected but insufficient or inadequate biopsy specimens have been received initially, a second endoscopy could be necessary.

20.3.2 Studies for Diagnosis

Lymphoepithelial lesions represent the histomorphological characteristic of gastric MALT lymphoma [20] (Fig. 20.3a, b). Immunophenotypically, these cells express pan-B-cell markers, CD20, while they show no immunoreactivity for CD5, CD10, and CD11c (Fig. 20.3c). Staining for Ki67 may help in identifying large cell components [21]. There is a widely accepted consensus that the diagnosis of gastric MALT lymphoma is based on histomorphological criteria according to the WHO classification [22] (Table 20.1).

Although gastric MALT lymphoma has an indolent course with a low morbidity and mortality, an accurate staging procedure is mandatory. It has been found that low-grade gastric lymphomas diagnosed in an advanced stage (III or IV) in nearly 10 % of cases, with localization in both lymph nodes and other organs, particularly the bone marrow, lungs, and liver [23]. Therefore, comprehensive staging procedure, with a complete physical examination including Waldeyer's ring; routine laboratory tests (including lactate dehydrogenase and β2-microglobulin); computed tomography of the chest, abdomen, and pelvis, endoscopic ultrasonography; and bone marrow biopsies are mandatory. Indeed, bone marrow involvement (stage IV) has been reported in up to almost 10 % of cases, requiring chemotherapy. However, since the bone marrow biopsies were a

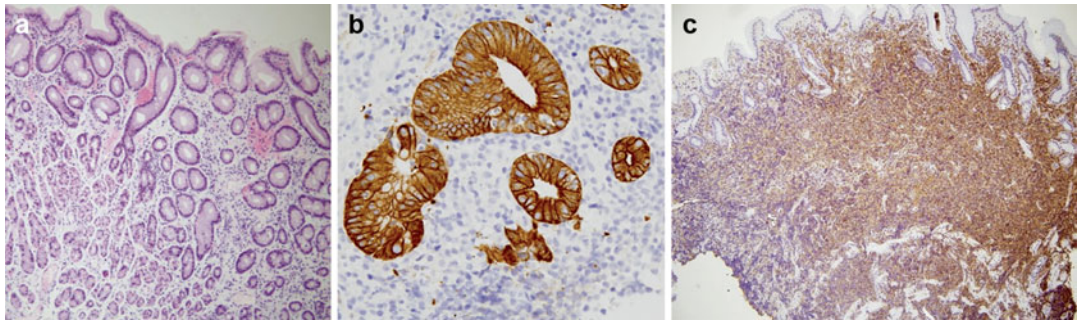

Fig. 20.3 Infiltration patterns of gastric mucosa-associated lymphoid tissue (MALT) lymphoma. (**a**) As a hallmark of gastric MALT lymphoma, lymphoepithelial lesions were detected in H&E staining. (**b**) Lymphoepithelial lesions stained by cytokeratin (*brown*). (**c**) Diffuse stain with pan-B-cell markers CD20 immunohistochemistry (*brown*)

Table 20.1 Histological scoring system for gastric MALT lymphoma (WHO/Wotherspoon score)

Score	Diagnosis	Histological features
0	Normal	Scattered plasma cells in lamina propria. No lymphoid follicles
1	Chronic active gastritis	Small clusters of lymphocytes in lamina propria
		No lymphoid follicles. No lymphoepithelial lesions
2	Chronic active gastritis with florid lymphoid follicle formation	Prominent lymphoid follicles with surrounding mantle zone and plasma cells
		No lymphoepithelial lesions
3	Suspicious lymphoid infiltrate, probably reactive	Lymphoid follicles surrounded by small lymphocytes that infiltrate diffusely in lamina propria and occasionally into epithelium
4	Suspicious lymphoid infiltrate, probably lymphoma	Lymphoid follicles surrounded by marginal zone cells that infiltrate diffusely in lamina propria and into epithelium in small groups
5	MALT lymphoma	Presence of dense infiltrate of marginal zone cells in lamina propria with prominent lymphoepithelial lesions

Adapted from Wotherspoon et al. [22]

quite invasive procedure compared to other imaging tests, performing the test in all of the diagnosed cases is not easy in practice. When the advanced stage is suspected by other studies or large cell compound is found, the bone marrow biopsy should be considered.

The endoscopic ultrasound (EUS) allows to accurately assessing both the infiltration of lymphoma in the gastric wall and the regional lymph nodes involvement [24]. The depth of invasion into the different layers of gastric walls is a predictive factor of lymphoma remission following the therapy.

20.3.3 Staging

The histological criteria for the diagnosis of gastric MALT lymphoma are using the 5-grade system (Table 20.1) which Wotherspoon et al. reported [22]. Based on the system, the lesion with a score of 5 is defined as MALT lymphoma, but lesions with a score of 3 or 4 are defined as MALT lymphoma when the monoclonality is demonstrated. For staging, the Ann Arbor classification with Musshoff's modification [25] is still used (Table 20.2), but the clinical relevance of TNM stage has not been validated.

Table 20.2 Ann Arbor classification of extranodal lymphoma

IE: Lymphoma restricted to GI tract on one side of diaphragm
IE1: Infiltration limited to mucosa and submucosa
IE2: Lymphoma extending beyond submucosa
IIE: Lymphoma additionally infiltrating LNs on same side of diaphragm
IIE1: Infiltration of regional LNs (compartment I+II)
IIE2: Infiltration of intra-abdominal distant LNs
IIIE: Lymphoma infiltrating the GI tract and/or LNs on both sides of the diaphragm
IV: Diffuse or disseminated infiltration of distant or extra-gastrointestinal organs

Modified from Musshoff and Schmidt-Vollmer [25]
GI gastrointestinal, *LN* lymph nodes

20.4 Therapeutic Options

The goal of gastric MALT lymphoma should always be a complete cure. *H. pylori* infection causes most cases of gastric MALT lymphoma. Therefore, diagnosis and treatment of *H. pylori* infection is the first step in the management of gastric MALT lymphoma independent on the stage of disease [21].

20.4.1 Anti-*H. pylori* Therapy

20.4.1.1 Indication and the Effect

For stage I and II diseases, *H. pylori* eradication is the initial treatment of choice, with a curative intent. According to a recent meta-analysis with 1,408 patients from 32 studies, 77.5 % of patients with gastric MALT lymphoma achieve complete regression after successful eradication of *H. pylori*. A relapse occurred in 7.2 % of patients (2.2 % per year) [2]. High-grade transformation into aggressive lymphoma was very rare (0.05 %) [2]. Thus, *H. pylori* eradication is now regarded as the first-line treatment for gastric MALT lymphoma. Regimens for the anti-*H. pylori* therapy can be chosen based on the condition of each region or country. In a pooled data analysis, Zullo et al. reported a success rate of first-line eradication therapy as 91 % [26]. Including second- to fifth-line treatment protocols, the eradication rate was 98.3 % [26].

20.4.1.2 The Predictive and Prognostic Factors for Regression After Anti-*H. pylori* Therapy

Different predictive factors of lymphoma remission have been found, including stage, depth of penetration in the gastric wall, *API2-MALT1* translocation status, or different ethnic. Complete regression of lymphoma was more often observed in stage I than in stage II tumors (78.4 % vs. 55.6 %) and was more common in mucosal and submucosal lesion than in lesions beyond the submucosal layer [2]. The presence of *API2-MALT1* translocation in lymphoma cells reduces a patient's probability of remission to less than 25 % [2]. Having t(11;18)(q21;q21) was a predictive factor for nonresponsiveness for *H. pylori* eradication in our previous study [27]. Patients with a major neoplastic lesion in the proximal stomach have a significantly lower remission rate than those with neoplasms of the distal stomach. Remission after eradication of *H. pylori* is more common in Asian than in European populations (84 % vs. 55 %). In summary, when the lesion is stage IE1 without the translocation of t(11;18)(q21;q21), more than 80 % of probability of remission after eradication of *H. pylori* could be expected.

20.4.1.3 Judgments of the Response to Treatment

It takes time for the neoplasia to disappear and be replaced with normal mucosa. There has been no definite consensus about how often the patients with gastric MALT lymphoma undergo upper gastrointestinal endoscopy after anti-*H. pylori* therapy. The remission is usually achieved 3–12 months after termination of anti-*H. pylori* therapy. Therefore, it can be recommended that the first follow-up endoscopy could be done 3–6 months after termination of the treatment, and it can be repeated every 4–6 months until the remission is demonstrated [21, 28].

Table 20.3 GELA histological grading system for posttreatment evaluation of gastric MALT lymphoma

Category	Lymphoid infiltrate	LEL	Stromal changes
Complete histological remission (ChR)	Absent/almost absent	Absent	Normal
Probable minimal residual disease (pMRD)	Aggregates of lymphoid cells in the LP/MM/SM	Absent	Regression
Responding residual disease (rRD)	Dense, diffuse, or nodular	Focal LEL/absent	Regression
	Extending around glands in LP		
No change (NC)	Dense, diffuse, or nodular	Present, "may be absent"	No change

Adapted from Copie-Bergman et al. [29]
MM muscularis mucosa, *LP* lamina propria, *SM* submucosa, *LEL* lymphoepithelial lesions

Same as the initial diagnosis, the histological evaluation is also essential for diagnosing remission or residual disease in posttreatment lesions. In order to standardize the histological criteria for evaluating lymphoma response to therapy, pathologists from the Groupe d'Etude des Lymphomes de l'Adulte (GELA) established an ad hoc histological grading system, named the GELA score [29]. Based on these criteria, the posttreatment lesions were divided into four categories: complete histological remission, probable minimal residual disease, responding residual disease, and no change [29] (Table 20.3). Therefore, careful biopsies both in the mucosa where the lesion used to be and in surrounding normal mucosa are still needed during the follow-up endoscopy in patients with endoscopic remission.

20.4.1.4 Long-Term Outcome After Successful Eradication

Recent follow-up studies have demonstrated that *H. pylori* eradication also provides a favorable long-term outcome, with a genuine chance of cure or at least of long-lasting complete remission in the majority of patients. A study with 420 patients with low-grade gastric MALT lymphoma who had undergone successful *H. pylori* eradication and been followed up was published in Japan in 2011 [30]. The mean follow-up duration was 6.5 years. When the characteristics of subjects were analyzed, 90 % of patients had clinical stage I disease. Among 341 patients who underwent EUS, the depth of lymphoma infiltration in the gastric wall was mucosa in 52 %, submucosa in 38 %, and muscularis propria or beyond in 10 % of cases. Translocation of t(11;18)/*API2-MALT1* was positive in 30 of 206 patients (15 %) who were examined [30]. As a result, 323 patients (77 %) responded to *H. pylori* eradication, and the median period from the end of treatment to remission was 4 months. Probabilities of freedom from treatment failure, overall survival, and event-free survival after 10 years were 90 %, 95 %, and 86 %, respectively. Among finally determined 323 responders (complete histological remission and probable minimal residual disease; ChR/pMRD), lymphoma relapse was observed in 10 patients (3.1 %), and the time duration from ChR/pMRD varied from 1 to 131 months. Only one patient showed transformation into DLBCL. Consequently, patients with stage I disease who achieved complete remission after *H. pylori* eradication can be managed only by follow-up endoscopy with multiple biopsies every 1 or 2 years with the excellent long-term clinical outcome.

20.4.1.5 Histological Residual Disease

Patients not responding to eradication therapy are usually referred for radiation or sometimes for chemotherapy, immunotherapy, or combinations of these. Up to now, the same established options are offered to patients with persistent residual lymphoma infiltrates on histology ("minimal residual disease") despite successful *H. pylori* eradication and normalization of the endoscopic findings. However, there has been accumulating evidence that patients with minimal residual

disease can also have a favorable prognosis [31]. For them, oncological therapy probably means overtreatment. According to a large series of 108 patients with a stage I gastric lymphoma, who underwent *H. pylori* eradication therapy [31], residual lymphoma was still present histologically 12 months after successful *H. pylori* eradication. Despite the patients not receiving any further therapy, 35 of them (32 %) developed late (>1 year) complete remission, while 67 patients (62 %) maintained histological residuals stable during the median follow-up of almost 3 years. Patients with successful eradication of *H. pylori* and normalization of the endoscopic findings but persisting histological infiltrates can no longer be regarded as treatment failures and regular candidates for radiation or chemotherapy.

Abovementioned long-term follow-up study in Japan [30] also reported same results. Fourteen nonresponders without progressive disease have been followed up as a "watch-and-wait" strategy since they rejected further rescue treatment. In the follow-up, any of them have not showed a progressive disease. The other two patients with relapse after the eradication of *H. pylori* achieved the second remission without additional therapy 1 year after the relapse. It took more than 2 years for the ten responders to achieve the remission to the anti-*H. pylori* therapy. A recent European consensus report recommends that patients with responding residual disease or no change can be followed for up to 2 years by a "watch-and-wait" strategy [21]. It seems that "watch-and-wait" follow-up can be prolonged for more than 2 years unless progressive disease or relapsed endoscopic lesions. The decision to continue a "watch-and-wait" strategy or to start oncological treatment should be made based on multiple factors including *H. pylori* status, clinical stage, presence of t(11;18), or patients' compliance to follow-up.

20.4.2 Nonresponder to Anti-*H. pylori* Therapy

Patients revealing no change or progression of lymphoma despite successful *H. pylori* eradication should be referred for radiation or chemotherapy. Both radiation and chemotherapy are considered as curable modalities for localized gastric MALT lymphoma [21].

20.4.2.1 Radiotherapy

MALT lymphomas have been reported to be highly sensitive to radiotherapy. Unlike other mucosal tumor, most of low-grade MALT lymphomas are infiltrated within the stomach wall, and metastasis of lymph node is also confined in the perigastric area [32]. This characteristic of gastric MALT lymphoma allows the radiotherapy to be a curative option for the patients in stages I and II. For cases of stage IE and IIE1 MALT lymphoma, 5-year event-free survival rate reached 80–90 % by 36–45 Gy of the radiation [33, 34]. Moreover, radiation doses have been reduced over the past few decades. Modest dose (30.6 Gy) of radiation is highly efficacious and has fewer complications [35]. The side effects of irradiation are strongly dependent on the radiation field and radial dose. The acute side effects of radiation to the stomach mainly consist of transient anorexia, nausea, and vomiting, which can be usually managed with antiemetics and proton-pump inhibitors [36].

20.4.2.2 Chemotherapy

In other countries, chemotherapy is restricted to MALT lymphoma in disseminated stages III and IV. There is currently no generally accepted standard chemotherapy in gastric MALT lymphoma. The anti-CD20 antibody rituximab with cyclophosphamide, vincristine, and prednisone (COP); combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP); bendamustine; and fludarabine plus cyclophosphamide are possible choices [21].

20.4.3 *H. pylori*-Negative Gastric MALT Lymphoma

An initial therapeutic option with *H. pylori* eradication therapy could be attempted even in *H. pylori*-negative patients. Complete lymphoma remission following eradication therapy in some uninfected patients has been observed. A recent

systematic review, including data of 11 studies with 110 patients with low-grade gastric MALT lymphoma, showed that eradication therapy achieved complete lymphoma regression in 17 (15.5 %; 95 % confidence interval [CI], 8.7–22.2) patients, although *H. pylori* infection was initially excluded with at least three different diagnostic tests [37]. The author also reported that the two patients had reached the remission among five uninfected patients after *H. pylori* eradication [27].

It has been interpreted that either *H. pylori* infection is present, despite the negative results of all the three to five diagnostic tests (i.e., false negative) or antibiotic therapy may act against other bacteria (e.g., *H. heilmannii*), potentially involved in driving lymphoma cell proliferation.

20.4.4 Stage IIIE/IV Gastric MALT Lymphoma

Chemotherapy with or without immunotherapy should be given to these stages of disease. Although there is few evidence from the literature that *H. pylori* eradication may also cure gastric MALT lymphoma of stages III and IV, eradication therapy is now recommended in all stages to eliminate a possible immune-proliferative stimulus.

Figure 20.4 summarizes the treatment recommendations based on the European EGILS consensus report of 2011 [38].

20.5 Plans for Follow-Up After Remission

Patients having achieved complete remission after *H. pylori* eradication or oncological treatment should continue to be followed, although the exact time interval and the duration of surveillance are not yet known [21].

Experts in the clinical fields recommend annual endoscopic follow-up with biopsy. The rationale for follow-up endoscopies is to detect local relapse of MALT lymphoma relatively early; this occurs in about 7 % of cases [2].

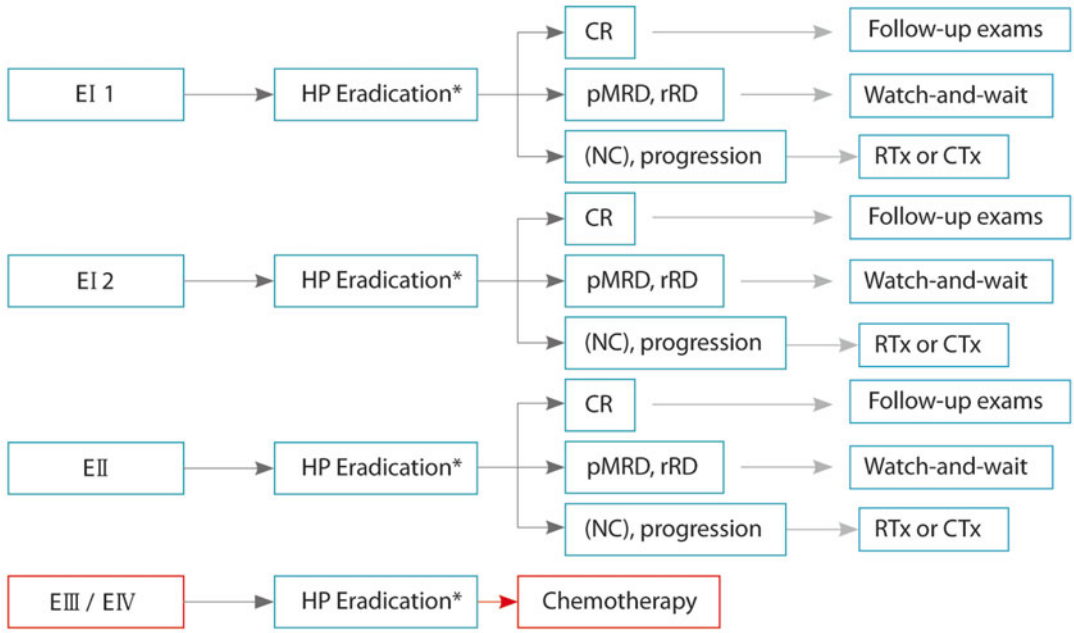

Fig. 20.4 Therapeutic options of gastric MALT lymphoma. *CR* complete remission, *CTx* chemotherapy, *Hp H. pylori*, *NC* no change, *pMRD* probable minimal residual disease, *rRD* responding residual disease, *RTx* radiotherapy. Independent of *H. pylori* status (*asterisk*) (Adapted from Fischbach [38])

Moreover, an elevated risk for gastric carcinoma has been reported for patients with MALT lymphoma in epidemiologic surveys [39, 40]. In a prospective multicenter trial published recently, there was a significantly higher incidence of gastric cancer (standardized morbidity ratio 8.6; 95 % CI, 3.6–20.6) or non-Hodgkin lymphoma (standardized morbidity ratio 18.6; 95 % CI, 8.4–41.5) compared with the general German population [40].

Conclusions

Low-grade gastric MALT lymphoma is a relatively rare disease. Since symptoms or endoscopic findings are nonspecific, it is difficult to diagnose this disease in the early stage. Since *H. pylori* infection is the most important etiologic factor for low-grade gastric MALT lymphoma, an initial therapeutic attempt with *H. pylori* eradication therapy could be attempted even in *H. pylori*-negative patient. If there is a suspicion of this disease during the endoscopy, multiple biopsies should be done with diagnostic tests for *H. pylori* infection. Patients not responding to eradication therapy were usually referred for second therapy, but there has been a new therapeutic trend that patients with responding residual disease or no change can be followed for up to 2 years by a "watch-and-wait" strategy. Consequently, the treatment guideline of gastric MALT lymphoma has been changing, and the therapeutic options and follow-up strategy deserve further study.

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Gastric Cancer: Synopsis and Epidemiology of Gastric Cancer

21

Ernst J. Kuipers

Abstract

Gastric cancer is the fifth most common cancer accounting for close to 7 % of all human cancers. Despite the decrease in incidence, gastric cancer remains the most common cause of gastrointestinal cancer-related death, with more than 800,000 fatalities annually. The disease is more common in men than in women, and non-cardia gastric cancer is twice as common as cardia cancer. More than two-thirds of gastric cancer occur in East Asia, in particular, in China, Japan, and Korea. There are large regional and racial differences in the incidence of gastric cancer. These differences are related to prevalence of *Helicobacter pylori* (*H. pylori*), diet, and other risk factors. The mortality of gastric cancer closely matches the regional differences in incidence. The age-standardized incidence and mortality rates of gastric cancer are expected to further decrease due to improvement in socioeconomic conditions and decreasing prevalence of *H. pylori*. Population screening and intervention, as well as general health measures such as antismoking campaigns, can accelerate the changing epidemiology of gastric cancer. In the absence of such measures, gastric cancer will for long remain a very common and lethal disease. This chapter reviews the epidemiology of gastric cancer, with focus on regional differences in incidence and mortality, risk factors for gastric cancer. It further summarizes the changing epidemiology of gastric cancer in recent decades and the expected future trends.

Keywords

Gastric cancer • Epidemiology • Incidence • Mortality

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21.1 Introduction

The first cancer registries started in the late nineteenth century among others in Germany. At that time, 30–40 % of all cancers were of gastric origin [1]. That vast predominance of gastric cancer was strongly related to the ubiquitous colonization of *Helicobacter pylori* (*H. pylori*) from early childhood onwards. Similar findings were observed elsewhere. For instance in the 1930s, gastric cancer was almost twice as common as cause of cancer-related death than any other individual malignancy [2]. Since then, we have seen significant changes that are still ongoing. Between 1990 and 2014, the average life expectancy of the world population increased by 6.3 years [3]. This marked change was most marked in Southeast Asia where life expectancy increased by more than 8 years. These improvements were firstly due to prevention and improved outcome of infectious diseases, among others, as a result of further implementation of vaccination programs and clean water supply. Other marked improvements were noted in the outcome of treatment of patients with cancer. Both these factors have changed the epidemiology of gastric cancer.

21.2 Incidence and Mortality

21.2.1 Incidence

The incidence of non-cardia gastric cancer has in Western countries continuously decreased over the past 80 years; this decrease was more than 80 % in the United States [2]. In Asia, these changes occurred later and were so far less pronounced [4, 5]. Gastric cancer remained the most common malignancy worldwide until 1975. Nowadays, gastric cancer is the fifth most common cancer after cancers of the lung, breast, colorectum, and prostate, making up for 6.7 % of all human cancers [6]. The annual incidence worldwide approximates 951,000 cases [7]. This includes 260,000 patients with cardia gastric cancer, and 691,000 with non-cardia gastric cancer. These match age-standardized incidence rates of, respectively, 3.3 and 8.8 per 100,000 worldwide. More than two-thirds of gastric cancer occur in East Asia, in particular, in China, Japan, and Korea [4] (Fig. 21.1). In 167 (91 %) of 184 countries for which there are data, non-cardia is more common than cardia gastric cancer with an average ratio of 2:1. The few countries in which the incidence of cardia gastric cancer is higher than or equal to non-cardia cancer can, in particular, be found in Northwestern Europe and include Denmark, Finland, and Sweden. These countries are characterized by high socioeconomic status, low *H. pylori* prevalence, and relatively low proportion of the population consisting of first- and second-generation immigrants. In other Western countries such as the United States and Canada, the incidence of non-cardia gastric cancer is only marginally higher than that of cardia gastric cancer. This pattern markedly differs from that in most Asian and South American countries where non-cardia gastric cancer tends to be considerably more common than cardia gastric cancer. In Eastern and Southeastern Asia, for instance, the age-standardized incidence rates for cardia and non-cardia gastric cancer approximate 9 and 22 cases per 100,000 per year [7]. A similar pattern existed in most Western countries until recently. Within individual countries, marked differences in the incidence of gastric cancer are often observed. One consistent observation is the considerably higher incidence in various indigenous populations [8]. These differences are observed in many different countries with indigenous populations often suffering from an at least fourfold increased standardized incidence ratio of gastric cancer compared with the national average. Other groups also differ in the incidence of gastric cancer. In the United States, for instance, the incidence of non-cardia gastric cancer is considerably higher among African-Americans and Hispanics than among Caucasians [9]. In other countries, these differences among population subgroups can even be larger. In Malaysia, for instance, the incidence is more than fivefold higher among Chinese and Indian men and women than among Malay men and women [10].

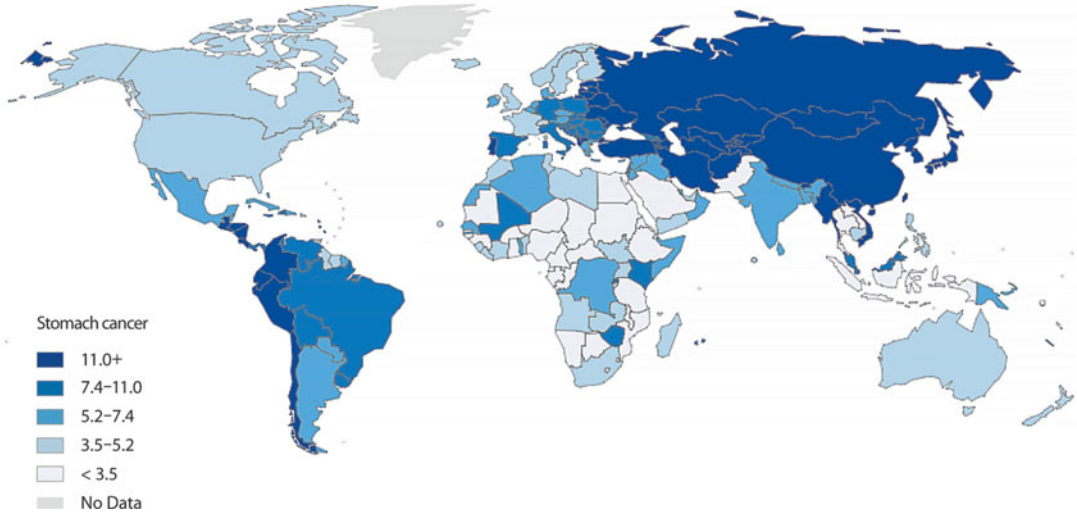

Fig. 21.1 Age-standardized incidence rates of gastric cancer (Adapted from http://globocan.iarc.fr/Default.aspx)

The highest incidences of both cardia gastric cancer are found in China, Central Asia, parts of Eastern Europe and Baltic countries, Central America, and parts of South America [7] (Fig. 21.1). The lowest incidences are observed in the Sahel region and sub-Saharan Africa. The pattern for non-cardia gastric cancer shows significant similarity, with minor differences. The highest incidences are observed in the countries mentioned with inclusion of Russia, the lowest in central and some parts of southern Africa, Northwestern Europe, and North America [7]. The variation in age-standardized incidence rates per 100,000 person-years is marked. It ranges in men from approximately 1 in Botswana and Mozambique to more than 40 in parts of Central Asia such as Mongolia. In women, this range reaches from 0.5 in Botswana and Mozambique to 20 in Guatemala. The latter together with Peru is one of the few countries where the incidence of non-cardia cancer is slightly higher in women than in men. Globally, approximately 631,000 gastric cancer cases diagnosed in 2012 were male and 320,000 were female. The global male-female ratio is most marked for cardia cancer ranging from 1.5 in Southern Africa to 4 in North America with an average of 3 [7]. For non-cardia cancer, the global male-female ratio was approximately 2 with the highest difference in Southeast Asia.

21.2.2 Mortality

The developments in the incidence of gastric cancer are closely matched by geographic differences and changes over time in mortality due to the disease [6] (Fig. 21.2). Between 1990 and 2014, the global age-standardized death rate due to gastric cancer declined by 36 % from 21.7 to 13.8 per 100,000 population [3]. Although this decline was more marked than for any other gastrointestinal malignancy, the age-standardized death rate for gastric cancer remained the highest of any of gastrointestinal cancer. Over the same time period, the number of people dying of gastric cancer actually increased from 763,000 to 841,000 per year [3]. This rise was due to the increase in numbers and life expectancy of the world population, in particular, in areas with a high gastric cancer incidence. The comparison between annual numbers of newly diagnosed patients (see below) and those dying of the disease shows that even today the global disease fatality rate may amount to 85 %. This fatality rate is significantly lower in countries with population screening programs aiming for early detection of gastric cancer [11]. In Japan, the 5-year survival rate is now approximately 69 % [12], while even better results are achieved in high-volume centers in Korea [13]. In Western

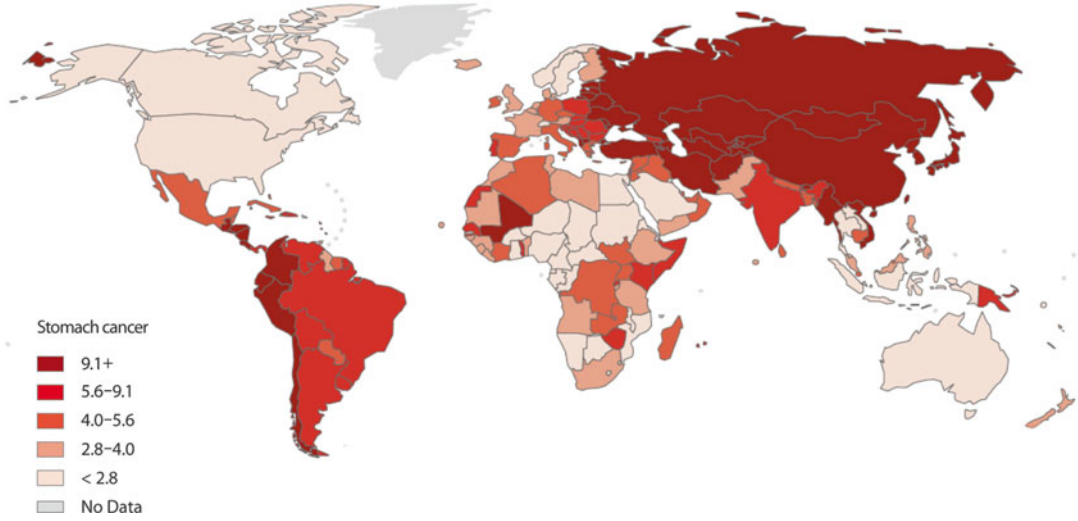

Fig. 21.2 Age-standardized mortality rates of gastric cancer (Adapted from http://globocan.iarc.fr/Default.aspx)

countries, the majority of cases are detected at a more advanced stage. Survival rates for non-cardia cancer reach 30 %, while survival rates for cardia cancer remain as low as 20 % [14], with multimodality treatment contributing to this effect [15]. Observations in the Surveillance Epidemiology and End Results (SEER) database suggest that the difference in gastric cancer survival may also be related to host and disease factors, since patients of Asian descent had a 12 % better survival than Caucasian patients [16]. This effect persisted after correction for tumor grade and number of lymph nodes investigated. Depending on stage, Asian patients had a 13–72 months longer median survival than corresponding Caucasian patients.

21.2.3 Incidence and Mortality in Lynch Syndrome Carriers

The previous observations were also reflected in the patterns of gastric cancer among carriers of a hereditary cancer syndrome. For instance, in the first description of Lynch syndrome in 1913 in the United States, gastric cancer was the predominant cancer affecting family members [17]. This usually already occurred around the age of 40 years. This is thought to have resulted from early exposure to *H. pylori*, most likely in combination with a diet rich in salt and nitrosamines. With changes in diet and a decrease in exposure to *H. pylori*, the predominant cancer in Lynch carriers became colorectal cancer, usually at an age above 40 years. In a nationwide cohort of Lynch carriers in the Netherlands, we observed a 50 % decline in gastric cancer incidence between 1970 and 2000 [18].

21.3 Risk Factors

21.3.1 *Helicobacter pylori*

The most important risk factor for non-cardia gastric cancer is *H. pylori* gastritis. The International Agency for Research on Cancer (IARC) thus classified *H. pylori* as a class I carcinogen [19]. Based on nested case-control in longitudinal cohorts [20] as well as retrospective case-control studies, *H. pylori* gastritis has been estimated to be associated with a 17-fold increased risk for development of non-cardia gastric cancer [21]. Based on this relative risk and the high global prevalence of *H. pylori*, 89 % of all non-cardia gastric cancers are thought to be attributable to *H. pylori* colonization and the resulting gastritis in all *H. pylori*-positive subjects

[22]. This risk depends on the severity of gastritis and is thus higher in those who have more severe chronic active mucosal inflammation as a result of colonization with a more pathogenic *H. pylori* strain [21, 23, 24]. Likewise, gastric cancer in *H. pylori*-positives occurs also more frequently in hosts with a more pro-inflammatory genotype [25, 26]. These observations form the basis for the recommendation to consider *H. pylori* eradication either as a population approach in regions with high gastric cancer incidence [27, 28] or as targeted approach, for instance, in subjects with a positive family history for gastric cancer [29, 30]. When applied prior to the stage of development of intestinal metaplasia, this intervention can strongly reduce the risk of cancer development [31], but it appears to have little preventive effect in those with intestinal metaplasia [31]. In those subjects, cancer can occur even more than a decade after *H. pylori* eradication [32] (see also Chap. 53).

21.3.2 Other Risk Factors

There are a range of other factors that either increase or decrease the risk of gastric cancer, in particular factors related to diet [33]. A high intake of salt is associated with an estimated up to threefold increased risk of gastric cancer [34]. In a Japanese study that followed 2476 subjects for 14 years, those in the highest quartile of salt intake had a 2.98-fold increased risk of gastric cancer compared with those in the lowest quartile [35]. This relative risk remained after correction for confounders such as age, sex, and presence of atrophic gastritis. In a retrospective study from Portugal comparing salt intake in 422 patients with gastric cancer versus 649 community controls, the highest tertile of salt intake was associated with a 2.6-fold increased risk for gastric cancer [36]. Very similar data were observed elsewhere, such as in China and Latin America [37, 38].

A diet that is short on fresh fruits and vegetables also increases the risk of gastric cancer. In a retrospective case-control study from Portugal, the highest tertile of fruit intake in the population was associated with an odds ratio (OR) of 0.47 and 0.53 for, respectively, cardia and non-cardia gastric cancer [39]. For the highest tertile of vegetable intake, OR were 0.59 and 0.60, these results were in line with a meta-analysis of literature findings [39]. A meta-analysis of a range of studies from South America reported that high intake of fruits and vegetables was associated with a respective 32 and 42 % decrease in risk of gastric cancer, without specification of tumor subsite [38]. The same meta-analysis also reported a 2.3-fold increased risk for gastric cancer in those with high intake of chili peppers, but also noted that the data were limited. An increase of risk for gastric cancer has also been reported with high intake of processed and red meat, with relative risks ranging from 1.5 to 1.6 in different studies and a meta-analysis from South America [38], while another meta-analysis reported a 15–38 % increased risk with every 30 g/day increase in meat consumption [40]. A large European population study following 521,000 men and women for a median 6.5 years concluded that the increased risk with high meat intake was most pronounced in *H. pylori*-positives, with an OR of 5.3 with every 100 g/day increase in meat intake [41].

The risk of gastric cancer is increased in smokers. In a European prospective cohort, smoking increased the risk of gastric cancer with approximately 80 % after adjusting for other factors such as intake of fruit and vegetables [42]. Other studies confirm this correlation [38, 43, 44]. A US population microsimulation model showed that reduction in smoking significantly contributed to the decline in the incidence of non-cardia gastric cancer and can further do so with in the coming period on top of changes in *H. pylori* prevalence [45].

Other factors that have been associated with gastric cancer risk, but less consistent, include alcohol and green tea consumption. In a systematic review of six cohort studies on green tea intake, a limited protective effect was noted on non-cardia gastric cancer but only in women who drank at least five cups of tea per day [46]. There is no convincing evidence for a modulation of gastric cancer risk by coffee consumption [33, 47]. The evidence for a link between both cardia

and non-cardia gastric cancer has not been consistently reported, with a recent meta-analysis concluding that the relative risks were not significantly increased [48].

Together, the abovementioned risk factors have been adequately used for implementation in a prediction model for gastric cancer in Korea as a high-incidence country [49]. In Western Europe, as a low-incidence region, it has been estimated that 19 % of all gastric cancers and even 62 % of all cardia cancers could be prevented by universal adoption of a healthy lifestyle including use of a healthy diet and refraining from smoking and alcohol consumption [50].

21.4 Future Trends

Continuation of the trends of recent decades will likely imply a significant decrease in the number of newly diagnosed patients as well as mortality rates due to gastric cancer despite an expanding and aging world population. The largest changes are likely to occur in current high-incidence countries, in particular, with changing epidemiology of *H. pylori*. This effect may in some areas such as Japan and Korea be augmented by gastric cancer screening programs [51, 52], and further among others in Japan, and parts of China, and Taiwan by population screening programs aiming to screen and treat for *H. pylori* and/or atrophic gastritis [27, 31, 53].

A similar effect may occur in Western countries, even in those that already have low incidences of gastric cancer. It has, for instance, been estimated that the decrease would be 66 % for mortality and 50 % for incidence within Europe when comparing 2030 with 2005 [54]. This estimate is supported by the observation of a marked decrease in the prevalence of atrophic gastritis and intestinal metaplasia in the general population [55]. Since these lesions are predominant risk factors for later development of gastric cancer, their prevalence is a looking glass for the incidence of gastric cancer in a population in the following two decades [56]. However, despite the decrease, atrophic gastritis and intestinal metaplasia remain common conditions in those above the age of 50 years even in Western Europe [55, 57].

Some effects may however interfere with this trend. These include the observation that cardia cancer is becoming predominant in Western countries [7]. The incidence of this tumor shows much less of a change over the past decades. For instance, in the United States, the incidence of cardia cancer actually tended to increase between 1976 and 2007 in all racial groups [9]. Over the same time period, the incidence of non-cardia gastric cancer stabilized or even slightly increased in men and women below the age of 50 years [58]. A similar stabilization of non-cardia gastric cancer has recently been observed in the Netherlands [59]. Furthermore, there are some observations that the decreasing prevalence of *H. pylori* in subsequent birth cohorts may have slowed or stopped in a Western country such as the Netherlands for reasons which are not yet fully understood, but may include a marked increase in the use daycare at very young age [60, 61].

Conclusions

Gastric cancer has for long been the most common human malignancy. Despite a marked decline in incidence during the past decades, it still is the fifth most common cancer accounting for close to 7 % of all human cancers. The disease is more common in men than in women, and more often affects the body and antrum of the stomach than the cardia. Despite the decrease in incidence, gastric cancer remains the most common cause of gastrointestinal cancer-related death, with more than 800,000 fatalities annually. There are large regional and racial differences in the incidence of gastric cancer. These differences are related to the prevalence of *H. pylori*, diet, and other risk factors. The mortality of gastric cancer closely matches the regional differences in incidence. Globally, the majority of patients affected by gastric cancer still die as a result of the disease. The high incidences and fatality rates are remarkable in view of the fact that gastric cancer is largely a preventable disease and can also be detected and treated with

excellent outcome at a precursor or early cancer stage. The age-standardized incidence and mortality rates of gastric cancer are expected to further decrease due to improvement in socioeconomic conditions and decreasing prevalence of *H. pylori*. Population screening and intervention, as well as general health measures such as antismoking campaigns, can accelerate the changing epidemiology of gastric cancer. In the absence of such measures, gastric cancer will for long remain a very common and lethal disease.

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22 Gastric Cancer: Genetic Alternations Induced by *H. pylori* Infection: The Role of Activation-Induced Cytidine Deaminase

Cheol Min Shin

Abstract

Oxidative stress and aberrant activation-induced cytidine deaminase (AID) expression induced by chronic inflammation play an important role in inflammation-associated carcinogenesis. While AID plays a physiological role in B lymphocytes to induce somatic hypermutation and class switch recombination of immunoglobulin gene, it appears to promote mutagenesis in other cells than B lymphocytes. The impact of AID on carcinogenesis has been reported in numerous studies on colon, liver, lung, and gastric cancers, as well as hematologic malignancies. Chronic active gastritis by *Helicobacter pylori* (*H. pylori*) infection leads to aberrant AID expression, followed by mutations of tumor-related genes such as *p53* and *CDKN2B-CDKN2A*, which suggests one possible mechanism how *H. pylori* infection induces gastric carcinogenesis.

Keywords

Helicobacter pylori • Mutation • Reactive oxygen species • Activation-induced cytidine deaminase

22.1 Introduction

Helicobacter pylori (*H. pylori*)-associated chronic gastritis induces altered cellular signaling, imbalanced proliferation, and apoptosis of gastric epithelial cells via genetic mutations and/or epigenetic changes (aberrant DNA methylation and microRNA dysregulation), both of which modulate gene expression and genomic instability, driving the progression from precancerous lesions to cancer. As a matter of fact, *H. pylori*-induced gastric carcinogenesis is a complex process and could not be easily explained by a single mechanism. In this chapter, how chronic inflammation can induce mutagenesis and cancer will be highlighted. In addition, how *H. pylori* manipulates the host nucleotide-editing enzyme activation-induced cytidine deaminase (AID) and

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promotes genomic mutations in the gastric epithelium will be discussed.

22.2 Genetic Alternations in Gastric Carcinogenesis

Carcinogenesis can be defined as a stepwise accumulation of genetic and epigenetic alterations that drives the progressive transformation of normal cells into malignant derivatives [1]. Mutagenesis in gastric carcinogenesis includes microsatellite instability (MSI) resulting from altered DNA mismatch repair, point mutations, and genomic instability including loss of heterozygosity (LOH), gene amplifications, rearrangements, insertion and deletion mutations, chromosomal losses and duplications, and DNA copy number variation.

The spectrum of mutations in gastric cancer has been explored by massive parallel sequencing approaches. A recent study performed whole exome sequencing in gastric cancer, and it identified frequently mutated genes and involved molecular pathways [2]. It was found that chromatin modification and cell junction organization were the pathways with the most significant enrichment of mutated genes [3]. That is, mutations were found in members of the SWI-SNF complex (*ARID1A*, *PBRM1*, and *SMARCC1*), ISWI complex (*SMARCA1*), and NuRD complex (*CHD3*, *CHD4*, and *MBD2*), and other genes encoding histone-modifying proteins (*SIRT1* and *SETD2*), in 59 % of gastric cancer samples. In addition, 59 % of gastric cancer samples had mutations in genes involved in cell adhesion, including *CDH1* and other cadherin family members, which shows the infiltrative nature of gastric cancer and the importance of the loss of cell-to-cell adhesion in gastric carcinogenesis. Genes involved in cell cycle regulation such as *TP53*, *PTEN*, and *TTK* were mutated in 77 % of gastric cancers. Other signaling pathways frequently mutated in gastric cancers included the Wnt-BMP-TGFβ, axon guidance, MAPK, DNA replication, focal adhesion, ERBB, ATR-BRCA, and Rb pathways. Mutations or protein deficiency of *ARID1A* was frequent in 83 % of gastric cancers with MSI and 73 % with Epstein-Barr virus (EBV) infection, but it was infrequent in 11 % that were microsatellite stable (MSS) without EBV infection. Thus, the frequency of *ARID1A* mutation was different between molecular subtypes of gastric cancer, and it was negatively associated with that of *TP53* mutation [2]. Another study also performing exome sequencing has reported that cell-to-cell adhesion was the pathway most enriched for mutations [3]. *TP53* was mutated in 66.7 %, and *PIK3CA* and *ARIDI1A* were mutated in 20 % of gastric cancers. Frequent mutations in chromatin remodeling genes (*ARIDI1A*, *MLL3*, and *MLL*) were found in 47 % of gastric cancers. *ARIDI1A* mutations were associated with *PIK3CA* mutations and MSI [3].

22.3 Chronic Inflammation and Genetic Alternations

As mentioned above, genomic mutations accumulate during the steps of gastric carcinogenesis in cells representing intestinal metaplasia, dysplasia/adenoma, and adenocarcinoma and probably in epithelial progenitors and stem cells. In the multistep gastric carcinogenesis, genetic alternation of tumor suppressor gene *TP53* and *APC* is most frequently observed [4]. Mutation of *TP53* is common in noncancerous gastric mucosae with chronic inflammation or precancerous lesions [5]. *APC* mutations, including stop-codon and frameshift mutations, were reported in 40 % of gastric flat adenomas [6].

Among digestive organ cancers, gastric cancers are among the class of cancers that arise in association with chronic inflammation (chronic active gastritis), like other gastrointestinal tract cancers such as esophageal adenocarcinoma associated with reflux esophagitis and Barrett's esophagus and colorectal cancers in patients with inflammatory bowel disease [7]. To date, there are two possible intrinsic mutagens responsible for genetic aberrations in the inflammatory condition: (1) free radicals and (2) intrinsic DNA mutator enzymes [7].

22.3.1 Free Radicals

Chronicammation induces reactive oxygen species (ROS) and reactive nitrogen species, and oxidative stress caused by ROS increases DNA damage of epithelial cells. ROS generate various modified bases of oxidatively altered purines or pyrimidines, which induces the putative DNA damages such as single- or double-stranded DNA breaks, DNA intra-strand adducts, and DNA protein cross-links [8, 9]. In addition, ROS can also induce mismatch repair function and mutation of microsatellite sequences, and the genetic instability is closely related to not only cancers but precancerous lesions. In this regard, ROS is a putative mediator that links excessive activity of oncogene products and DNA damage [7].

22.3.2 Activation-Induced Cytidine Deaminase

However, oxidative stress induced by ROS cannot fully explain the mutagenesis observed in many human cancers, particularly in inflammation-associated cancers [7]. Recently, several human enzymes that are capable of inducing nucleotide alterations have been identified, providing a new concept for understanding the mechanisms of mutagenesis. Among them, AID is a well-defined molecule involved in DNA mutations in the human genome [10]. Through its enzymatic activity, AID can deaminate cytidine (C) on target DNA to a uracil (U), leading to the change of a DNA C:G pair into a U:G mismatch. When DNA replication starts before being recognized by the repair system, a U:G mismatch gives rise to C/G to T/A transition [11].

Under physiological conditions, AID contributes to promoting antibody gene diversification in germinal center B cells by inducing somatic hypermutation and class switch recombination of immunoglobulin gene [12]; it leads to the maintenance of the immune system diversity. While AID plays a favorable role in the immune system, aberrant AID activity might affect non-immunoglobulin genes, including tumor-related genes in non-lymphoid cells.

The role of AID in tumorigenesis is the induction of genetic instability, which was first suggested in hematopoietic malignancies [13]. A number of studies have shown that increased AID expression was associated with unfavorable mutations and chromosomal translocations in various malignancies of the B lymphocytic lineage [14, 15]. AID has been shown to be responsible for the chromosomal breaks in c-MYC, leading to a c-MYC/IgH translocation in B-cell lymphoma [16]. Moreover, AID induces breakpoint cluster region-Abelson murine leukemia viral oncogene homolog 1 mutations, resulting in the imatinib resistance in chronic myeloid leukemia cells [17]. Because the target of AID-mediated genotoxic effects was not only immunoglobulin genes but numerous other genes, aberrant AID expression induced genetic alterations in various tumor-related genes, leading to the transformation of the hematopoietic cells. Recently, there have been several reports on the impact of AID expression in gastric and colorectal cancers and HCV-related hepatocellular carcinoma. Especially, AID transgenic mice developed neoplasia in epithelial tissues including the lung, liver, and stomach, related to *Trp53* mutations. This result indicates that aberrant AID expression in epithelial cells can promote carcinogenesis by genetic instability [18]. Interestingly, the frequently mutated tumor-related genes induced by aberrant AID expression differ among different cancers. For example, while the mutations of *c-Myc* were usually detected in lung cancer, *K-ras* mutations were frequently observed in gastric cancer [19], which indicates the diversity of organ-specific oncogenic pathways in various epithelial tissues.

Nuclear factor (NF)-κB can be activated in response to various inflammatory stimulations, which is closely related to the multiple processes of initiation and progression in carcinogenesis. NF-κB is known as a major transcription factor for AID [20]. Increased expression of AID induced by tumor necrosis factor (TNF)-α stimuli has been reported in *H. pylori*-related gastric carcinogenesis [21], as well as colitis-associated colorectal carcinogenesis [22]. In human colon

cancer cell lines, AID expression could be induced in STAT6-dependent manner.

22.4 Genetic Alternations in *H. pylori*-Associated Gastric Carcinogenesis

In *H. pylori*-associated gastric carcinogenesis, DNA damage of epithelial cells is attributed to the oxidative stress caused by ROS and reactive nitrogen species generated by inflammatory cells as well as by gastric epithelial cells activated by *H. pylori*. In addition, epithelial expression of AID in *H. pylori*-associated gastritis may induce C/G to T/A transitions by its cytidine deaminase activity [21], which is regarded as one of the mechanisms in gastric carcinogenesis.

While endogenous AID expression is not detected in normal epithelial cells under physiological conditions, it has been observed in the *H. pylori*-infected gastric epithelium. As mentioned above, NF-κB is an important transcription factor for AID expression. According to the previous studies, AID expression in human gastric cancer cell lines could be induced by NF-κB activation stimulated by TNF-α, but was not observed if there was no TNF-α stimulus. That is, *H. pylori* infection promotes aberrant AID expression in gastric mucosae via increased production of inflammatory cytokines such as TNF-α and interleukin (IL)-1β. In addition, pathogenic *H. pylori* strain per se is able to induce AID expression, too; there is evidence that cytotoxin-associated gene (*cag*) pathogenicity island (PAI)-positive strains could activate the NF-κB pathway via type IV secretion system; cag PAI-positive *H. pylori*-derived virulence factors (peptidoglycans) injected into the gastric epithelial cells induce the NF-κB activation [23] (Fig. 22.1). AID in the gastric epithelium in the setting of *H. pylori* infection can lead to increased U:G mismatches further contributing to the number of unrepaired mismatches and increased T/A transitions. Gene targets of AID-enhanced mutagenesis in gastric cells include *TP53* and *CDKN2B-CDKN2A* (encoding p16, p15, and p14 suppressor proteins) and caused submicroscopic deletions with chromosome copy number losses involving the *CDKN2B-CDKN2A* locus [21, 24]. Interestingly, one recent study showed that *H. pylori* eradication attenuated aberrant AID expression in the gastric epithelium, which suggests one possible mechanism that anti-*H. pylori* treatment can reduce the risk of gastric cancer [25].

In contrast to the significant role in gastric adenocarcinoma, AID appears to play a limited role in *H. pylori*-associated mucosa-associated lymphoid tissue (MALT) lymphoma. According to the previous reports on non-gastric MALT lymphomas, aberrant AID expression was confined to the reactive areas; AID was rarely expressed in neoplastic marginal zone B cells [26, 27]. In addition, it remains unknown whether *H. pylori* infection enhances aberrant AID expression in gastric B lymphocytes [23]. Therefore, further study is necessary in this issue.

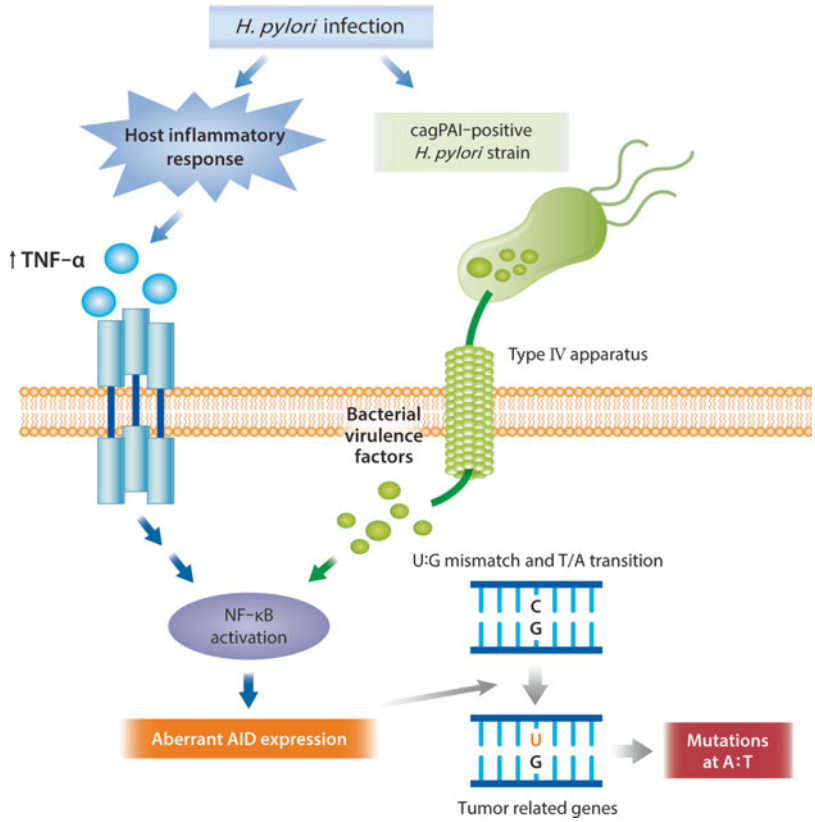

Fig. 22.1 The role of *Helicobacter pylori* infection in gastric carcinogenesis in view of aberrant activation-induced cytidine deaminase (*AID*) expression. AID acts as a cytidine deaminase that is capable of inducing nucleotide alterations in human DNA sequences. *H. pylori* infection can induce AID expression in gastric epithelial cells via two distinct pathways. First, *cag* PAI-positive *H. pylori* strains per se possess type IV machinery and can inject bacterial virulence factors into gastric epithelial cells, leading to the activation of the host transcriptional factor NF-κB. Second, the host inflammatory response triggered by *H. pylori* infection can also activate NF-κB in the gastric epithelium. As a result, AID is transcriptionally upregulated in gastric epithelial cells and can contribute to the mutagenesis of tumor-related genes (Modified from Marusawa and Chiba [23])

Conclusions

Chronic inflammation plays important roles in gastric carcinogenesis. During chronic inflammation, intrinsic mediators of inflammatory responses, including pro-inflammatory cytokines and reactive oxygen and nitrogen species, can induce genetic mutations. Recently, the role of AID as a genomic modulator has been highlighted in *H. pylori*-associated gastric cancer development.

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23 Gastric Cancer: Epigenetic Mechanisms: Aberrant DNA Methylation and Dysregulation of MicroRNA

Cheol Min Shin

Abstract

Like many human cancers, global DNA hypomethylation and promoter CpG island hypermethylation in tumor suppressor or tumor-related genes are frequently observed in gastric cancer, and aberrant DNA methylation occurs in a gene-specific manner during the multistep gastric carcinogenesis. Chronic *Helicobacter pylori* (*H. pylori*) infection induces pro-inflammatory cytokines and reactive oxygen and nitrogen species in the gastric mucosa, which is known to be associated with the accumulation of aberrant DNA methylation. Aberrant DNA methylation caused by *H. pylori*-associated gastritis persists even after the disappearance of *H. pylori*, and epigenetic alterations induced by *H. pylori* correlate with the risk for gastric cancer. Numerous microRNAs (miRNAs) are dysregulated during the gastric carcinogenesis, and some of these miRNAs are known to be also dysregulated by *H. pylori* infection. miRNAs dysregulated by *H. pylori* infection play an important role in gastric carcinogenesis by modulating inflammation and immune response of the host, cell cycle progression, apoptosis and proliferation, and tumor invasion and metastasis.

Keywords

Helicobacter pylori • Epigenetics • Gastric cancer • Methylation • MicroRNA

23.1 Introduction

Aberrant DNA methylation is a major epigenetic mechanism associated with gene silencing; it is observed in many human cancers [1]. Global DNA hypomethylation and promoter hypermethylation in the specific genes have been associated with genomic instability and inactivation of tumor sup-

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pressor genes, respectively. Global hypomethylation mostly occurs in the intergenic region, and it precipitates chromosomal instability which leads to chromosomal mutations such as recombination, translocation, deletion, and rearrangement [2, 3]. On the other hand, promoter CpG island hypermethylation induces carcinogenesis by gene silencing of mostly tumor suppressor genes. These epigenetic changes are known to be replicated with a high fidelity in mammalian cells, mediated by DNA methyltransferases (DNMTs) which add methyl groups to cytosines and in result serve as a long-term memory of cells.

In gastric cancer, tumor suppressor or tumor-related genes are more frequently inactivated by CpG island hypermethylation than by mutations [4]. To date, promoter hypermethylation of such genes as *p16*, *CDH1*, *MGMT*, *MLH1*, *APC*, and *RUNX3* has been reported in gastric cancers. Global DNA hypomethylation is frequently observed in gastric cancer cells [5]. Interestingly, promoter CpG island hypermethylation has also been found both in the adjacent noncancerous tissues of patients with gastric cancer and in nonneoplastic gastric mucosae of subjects without gastric cancer. From the results of previous studies, aberrant DNA methylation occurs in a gene-specific manner along the multistep gastric carcinogenesis [6].

MicroRNA (miRNA) is a small noncoding RNA molecule (containing approximately 21–23 nucleotides) that functions as RNA silencing and posttranscriptional regulation of gene expression. miRNAs can inhibit translation of the target messenger RNAs (mRNAs) by binding to complementary sequences in the 30 untranslated regions (UTRs) of the mRNAs. Approximately more than one-third of human genes are known to be regulated by ~1,000 miRNAs, and a single miRNA can regulate hundreds of unique mRNAs [7, 8]. miRNAs play important roles in the regulation of almost all biological processes, including proliferation, apoptosis, cell differentiation, metabolism, and epithelial-to-mesenchymal transition [9]. Therefore, miRNA dysregulation has been determined to correlate with cancer development and progression. The first report on miRNA dysregulation in cancer was in chronic lymphocytic leukemia [10]. To date, specific miRNAs have subsequently been found to have links with various malignancies including lung, colorectal, and breast cancers.

The role of miRNAs has also been addressed in gastric carcinogenesis. According to the results of miRNA chip studies, numerous miRNAs were demonstrated to be associated with gastric cancer. For example, miR-21, miR-17-92 cluster, miR-106b-25 cluster, and miR-150 were overexpressed in gastric cancer. On the other hand, miR-451, miR-141, miR-31, miR-218, and miR-9 were reported to be downregulated in gastric cancer tissue [11]. Alterations of miRNAs in gastric mucosa or blood or gastric juice may provide important data on early diagnosis and prognostication of gastric cancer [12]. More studies are warranted in the future.

23.2 *H. pylori*-Induced Gastric Carcinogenesis and Aberrant DNA Methylation

Helicobacter pylori (*H. pylori*) infection is an established risk factor for gastric cancer and is known to be associated with the accumulation of epigenetic alterations such as aberrant DNA methylation in gastric mucosa. A recent study on genome-wide methylation profiling shows that a number of genes were differentially methylated by *H. pylori* infection [13] (Fig. 23.1). Previous studies have shown that *H. pylori* infection can induce promoter hypermethylation of *CDKN2A* (*p16*), *CDH1*, and *MLH1* [4]. Since the aberrant DNA methylations of these genes are closely related to gastric carcinogenesis, *H. pylori* infection seems to be associated with promoter hypermethylation with gene type-specific methylation profiles in the multistep process of carcinogenesis [14, 15].

23.2.1 Underlying Mechanisms of Induction of Aberrant DNA Methylation by *H. pylori* Infection

Animal studies may address the underlying mechanisms regarding how *H. pylori*-induced chronic

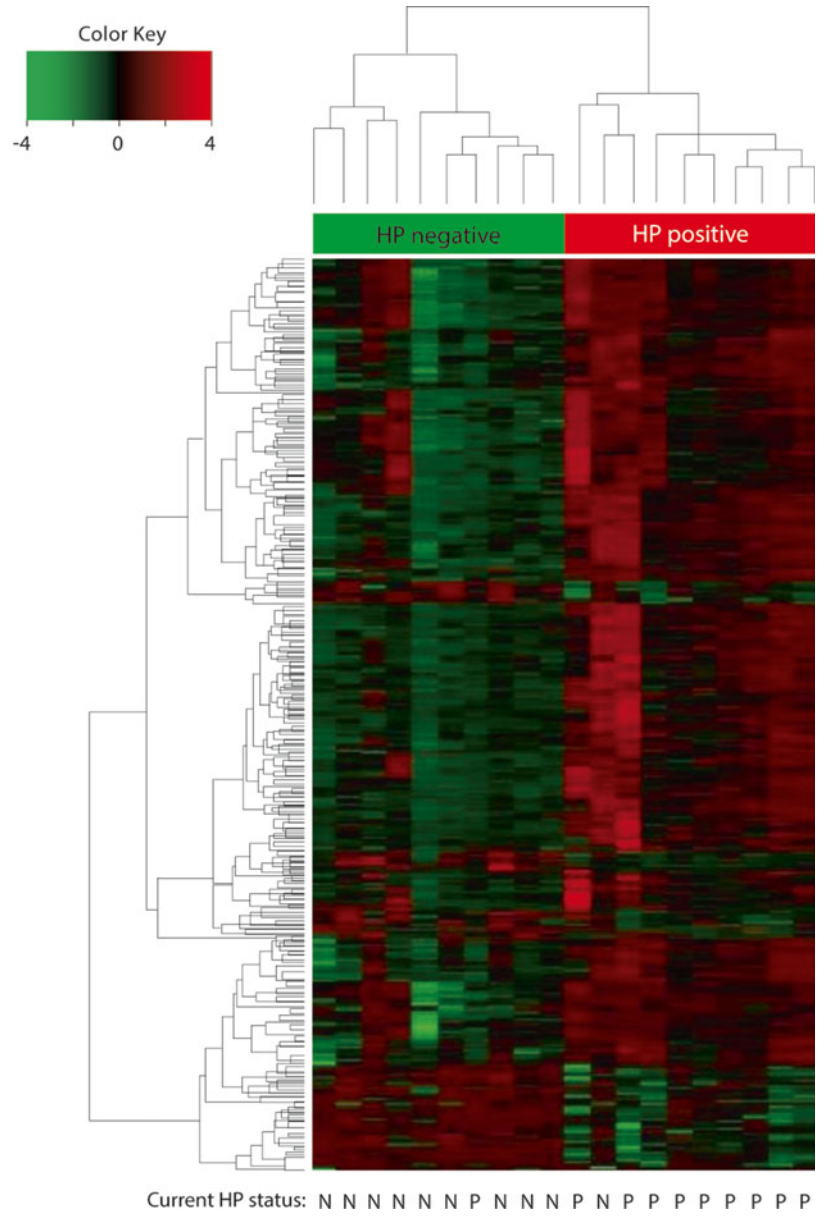

Fig. 23.1 Genome-wide DNA methylation profiles obtained from 10 *H. pylori*-positive patients and 10 *H. pylori*-positive controls. *Hp Helicobacter pylori*, *N H. pylori* negative, *P* current *H. pylori* infection positive (Adapted from Shin et al. [13])

inflammation induces aberrant DNA methylation. If chronic active inflammation induced by *H. pylori* infection was suppressed by cyclosporin A in Mongolian gerbils, aberrant DNA methylation was significantly reduced, while the number of *H. pylori* was unaffected in gastric mucosae [16]. This study indicates that not *H. pylori* itself but inflammation is important in the induction of aberrant DNA methylation [17]. However, repeated induction of acute inflammation by ethanol or high salt diet could not induce aberrant DNA methylation [18]. *Il-1β*, *Nos2*, and *Tnf-α* were specifically upregulated by the *H. pylori*-induced inflammation. Probably, chronic active *H. pylori* infection activates macrophage, and its secretion of interleukin (IL)-1β and tumor necrosis factor (TNF)-α, as well as the production of active oxygen species by nitric oxide synthase (NOS), may induce DMNT1 and in result aberrant DNA methylation in gastric mucosae [17].

23.2.2 Reversibility of Aberrant DNA Methylation Following *H. pylori* Eradication

To date, changes of DNA methylation levels in gastric mucosae after anti-*H. pylori* treatment have not been clarified yet. Previous studies using nonquantitative methods have reported that hypermethylation in several tumor suppressor genes such as *CDH1* in gastric mucosae decreases following *H. pylori* eradication [19, 20]. On the other hand, more recent studies using quantitative methods have shown that aberrant DNA methylation induced by *H. pylori* seems to be partially reversible by *H. pylori* eradication [21, 22].

23.2.3 Epigenetic Fingerprint of *H. pylori* Infection and Epigenetic Field for Cancerization

In viewpoint of the prediction for gastric cancer using methylation biomarkers, DNA methylation profiles obtained from noncancerous gastric mucosae may be useful in identifying a high-risk group for gastric cancer. As mentioned above, *H. pylori* infection is recognized as one of the most important risk factors for gastric cancer. As mucosal atrophy and intestinal metaplasia progress, however, the bacteria are slowly removed from the gastric mucosa and active inflammation gradually decreases. Thus, it is difficult to demonstrate a causal relationship between *H. pylori* infection and gastric cancer. From the epigenetic point of view, *H. pylori*-associated chronic inflammation is responsible for the promoter CpG island hypermethylation and global DNA hypomethylation. DNA hypermethylation caused by *H. pylori*-associated gastritis persists even after the disappearance of *H. pylori* (epigenetic fingerprint of *H. pylori* infection). The duration of *H. pylori* exposure and the epigenetic alterations induced by *H. pylori*, not *H. pylori* infection *per se*, are known to be correlated with the future risk for gastric cancer (epigenetic field for cancerization) [23] (Fig. 23.2). From this background, it has been suggested that the DNA methylation levels in the specific CpG loci obtained from blood or gastric mucosae or gastric fluid might be used as a marker for gastric cancer.

23.3 *H. pylori*-Induced Gastric Carcinogenesis and miRNA

The role of miRNAs in *H. pylori*-induced chronic inflammation has been evaluated in recent studies. According to a miRNA profiling study of 470 human miRNAs in noncancerous gastric mucosae of *H. pylori*-positive and *H. pylori*-negative subjects, a total of 30 miRNAs were significantly downregulated with *H. pylori* infection, while only one miRNA, miR-223, was upregulated with *H. pylori* infection [24]. Interestingly, eradication of *H. pylori* normalized 14 of 30 miRNAs of which the expressions were downregulated [24]. One recent study has reported that 219 of 3,523 miRNAs showed at least two-fold increased or decreased expressions in *H. pylori*-positive gastric cancer tissues compared with *H. pylori*-negative gastric cancer tissues [25]. According to a review article, miRNAs such as miR-17, miR-20a, miR-21, miR-25, miR-146a, miR-155, miR-196, and miR-223 were upregulated both with *H. pylori* infection and gastric cancer, while miRNAs such as let-7a, miR-31, miR-34b, miR-34c, miR-101, miR-141, miR-203, miR-210, miR-218, miR-375, and miR-449 were downregulated both with *H. pylori*-infected gastric mucosae and gastric cancer tissues [16] (Tables 23.1 and 23.2). However, miRNAs that are dysregulated in response to *H. pylori* infection may not be the same miRNAs that are dysregulated in later stages of gastric carcinogenesis. For example, miR-106b is known as an oncogenic miRNA and is upregulated in gastric cancer, but its expression was reported to be suppressed in *H. pylori*-infected gastric mucosa [68, 69]. Likewise, miRNAs such as miR-34b, miR-34c, miR-103, miR-200a, miR-214, and miR-372 have been

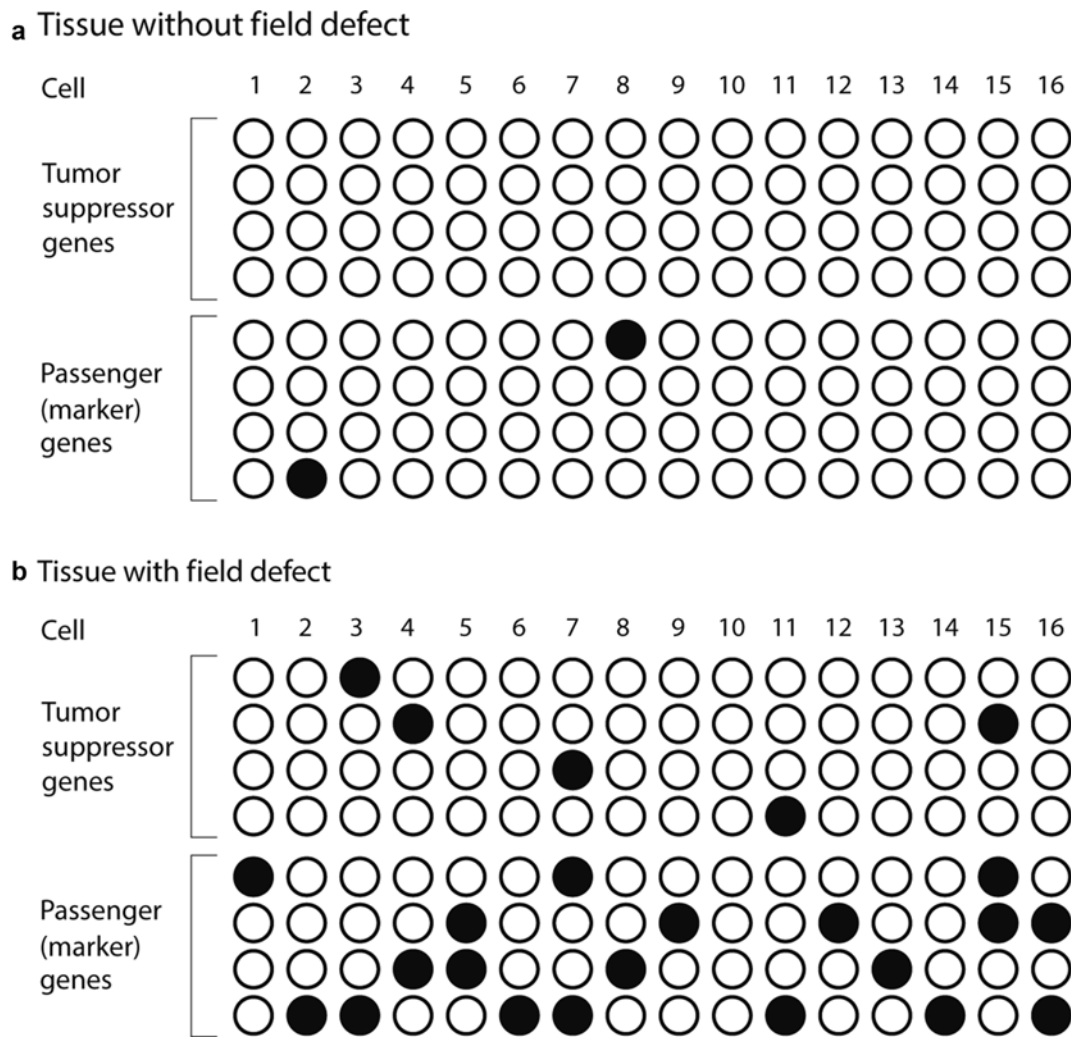

Fig. 23.2 Methylation of tumor suppressor and passenger (marker) genes. (**a**) Gastric mucosae without epigenetic field defect. (**b**) Gastric mucosae with field defect. In gastric mucosa with field defect, tumor suppressor genes may have low methylation levels, but passenger genes showed high levels of DNA methylation. Although methylation of passenger genes is not directly involved in carcinogenesis, their methylation levels correlate with those of tumor suppressor genes and reflect the future risk for gastric cancer. *Open circles* unmethylated genes, *closed circles* methylated (Modified from Ushijima [4])

reported to be overexpressed in gastric cancer, but these are downregulated in *H. pylori*-infected gastric mucosa [16]. MiR-146a was reported to be upregulated in *H. pylori*-infected gastric mucosae [18, 58, 70]. However, its expression has been reported to be upregulated with gastric cancer in one study but downregulated in the other studies [71–74].

23.3.1 *H. pylori* and miRNA: Underlying Mechanisms

Underlying mechanisms are still unclear regarding how miRNAs dysregulated by *H. pylori* infection can be involved in the gastric carcinogenesis. Nevertheless, it appears to be related to (1) modulating host inflammatory immune

Table 23.1 MicroRNAs downregulated in response to *Helicobacter pylori*

MicroRNAs	Downregulated in gastric cancer	Target mRNAs	Biological process targeted	Reference
let-7a	Yes	RAB40C	Cell cycle progression, proliferation	[24, 26, 27]
		HMGA2	Invasion	
let-7b		HMGA2	Invasion	[24, 26, 28, 29]
		TLR4, NF-kB, COX-2, cyclin D1, IL1B	Immune response	
let-7d		HMGA2	Invasion	[24, 26]
let-7e		HMGA2	Invasion	[24, 26]
let-7f		HMGA2	Invasion	[24, 26]
miR-1		ND	Proliferation	[30]
miR-31	Yes	ND	ND	[24]
miR-32		ND	ND	[24]
miR-34b	Yes	ND	ND	[31, 32]
miR-34c	Yes	ND	ND	[31, 33]
miR-101	Yes	COX-2, FOS	Proliferation	[24, 34, 35]
		MCL1	Apoptosis	
		EZH2	Invasion, migration	
miR-103		ND	ND	[24]
miR-106b		p21	Cell cycle progression	[24, 36, 37]
			Proliferation	
		BIM	Apoptosis	
miR-125a		ERBB2	Proliferation	[24, 38]
miR-130a		ND	ND	[24]
miR-133		ND	Proliferation	[30]
miR-141	Yes	FGFR2	Proliferation	[24, 39]
miR-200a		ZEB1, ZEB2	EMT	[24, 40, 41]
miR-200b		BCL2, XIAP	Apoptosis	[24, 40–42]
		ZEB1, ZEB2	EMT	
miR-200c		BCL2, XIAP	Apoptosis	[24, 41, 42]
			EMT	
miR-203	Yes	ABL1	Proliferation	[24, 43]
			Invasion	
miR-204		EZR	Proliferation	[24, 44]
miR-210	Yes	ND	ND	[24]
miR-214		ND	ND	[24]
miR-218	Yes	ECOP	Proliferation	[45, 46]
			Apoptosis	
		ROBO1	Invasion, metastasis	
miR-320		ND	ND	[24]
miR-370		FOXM1	Proliferation	[47]
miR-371-5p		LATS2	Cell cycle progression	[48]
miR-372		LATS2	Cell cycle progression	[48]
miR-373		LATS2	Cell cycle progression	[48]

Table 23.1 (continued)

MicroRNAs	Downregulated in gastric cancer	Target mRNAs	Biological process targeted	Reference
miR-375	Yes	PDK1, 14-3-3	Apoptosis	[24, 49, 50]
		JAK2	Proliferation	
miR-377		ND	ND	[24]
miR-379		ND	ND	[24]
miR-429		BCL2, XIAP	Apoptosis	[24, 42, 44]
		MYC	Proliferation	
miR-449	Yes	GMNN, CCNE2, MET, SIRT1	Cell cycle progression	[51, 52]
miR-455		ND	ND	[24]
miR-491-5p		ND	ND	[24]
miR-500		ND	ND	[24]
miR-532		ND	ND	[24]
miR-652		ND	ND	[24]

Modified from Noto et al. [16]
EMT epithelial-to-mesenchymal transition, *ND* target mRNA or biological process not determined

Table 23.2 MicroRNAs upregulated in response to *Helicobacter pylori*

MicroRNAs	Upregulated in gastric cancer	Target mRNAs	Biological process targeted	Reference
miR-17	Yes	p21	Cell cycle progression	[53]
miR-20a	Yes	p21	Cell cycle progression	[53]
miR-21	Yes	PTEN, PDCD4	Apoptosis	[54, 55]
		RECK	Metastasis, angiogenesis	
miR-25	Yes	ND	ND	[55, 56]
miR-93		ND	ND	[55]
miR-146a	Yes	IRAK1,TRAF6	Immune response	[18, 57, 58]
			Proliferation	
		SMAD4	Apoptosis	
miR-155	Yes	IKK-ε, SMAD2	Immune response	[59–62]
		FADD, PKIα	Apoptosis	
miR-194		ND	ND	[55]
miR-196	Yes	ND	ND	[55, 63, 64]
miR-200b		ZEB1	EMT	[65, 66]
miR-200c		ZEB1	EMT	[28, 65]
		ND	ND	
miR-222		RECK	Metastasis, angiogenesis	[67]
miR-223	Yes	EPB41L3	Invasion, metastasis	[24, 60]
miR-584		PPP2a, FOXA1	EMT	[42]
miR-1290		NKRF, FOXA1	EMT	[42]

Modified from Noto et al. [16]
EMT epithelial-to-mesenchymal transition, *ND* target mRNA or biological process not determined

response, (2) promoting cell cycle progression, (3) inhibiting apoptosis and promoting proliferation, and (4) promoting invasion and metastasis of gastric cancer [16].

23.3.1.1 Modulation of Host Inflammatory Immune Response

H. pylori can dysregulate miRNA expression to evade host defenses and successfully persist in the gastric niche. For example, *H. pylori* upregulates the expressions of miR-146a and miR-155, both of which modulate the innate and adaptive immune responses in a nuclear factor (NF)-κB-dependent manner [18, 57, 61, 62]. MiR-146a targets the Toll-like receptor (TLR) signaling adaptor molecules, interleukin-1 receptor-associated kinase (IRAK1), and TNF receptor-associated factor (TRAF6) [18, 57]. MiR-155 targets myeloid differentiation primary response gene (MyD88), the universal adaptor protein used by TLRs to activate NF-κB [61, 62]. As a result, both miR-146a and miR-155 overexpressions negatively regulate *H. pylori*-induced IL-8, TNF-α, IL-1β, growth-related oncogene (GRO)-α, and macrophage inflammatory protein (MIP)-3α expression, all key components to the pro-inflammatory innate and adaptive immune responses [16].

23.3.1.2 Promotion of Cell Cycle Progression

Several miRNAs dysregulated by *H. pylori* infection promote cell cycle progression by upregulating cyclin expression and/or downregulating expression of cyclin-dependent kinase (CDK) inhibitors (p15, p16, p18, p19, p21, p27, p28, p57) in gastric cancer [16]. The cell cycle consists of 4 distinct phases: G1, S, G2, and M. Two key classes of regulatory molecules, cyclins and CDKs, determine a cell's progress through the cell cycle. CDK inhibitors prevent the progression of cell cycle and function as tumor suppressors. miRNAs such as miR-106b and miR-449 target cyclins and CDKs as well as CDK inhibitors to disrupt normal cell cycle progression [36, 51, 52]. *H. pylori* is believed to modulate the expressions of cyclins, CDKs, and CDK inhibitors through dysregulation of host miRNAs. Thus it may induce gastric carcinogenesis [16].

23.3.1.3 Inhibition of Apoptosis and Promotion of Proliferation

miRNA dysregulation induced by *H. pylori* infection inhibits apoptosis and promotes cell survival. For example, miR-21 is overexpressed in gastric cancer tissues and cell lines, as well as in *H. pylori*-infected noncancerous gastric mucosae. It was also upregulated in cultured gastric epithelial cell lines cocultured with *H. pylori* [54]. Overexpression of miR-21 promoted cell proliferation and migration and inhibited apoptosis in this cell line. Activator protein (AP)-1 and the signal transducer and activator of transcription 3 (STAT3) can induce the expression of miR-21. *H. pylori* infection induces NF-κB and IL-6 secretion in gastric mucosae, which activate AP-1 and STAT3, respectively, which explains the upregulation of miR-21 during *H. pylori* infection. In addition, miR-21 targets phosphatase and tensin homolog (PTEN) and programmed cell death protein 4 (PDCD4) [26, 75–77].

23.3.1.4 Promotion of Tumor Invasion and Metastasis

H. pylori-induced dysregulation of specific miRNA may play a role in angiogenesis, invasion, and metastasis of gastric cancer. For example, *H. pylori* infection negatively regulates miR-449 during gastric carcinogenesis, which leads to the upregulation of Met, a known proto-oncogene. Upregulation of Met is known to promote not only proliferation but also angiogenesis, invasion, and metastasis of cancer [51, 52]. MiR-218 is known to be inhibited in gastric cancer, in relation to invasion and metastasis [78]. It might be attributed to roundabout homolog 1 (ROBO1) signaling pathway that has been implicated in the regulation of cell migration [46]. As mentioned above, *H. pylori* infection induces overexpression of miR-21. miR-21 has been reported to enhance the invasiveness of gastric cancer cells, which is attributed to inhibit reversion-inducing cysteine-rich protein with Kazal motifs (RECK), a tumor and metastasis suppressor that inhibits tumor

metastasis and angiogenesis through modulation of matrix metalloproteinases (MMPs) [54]. *H. pylori* infection is known to induce the expression of MMPs, including MMP-1, MMP-2, MMP-3, MMP-7, and MMP-9 [79]. So *H. pylori* has the potential to modulate expression of MMPs through dysregulation of host miRNAs [16].

Conclusions

Aberrant DNA methylation and the dysregulation of numerous miRNAs by chronic active *H. pylori* infection play an important role in gastric carcinogenesis. Alterations of these epigenetic changes in gastric mucosae or biological fluids have diagnostic and therapeutic potentials in gastric cancer, but further studies are necessary to validate these potentials in large clinical trials.

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Gastric Cancer: *H. pylori* and Macrophage Migration Inhibitory Factor

24

Kichul Yoon

Abstract

Macrophage migration inhibitory factor (MIF) is known to have an important role in angiogenesis, lymph node metastasis, tumor invasion, and distant metastasis according to various studies. As MIF has been reported to be upregulated in gastric cancer epithelial cell, measurement of the cytokine might be helpful for the diagnosis and the prediction of prognosis. *Helicobacter pylori* (*H. pylori*) is reported to promote the proliferation of gastric cancer cell line via stimulation of MIF expression; however, contradictory studies necessitate further investigation. Possible factors that could link *H. pylori* and MIF are cytokine receptor CD74, interleukin (IL)-6, cytokines for angiogenesis, reactive oxygen species, and autophagy. The role of MIF in carcinogenesis of gastric cancer in relation to *H. pylori* could be a potential research topic.

Keywords

Gastric cancer • *Helicobacter pylori* • Macrophage migration inhibitory factor

24.1 Introduction

Tumor microenvironment has become an important concept in explaining tumorigenesis. Among the factors known to be involved, macrophage migration inhibitory factor (MIF) has been shown to be related to various types of malignancy such as esophageal cancer, ovarian cancer, hepatocellular carcinoma, non-small cell lung cancer, and prostate cancer [1–3]. MIF has been reported to have role in angiogenesis, lymph node metastasis, and distant metastasis [2, 4, 5]. Increased epithelial and serum expression of MIF in gastric cancer suggested its role in diagnosis and prediction of prognosis. MIF as a possible link between *H. pylori* and gastric cancer is described here.

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24.2 Introduction of Macrophage Migration Inhibitory Factor

MIF is one of the first cytokines that have ever been discovered. It was described as a certain T-lymphocyte-derived factor that could inhibit the migration of monocyte and macrophage. Thereafter, MIF has been shown to be related to delayed-type hypersensitivity. As three-dimensional structure of MIF was revealed, CD74 – the receptor for MIF – was cloned and the co-receptor CD44 was found [6–8]. MIF is involved in signal transduction, and it continuously stimulates ERK1 and ERK2 MAP kinase, which property was related to carcinogenesis [9]. In addition to the important role in innate immunity, MIF-related continuous and repetitive inflammation has been associated with tumorigenesis as well [5].

24.3 Role of Macrophage Migration Inhibitory Factor in Tumorigenesis and Tumor Progression

There have been various evidences supporting the role of MIF in tumorigenesis and tumor progression. As shown in Table 24.1, the relationship between MIF and cancer such as non-small cell lung cancer, breast cancer, colorectal cancer, prostate cancer, esophageal cancer, hepatocellular carcinoma, and ovarian cancer has been suggested [10–16]. In gastric cancer, activity of MIF increases during the progression from normal epithelium to carcinoma [17–19]. Figure 24.1 shows the possible molecular biological pathway of MIF involved in tumorigenesis. MIF has a role in cell proliferation via signaling cascade, especially through Ras-related pathway. MIF also negatively affects the well-known tumor suppressor p53 by inhibiting its antiproliferative property and the role in apoptosis. High concentration of MIF expressed by dysplastic or inflammatory cells let the microenvironment bypass the p53 pathway, cumulating mutations via cellular proliferation, elongation of cellular life span, and inhibition of cell death [2, 5, 19, 37–42]. MIF inhibits activity of p21, cyclin G1, and Mdm2 [9]. MIF-deficient cells did not proliferate following the exposure to tumorigenic stimuli; it could be related to E2F and p53 tumor suppressor RB [43]. In Eμ-Myc lymphoma model, MIF deficiency led to delayed

Table 24.1 MIF and cancer

Cancer	MIF	References
Non-small cell lung cancer	Angiogenesis	[12, 20]
Breast cancer	Angiogenesis, interaction between breast cancer cell and stroma, autophagy regulation	[4, 11, 21]
Colon cancer	Promotor of the cancer; determinant of hypoxia-induced apoptosis	[22, 23]
Prostate cancer	Upregulated in prostate cancer	[24–28]
Esophageal cancer	Differentiation and lymph node status	[15]
Ovarian cancer	Prognostic factor; secreted from cancer cells	[13, 29, 30]
Hepatocellular carcinoma	Metastasis, angiogenesis	[2, 14]
Stomach cancer	Progressive increase from normal cells to cancer	[17, 18, 31–36]

MIF macrophage migration inhibitory factor

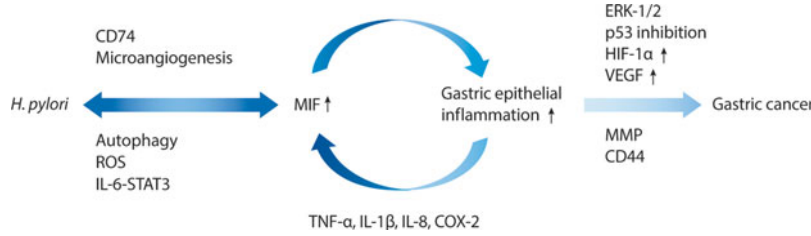

Fig. 24.1 Possible connection between *H. pylori*, macrophage migration inhibitory factor (*MIF*), and gastric cancer

lymphomagenesis [44]. Angiogenetic activity of MIF is well known. In B cell lymphoma animal model, administration of anti-MIF antibody inhibited tumor growth, especially the angiogenesis was affected [45]. Similar results were shown in non-small cell lung cancer and colorectal cancer model due to the interaction between MIF and CXC chemokine, interleukin (IL)-8, or vascular endothelial growth factor (VEGF) [2, 20].

MIF also enhances cell proliferation by upregulating HIF-1 and tumor invasion through matrix metalloproteinase. Its role in chronic inflammation has been associated with the receptor CD74 and co-receptor CD44 [3, 5, 18, 19, 37–39, 41].

24.4 *H. pylori* and Macrophage Migration Inhibitory Factor

The relationship between *H. pylori* and MIF has been sought in various aspects including cell line research and clinical study with human tissue. Increased expression of MIF by epithelial cells, T cells, and macrophages in stomach mucosa was reported to be associated with *H. pylori* infection. The difference in distribution of MIF-positive cells between antrum and corpus was also reported [46].

According to a cell line research, *H. pylori* directly stimulated MIF secretion from monocytes, resulting in gastric cell proliferation through its *cag* PAI. The effect was blocked with anti-MIF antibody, suggesting the role of MIF as a mediator of *H. pylori* 's tumorigenic effect [47–51]. A clinical study showed progressive increase of epithelial and serum MIF in *H. pylori*-induced gastritis, intestinal metaplasia, and gastric cancer. It suggested the potential role for MIF as a biomarker of gastric cancer [18]. Significant relationship between *H. pylori* and MIF is further supported by the report that eradication of *H. pylori* reduced MIF in the patients [49]. A clinical study showed a possibility of MIF as a biomarker for gastric cancer. Serum MIF level in the patients had diagnostic value better than carcinoembryonic antigen (CEA) and even correlated with the 5-year survival when combined with CEA [31]. MIF was also upregulated in acute gastric ulcer rat model [52]. MIF, released upon *H. pylori* infection, induced phosphorylation of epidermal growth factor receptor (EGFR) [40]. However, the relationship between *H. pylori* and MIF needs to be interpreted carefully. A report found that in human gastric epithelial cells, MIF expression and secretion did not directly increase after *H. pylori* infection, although IL-8 expression and secretion were upregulated [51].

24.5 Potential for Future Studies

Previous studies showed clues for the role of MIF in gastric tumorigenesis. However, exact mechanism remains elusive. MIF could potentially be an interesting topic especially in relation to *H. pylori*. Here are some plausible topics for future research. First, it is interesting that CD74 makes up the receptor for MIF, and it also serves as the receptor for *H. pylori* at the same time [53–55]. This protein molecule could be a target to prevent and treat gastric cancer. It is also well known that MIF helps microangiogenesis. To investigate whether *H. pylori* is involved in this specific process could be also meaningful [56].

Its role in IL-6/JAK2/STAT3 pathway could be another target of research, especially with Th17 (IL-17) as a positive regulator through Akt [38, 57]. According to a recent study on nasal polyp, inhibition of MIF enhanced suppressive effect of dexamethasone on IL-6 [37]. It suggested that MIF inhibitor could be a novel therapy for the disease. Positive relationship of IL-6 to MIF might be another plausible topic in gastric cancer research.

In addition, MIF with autophagy can be interesting object. In human hepatoma cell line, MIF was reported to induce autophagy through reactive oxygen species generation. On the contrary, MIF was found to inhibit autophagy in breast cancer. Their interaction in gastric cancer, especially through *H. pylori*, is not yet clear [58–61].

Conclusions

MIF has been suggested to be involved in tumorigenesis of various types of cancer. Its positive interaction with *H. pylori* could be an attractive research topic in gastric cancer.

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Gastric Cancer: Epithelial Mesenchymal Transition

25

Yoon Jin Choi and Hyeon Jang

Abstract

An epithelial-mesenchymal transition (EMT) induces loss of epithelial cell polarity and change of cell phenotype from typical cubical shape into fibroblast-like shape. Since the ability to migrate is obtained as a result of EMT, this has been an essential mechanism for cancer invasion and metastatic spreads. During EMT process, E-cadherin, a typical epithelial cell marker, is decreased, and hallmarks of mesenchymal cells are increased. Most studies about EMT in oncologic field have been focused on the role of EMT hallmarks as a prognostic marker. However, recent evidence has shown that EMT plays an important role in tumorigenesis and progression. In addition, EMT has taken center stage as the convergence point between inflammation and cancer.

This review will highlight the general concept of EMT, brief summary of the regulators and signal pathways involved in EMT, and the association between EMT and cancer including both carcinogenesis and progression. We also investigate the role of EMT in gastric cancer related with *Helicobacter pylori* infection.

Keywords

Helicobacter pylori • Epithelial-mesenchymal transition • Cancer stem cell

25.1 Introduction

Epithelial-mesenchymal transition (EMT) is a process that epithelial cells become mesenchymal stem cells. It is an example of cell plasticity, which is one cell could be changed to another type of cell. Epithelial cells have cell junctions: tight junctions, adherent junctions, desmosomes, hemidesmosomes, and gap junctions. And epithelial cells are tightly packed. As a result, epithelial

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cells exhibit apical-basal polarity and have role for protection, secretion, selective absorption, and cell-cell transduction [1]. But mesenchymal cells are detached and have cellular mobility with absence of polarity. Also it can be differentiated to functional cells: osteoblast, chondrocyte, and adipocyte [2, 3]. Cuboidal epithelial cell phenotype would lose cellular polarity and change into the type of fibroblast. And expressions of epithelial markers are decreased, and those of mesenchymal cell are increased.

E-cadherin is distributed in/out of cell membrane. It is typical epithelial cell marker and locates in adhering junction of epithelial cell and has several roles for cytoskeleton and maintenance of continuous sheet. Cadherin switch which occurs in early stage of EMT induces E-cadherin loss and increment of vimentin and N-cadherin. It results in phenotypical and functional change for weakening of cell-cell adhesion and enhancing cellular mobility [4]. Cellular microenvironment and tissue specificity can divide EMT into three types: embryogenesis, tissue regeneration and organ fibrosis, and carcinogenesis and metastasis [5].

This chapter explains overall regulation and inducers of EMT in specific type 3 EMT. The role for EMT in tissue invasiveness, metastasis, and gastric carcinogenesis is described as well. Then importance of *Helicobacter pylori* (*H. pylori*) infection as EMT inducers is shown.

25.2 Three Types of EMT

Sequential meetings in 2007 and 2008 had distinguished EMT into three types in tissue and function specific [6]. Type 1 EMT is involved with embryogenesis and appropriate organ development. Type 2 EMT is engaged in tissue regeneration and organ fibrosis [7]. Type 3 EMT is associated with cancer progression, tissue invasiveness, metastasis, recurrence, and resistance. Also metastatic cancer cell can get mesenchymal phenotype through type 3 EMT [8, 9]. While the distinct functions are apparent in different tissues, specific signals driven in these EMTs are similarly induced by genetic and biochemical factor in cells.

25.2.1 Type 1: Embryogenesis

Type 1 EMT regulates cellular growth of mesenchymal phenotype cell which associates implantation, embryogenesis, and organ development. Especially, secondary epithelium which can induce mesenchymal-epithelial transition (MET) would arise from this type 1 EMT [10, 11] (Fig. 25.1a).

25.2.2 Type 2: Tissue Regeneration and Organ Fibrosis

Type 2 EMT is engaged to tissue regeneration after wound and inflammatory tissue degradation. Fibroblast and inflammatory cells are involved in tissue regeneration, and after wound healing, type 2 EMT would be ceased. But chronic inflammation caused by recurrence of infection, autoimmune, and allergy would induce excessive tissue regeneration and results in tissue fibrosis [12–14] (Fig. 25.1b).

25.2.3 Type 3: Invasiveness and Metastasis of Cancer

Relationship between chronic inflammation and carcinogenesis informs that EMT would increase risk of carcinogenesis [13]. Mutation of oncogene and tumor suppressor gene induces serial changes of EMT signaling, and this distinguishes distinct physiological phenotypes from type 1 and 2 EMTs. After the initiation of type 3 EMT, tumor cell can invade into the circulatory system, metastasize in other organs, and finally promote newly formed tumor (Fig. 25.1c).

As EMT is progressed, tumor cell lose epithelial cell markers, E-cadherin and cytokeratin, and acquire mesenchymal cell markers, α-smooth muscle actin (α-SMA), N-cadherin, and vimentin [9] (Fig. 25.2). This newly changed tumor cells are observed in border between central region of cancer and surrounding tissues. Then tumor cell finally establishes micrometastasis after migration [15].

Many researches try to disclose the gene regulation and biochemical mechanism which cause

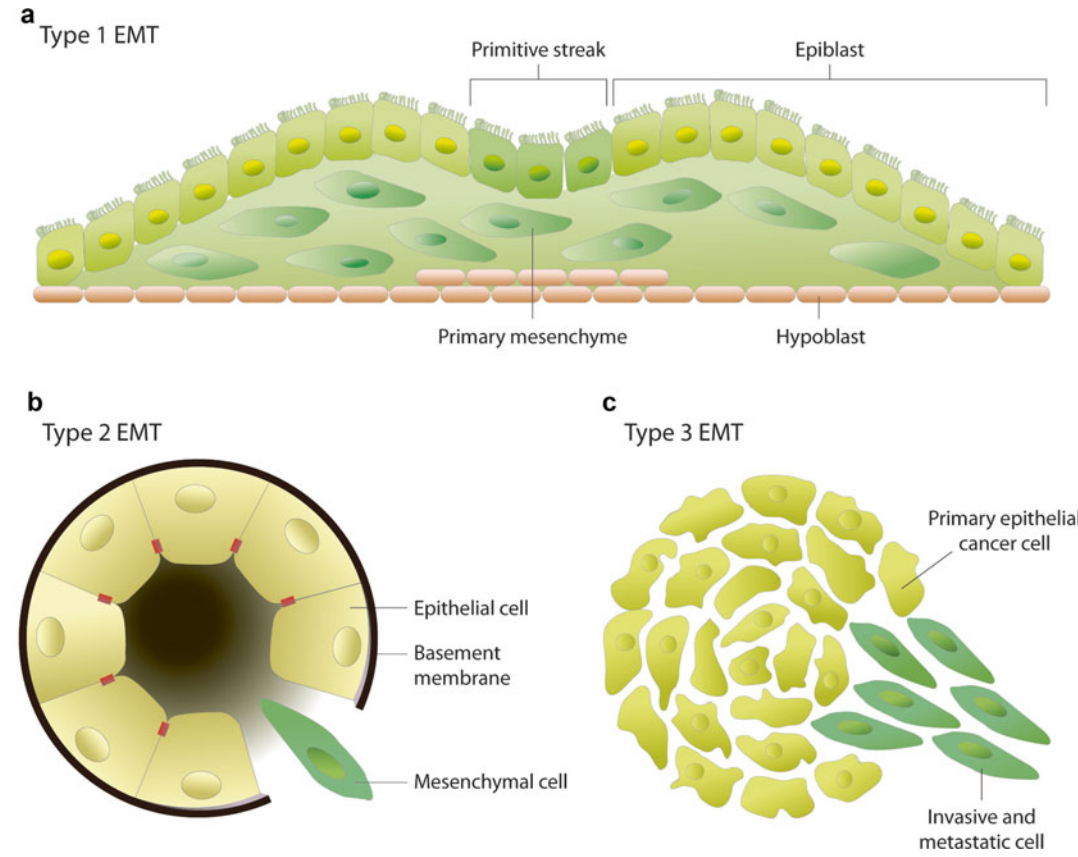

Fig. 25.1 Three types of EMT. (**a**) Type 1 EMT associated with embryogenesis and organ development. (**b**) Type 2 EMT associated with tissue regeneration and organ fibrosis. (**c**) Type 3 EMT associated with cancer progression and metastasis. *EMT* epithelial-mesenchymal transition (Adapted from Kalluri et al. [9])

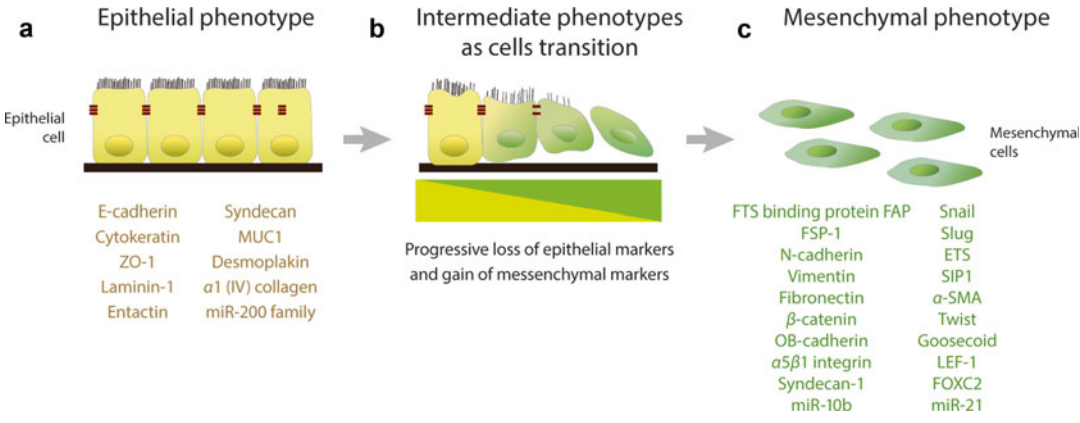

Fig. 25.2 Change of EMT marker expression. (**a**) Epithelial cell markers. (**b**) Epithelial cell markers decreased and mesenchymal cell markers increased in EMT. (**c**) Mesenchymal cell markers. *ZO-1* zona occludens 1, *MUC1* mucin 1, *SIP1* Smad-interacting protein 1, *α-SMA* α-smooth muscle actin, *FOXC2* forkhead box C2 (Adapted from Kalluri et al. [9])

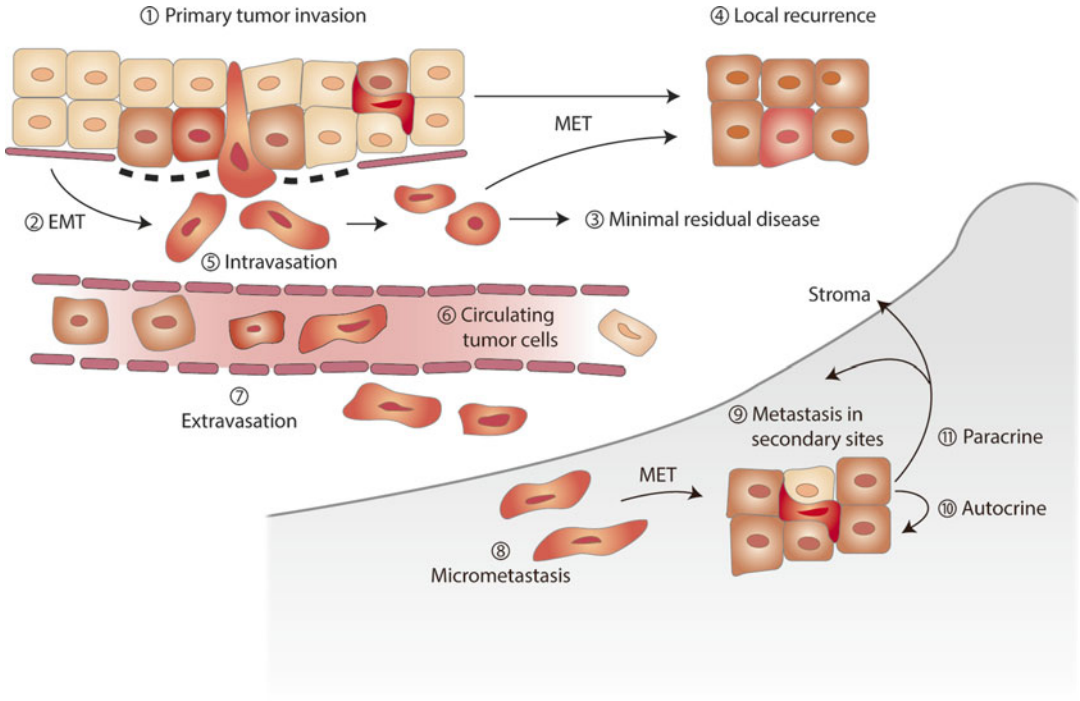

Fig. 25.3 Metastasis procedure of EMT and/or MET. (*1*) Primary tumor invasion. (*2*) Tissue invasion after EMT. (*3*) Minimal residual disease occurs as well. (*4*) MET occurs in residual mesenchymal cell. And local recurrence arises. (*5*) Mesenchymal cell easily achieves intravasation. (*6*) Circulating tumor cells can exist as epithelial cell and/or mesenchymal phenotype cell. (*7*) Mesenchymal cell easily achieves extravasation. (*8*) Tumor cell can exist as micrometastasis. (*9*) After incubation period, metastasis in secondary sites and MET occur. (*10*) Autocrine and (*11*) paracrine would promote metastatic tumor cell growth (Adapted from Thompson and Haviv [17])

proliferation of tumor cell, and most of those studies recommend that activation of EMT could be a reason for the malignant alteration in epithelial tumor cell [16]. Moreover, metastasized tumor cells do not have mesenchymal cell phenotype in secondary site after EMT progression, but they have the epithelial tumor cell phenotype which is shown in the primary site. Thus, in addition to the metastasis promotion ability of EMT, it is needed to comprehend that MET would induce loss of mesenchymal cell phenotypes after metastasis [17] (Fig. 25.3).

It is implicated that EMT progression in metastatic cancer cell would associate with cellular microenvironment, but further studies are needed [18]. Also the mechanisms for mesenchymal cell phenotype acquisition of non-epithelial cell are not yet clear. But EMT is regarded as principal mechanism for metastatic cancer progression as well [19].

25.3 Major Signal Pathways of EMT

It is impossible to understand the whole EMT signaling of tumor cells, but most persuasive hypothesis suggests that genetic and epigenetic signals which occurred in early stage of carcinogenesis would change tumor cells to be susceptible to the EMT. And inhibition of tumor cell senescence would associate with early step of carcinogenesis in EMT progression [20, 21]. Various EMT-inducing signals, hepatocyte growth factor (HGF), epithelial growth factor (EGF), platelet-derived growth factor (PDGF), and transforming growth factor (TGF)-β, can promote EMT in basal site of tumor and activate several transcription factors: Snail, Slug, zinc finger E-box binding homeobox 1 (ZEB1), and Twist [8, 22–25] (Fig. 25.4). Activation for transcription factors needs mitogen-activated protein

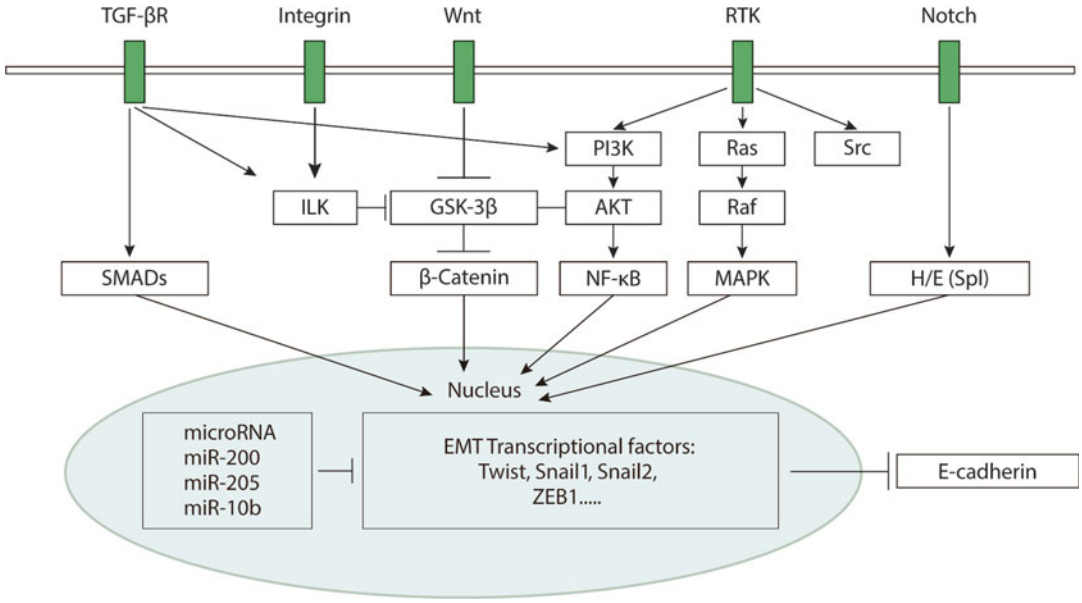

Fig. 25.4 Scheme of major EMT induction signal. TGF-β transduces through SMAD and PI3K/AKT. Wnt ligand inhibits β-catenin senescence. Accumulated β-catenin can enter into the nucleus and increase transcription factors Slug and Snail. Integrin signal induces hyperactivation of ILK, and this helps β-catenin invagination to nucleus. RTK signal induces EMT through Ras-Raf-MAPK or PI3K/AKT. *AKT* serine/threonine kinase, *GSK-3β* glycogen synthase kinase-3β, *H/E* (*Spl*) Hairy and enhancer of split, *ILK* integrin-linked kinase, *MAPK* mitogen-activated protein kinase, *NF-κB* nuclear factor-κB, *PI3K* phosphatidylinositol 3′ kinase, *RTK* receptor tyrosine kinase, *TGF-βR* transforming growth factor-β receptor, *ZEB1* zinc finger E-box binding homeobox 1 (Adapted from Iwatsuki et al. [8])

kinase (MAPK), phosphatidylinositol 3′ kinase (PI3K), serine/threonine kinase (Akt), glycogen synthase kinase-3β (GSK-3β), integrin-linked kinase (ILK), Smads, RhoA, Par6, β-catenin, and Ras signaling which interacts with signal transduction protein [26] (Fig. 25.5). Disruption of cell-cell adhesion or cell-basal adhesion by integrin also promotes EMT program activation [27–30].

TGF-β/Smad signaling, the central axis of type 3 EMT, and Wnt/β-catenin signaling and Notch signaling are introduced next.

25.3.1 TGF-β/Smad Signaling

TGF-β can inhibit unregulated proliferation of epithelial cells or induce carcinogenesis and metastasis in different environments. Also TGF-β is the most well-known EMT inducer. It would activate EMT via two kinds of cellular signaling (Smad/non-Smad) [26, 31–33] (Fig. 25.5).

25.3.1.1 Smad Signaling

When TGF-β attaches to type I, type II activin receptor-like-kinase (ALK) receptor and intrinsic serine/threonine kinase are activated. Smad2 and Smad3 are phosphorylated and attach with Smad4 and achieve the invagination into the nucleus [26] (Fig. 25.5). After that, transcription factors such as Snail expression would regulate the expression of connective tissue growth factor, α-SMA, plasminogen activator inhibitor-1, and collagen 1A2 [34–37].

25.3.1.2 Non-Smad Signaling

Non-Smad signaling induces EMT with various cells and microenvironment-specific proteins. Activation of these signal transduction proteins is overlapped in EMT. RhoA and MAPK mediate EMT which is induced by TGF-β in animal epithelial cell [37–40]. At the same time, PI3K activation after Ras would inhibit cellular apoptosis that is induced by TGF-β [41–44]. And COX-2 inhibits Smad signaling and causes

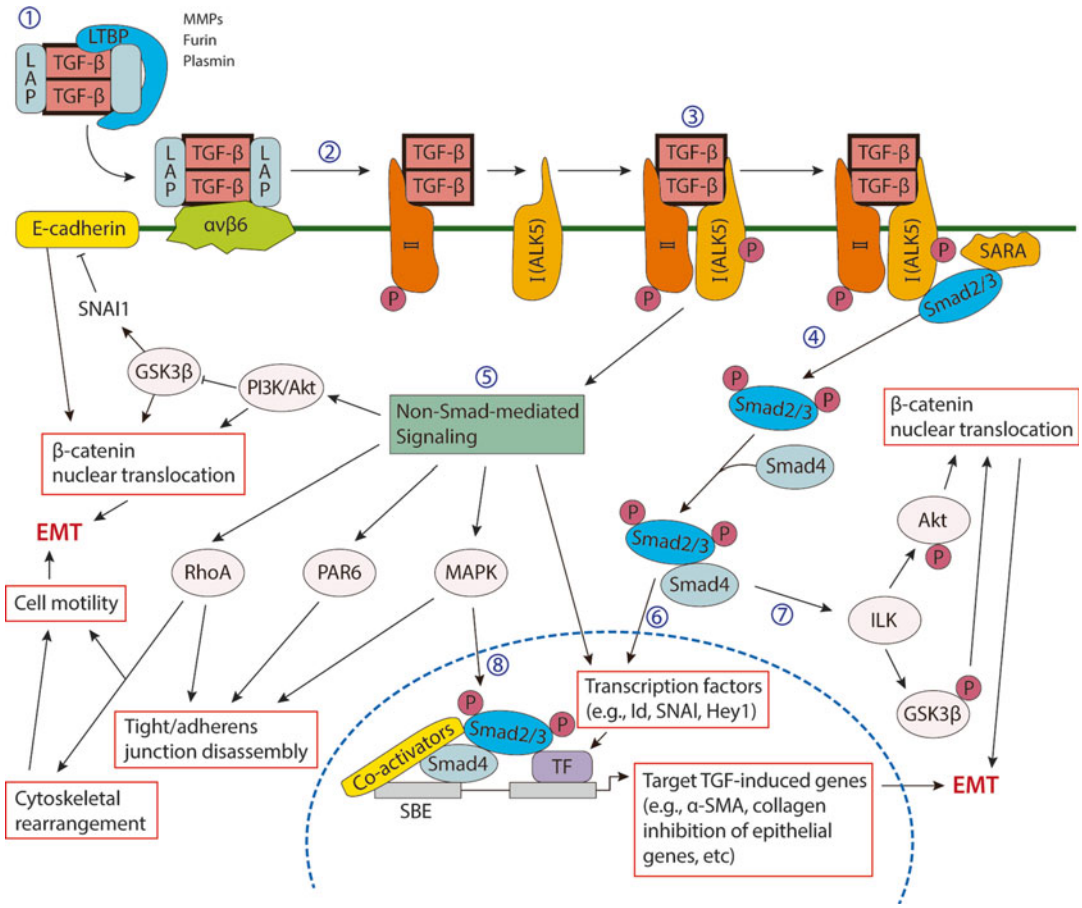

Fig. 25.5 EMT induced by transforming growth factor (TGF)-β1 signaling. (*1*) TGF-β is released. (*2*) TGF-β dimers fuse with TGF-β receptors (e.g., ALK-5). (*3*) Activation of receptor heterodimer promotes (*4*) Smad (Smad2/Smad3) and/or (*5*) non-Smad signaling. (*6*) Smad signaling activates Snail1, Snail2, Notch transcription factor, and TGF-β-induced target gene (e.g., α-smooth muscle actin, collagen, plasminogen activator inhibitor-1, connective tissue growth factor) and inhibits epithelial cell gene (e.g., E-cadherin). (*7*) Smad signaling also activates ILK, and this results in Akt/GSK3B activation. Finally β-catenin can enter into the nucleus and induce further EMT program activation. (*5*) Non-Smad signaling induces activation of PI3K/Akt, RhoA, PAR6, and MAPK and disruption of tight junction/adhering junction and rearrangement of cytoskeleton and inhibits E-cadherin and promotes of β-catenin invagination into nucleus. (*8*) Smad signaling and non-Smad signaling interact with MAPK. *SNAI1* snail family zinc finger 1, *LTBP* latent TGF binding protein, *LAP* latency-associated peptide, *GSK3β* glycogen synthase kinase-3β, *ILK* integrin-linked kinase, *SARA* Smad anchor for receptor activation, *RhoA*, Ras homolog gene family, member A, *PAR6* partitioning defective 6, *Id* inhibitor of DNA binding 1, *Hey1* Hairy/enhancer of split related with YRPW motif protein 1 (Adapted from Willis and Borok [26])

hyperexpression of prostaglandin E2. Then, this protein finally enhances EMT signaling induced by TGF-β [45, 46].

25.3.2 Wnt/β-Catenin Signaling

When Wnt signal is activated and β-catenin is dephosphorylated, β-catenin in cell-cell adhesion would achieve invagination into the nucleus. Then epithelial cells have mesenchymal cell phenotypes. Thus, expression of β-catenin in cytoplasm and inhibition of its activation are essential to keep the epithelial tumor cell phenotype [47]. Accumulation of β-catenin in nucleus is related with loss of E-cadherin expression and highly correlated with intravasation phenotype after EMT [48, 49].

25.3.3 Notch Signaling

Notch signaling has an important role in the birth of cancer stem cells. The hyperexpression and hyperactivation of Notch protein are reported in various solid cancers including gastric cancer. And Notch is associated with Snail, Slug, and TGF-β [49].

25.4 EMT and Gastric Cancer

25.4.1 Association Between Gastric Cancer and EMT

Although the incidence of gastric cancer is definitely decreasing, gastric cancer is the second most common cause for cancer-related death. Gastric adenocarcinoma accounts for 90 % of gastric cancer, and this is divided into intestinal type and diffuse type according to Lauren classification. Even though there is a study which reported that different mechanisms underlie for different Lauren types, downregulation of E-cadherin seems to be an essential component for EMT-related carcinogenesis. That is, (1) genetic abnormality, mutation, and deletion, (2) upregulation of E-cadherin suppressing transcription factor, and (3) epigenetic regulation (i.e., microRNA (miRNA) or hypermethylation of specific genes) result in a functional and morphological decline of E-cadherin [50]. In addition, amplification or mutation of receptors in other EMT signal pathways could contribute to a promotion of EMT leading to gastric cancer.

25.4.2 EMT Factors Related with Gastric Cancer

25.4.2.1 Regulation of E-Cadherin

Functional Loss Through CDH1 Mutation

CDH1 gene at human chromosome 16q22.1 encodes E-cadherin [50]. Mutation, deletion, and CpG hypermethylation of *CDH1* gene in gastric cancer have been reported [51]. Germline mutations of *CDH1* result in hereditary diffuse gastric cancer. This genetic mutation also leads to inactivation of other allele located in patients with familial gastric cancer of diffuse type.

Repression for the Transcription of CDH1

Snail, Slug, Smad-interacting protein 1 (SIP1), and Twist act as repressors for the transcription of *CDH1*, and this leads to EMT [24]. It is reported that there was an inverse correlation between the abovementioned transcription factors and CDH1 in various types of cancer. These facts indicated that Snail, Slug, SIP1, or Twist was implicated in the EMT in gastric cancer through the transcriptional repression of the *CDH1* gene:

Snail According to the study which evaluated the expression of Snail and E-cadherin in human gastric cancer mucosa, downregulation of E-cadherin (up to 40 %) and upregulation of Snail and N-cadherin were reported in diffuse type without of changes in SIP1 expression [52]. Contrary to this, approximately 60 % of patients with intestinal-type gastric cancer showed a decrease in E-cadherin expression and an increase in SIP1 without any change in expressions of Snail, Twist, or N-cadherin. These results suggested that expression of Snail was associated with suppression of E-cadherin in diffuse-type gastric cancer, while SIP1 inhibit E-cadherin expression. Different regulators may act according to histological type of gastric cancer [53, 54].

Slug Transcription factor Slug is included in Snail family. It represses the expression of E-cadherin as well. It is well known that Slug expression is considerably increased in intestinal type and diffuse type of gastric cancer. Also other studies elucidated that Slug expression in gastric cancer tissue is higher than that of normal tissue about 70 %, and it is statistically significant. Increased Slug expression is correlated with the loss of E-cadherin [52].

Twist The expression of helix-loop-helix transcription factor, Twist, and N-cadherin is mainly found in diffuse-type gastric cancer. Repression of Twist in gastric cancer cell resulted in loss of N-cadherin expression and decrease of cell

mobility. However, when N-cadherin expression is increased, cell mobility would increase, and E-cadherin expression is repressed [55]. Thus, it is identified that Twist is a key factor for induction of "cadherin switch" which shows the change of E-cadherin into N-cadherin, and it would increase cancer cell mobility [56]. The fibroblast-specific protein 1 and CXCL14 are highly expressed in fibroblast of gastric cancer tissue as Twist expression. Also Twist mRNA expression is correlated with PDGF receptor-α and receptor-β. This means that high expression of Twist would increase EMT sensitivity of gastric cancer tissue, and it consequently promotes cancer cell development and metastasis [57].

25.4.2.2 Epigenetic Regulation of EMT via miRNA

Small RNA, miRNA, consists of 17–22 nucleotides, and it represses gene expression by irreversible attachment on mRNA in mRNA transcription. Studies which identified role of miRNA in metastatic cancer also gave the information of EMT-regulating miRNAs [58] (Fig. 25.6 and Table 25.1). MiR-27 is mainly studied for its relation on gastric cancer. It is usually upregulated in gastric cancer tissue compared to normal tissue [59]. MiR-27 would increase the mobility of AGS, the cancer cell line, and induce the expression of ZEB1, ZEB2, Slug, and vimentin so miR-27 finally induced EMT [60]. Also miR-27 expression would be increased from Wnt signal transduction, and it represses the activation of tumor suppressor gene adenomatous polyposis coli (APC) to promote metastasis of gastric cancer [60]. Other EMT-regulating miRNAs are included in miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) [58]. Because those miRNAs induce MET and repress EMT by regulating ZEB1 and ZEB2, the members of miR-200 family are known as EMT markers [58].

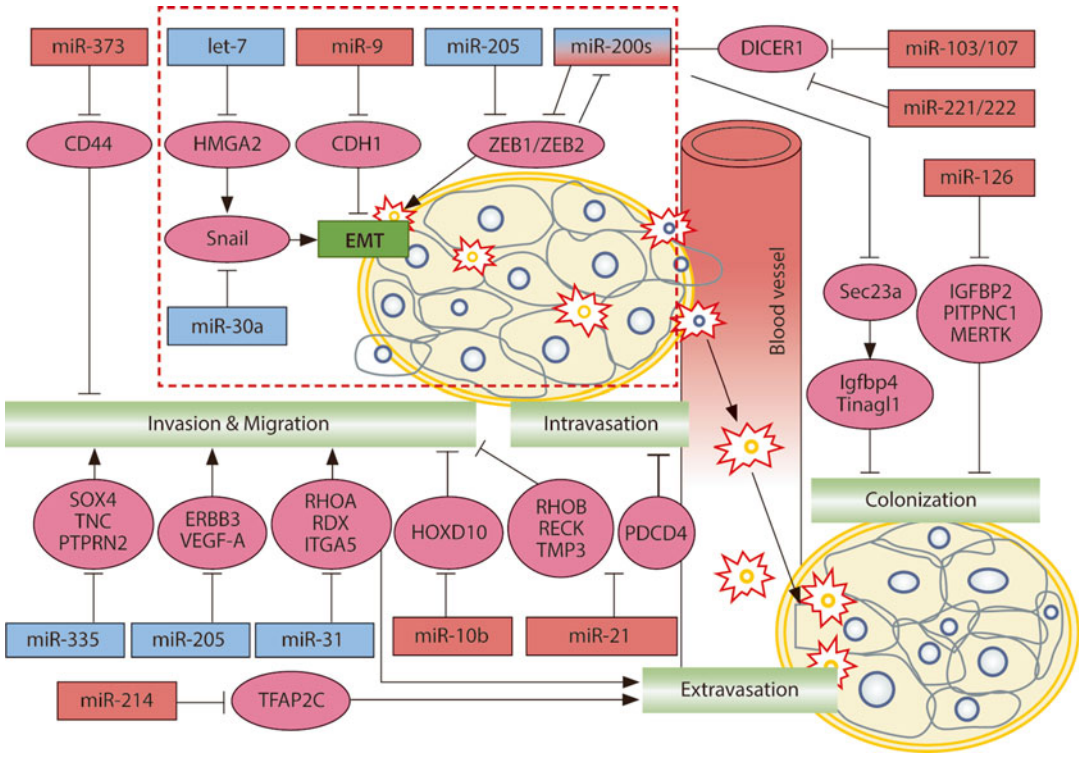

Fig. 25.6 miRNAs that regulate metastasis. Metastasis consists of multiple steps: epithelial-mesenchymal transition (*EMT*), local invasion, intravasation, extravasation, and colonization (as indicated by *green boxes*). MiRNAs and their target genes are indicated in *red/blue boxes* and *pink circles*, respectively. *Red box* metastasis-promoting miRNAs, *blue box* metastasis-suppressing miRNAs (Adapted from Zhang et al. [58])

Table 25.1 miRNAs involved in epithelial-mesenchymal transition/mesenchymal-epithelial transition

miRNA	Effect on EMT	Target
miR-9	Promote	CDH1
miR-15b	Suppress	BMI1
miR-27	Promote	APC
miR-29a	Promote	TTP
miR-30a	Suppress	Snail
miR-103/107	Promote	DICER1
miR-155	Promote	RHOA
miR-194	Suppress	BMI1
miR-200 family	Suppress	ZEB1/ZEB2, Sec23a
miR-205	Suppress	ZEB1/ZEB2
miR-204	Suppress	TGFβR2, SNAIL2
miR-221/222	Promote	TRPS1, ESR1, DICER1
miR-661	Promote	StarD10, Nectin-1

Adapted for Zhang et al. [58]

EMT epithelial-mesenchymal transition, *miR* microRNA

However, ZEB1 has double negative feedback structure so it can attach on the promoter of miR-200 [61]. Also upregulation of ZEB1/ZEB2 and reduction of miR-200 family resulted in decrease of E-cadherin expression within metastatic cancer tissue which has reduced E-cadherin expression [58]. In addition, miR-9 can repress mRNA of E-cadherin gene, *CDH1* [62], and miR-155 and induce EMT by upregulating ROHA [63]. But miR-204 is known as EMT repressor [64]. The most interesting part of gastric EMT is that *H. pylori* infection can repress miR-200 family and miR-204, but it increases miR-155 [65]. Therefore, it is implicated that *H. pylori* infection has an effect on the invasiveness and metastasis of gastric cancer with EMT-regulating miRNAs.

25.4.2.3 Other EMT Regulatory Factors

Vimentin

Vimentin is 58 kDa of intermediate filament protein, and it is not expressed in epithelial cell but in mesenchymal cell. Vimentin has a role for maintaining cytoskeleton. When vimentin is expressed, it interacts with other intermediate filament proteins (i.e., actin) by inducing EMT and changing epithelial cell into migratory mesenchymal cell.

Bone Morphogenetic Protein (BMP)

Bone morphogenetic protein (BMP) is included in TGF-β family, and it is known for its role for the production of osteoinductive cytokine and growth of bone and cartilage in embryonic development. BMP combines with phosphorylation domain of serine/threonine BMP I/II receptor, and it phosphorylates Smad 1/5/8. Normally phosphorylated Smad can intrude nucleus and interact with transcription factors to activate cellular signal transduction. However, abnormal regulation of BMP would induce metastasis, invasiveness, and resistance against apoptosis in gastric cancer cell. Especially, it is supposed that BMP-2 and BMP-4 are considerably related with tumor metastasis. Because BMP-2 makes and activates BMP-2 receptor by autocrine process, BMP receptors which are expressed on extracellular domain of cancer cell can bind with massive BMP-2 and promotes EMT of gastric cancer cell [66–68].

Claudin

Claudin is one of the constructive proteins which compose the tight junction. It can repress tumor growth by RUNX3/Smad signaling and usually hypermethylated in gastric cancer tissue. Claudin 1 expression is lowered in intestinal gastric cancer [69, 70].

Gastrokine

Gastrokine 1 is one of the gastric mucosal protective factors. Normally it acts as a repressor against gastric cancer. When the gastric cell lines were transformed to be overexpressed with gastrokine I, it repressed migration, metastasis of gastric cancer cell, and expression of reactive oxygen species (ROS) [71]. In addition, when gastrokine 1 is upregulated, it represses the PI3K/Akt signaling and EMT-related factors: β-catenin, Slug, Snail, fibronectin, and vimentin. And expression of gastrokine 1 would increase the expression of E-cadherin [71]. Thus, it is implicated that gastrokine 1 has a major role in gastric cancer development by regulating EMT and metastatic cancer cell repression.

25.4.3 EMT and *H. pylori*

Growing evidence that *H. pylori* induced the EMT process has been reported. In particular, it is supposed that cytotoxin-associated gene A (CagA) of *H. pylori* played an important role in triggering EMT in gastric epithelial cells. CagA can be injected into host gastric epithelial cells via a type IV secretion pilus [72], and this hampers the expressions of junctional adhesion molecules or E-cadherin leading to the destruction of desmosome or hemidesmosomes. The roles of *H. pylori* in the EMT process were reviewed in the following sections.

25.4.3.1 Promotion of Production of TGF-β- or TNF-α-Inducing Protein

TGF-β is one of the most important triggering factors for EMT, and *H. pylori* infection accelerates the production of TGF-β in human gastric mucosa or gastric epithelial cells [73–75]. *H. pylori* also promotes to produce TNF-α-inducing protein, which produces various cytokines including TNF-α, facilitates immigration of gastric cancer cells, and activates minting leading to the acceleration of EMT [76]. Consequently, chronic infection of *H. pylori* promotes the production of multiple cytokines and prepares the environment to favor EMT.

25.4.3.2 Activation of Snail, Twist, or β-Catenin

CagA binds GSK-3 in a manner similar to Axin and causes it to shift to an insoluble fraction, resulting in reduced GSK-3 activity [77] (Fig. 25.7). It was reported that the production of Snail protein is increased in *H. pylori*-infected epithelium. These results suggest that CagA induces a Snail-mediated EMT via the depletion of GSK-3 [77]. In gastric cancer cells cocultured

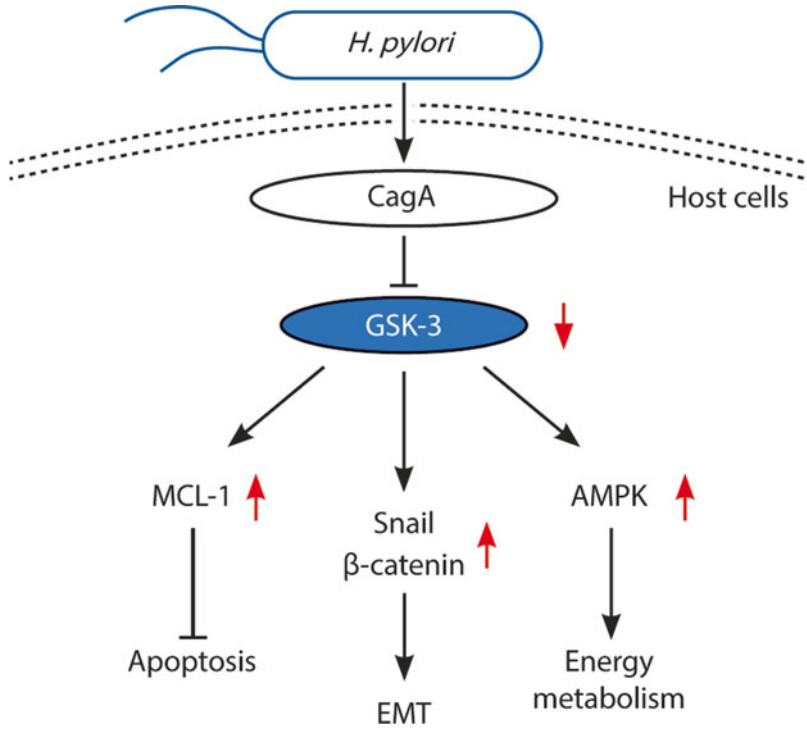

Fig. 25.7 Schematic diagram of CagA-mediated GSK-3 depletion and subsequent activation of EMT and other oncogenic pathways. Following transduction of CagA into the cells via a type IV secretion system, the cytoplasmic GSK-3 is rapidly insolubilized and downregulated, resulting in the activation of the GSK-3-dependent oncogenic pathway. *GSK3β* glycogen synthase kinase-3β, *MCL-1* myeloid cell leukemia, *EMT* epithelial-mesenchymal transition, *AMPK* AMP-activated protein kinase (Adapted from Lee et al. [77])

with CagA expression plasmid, CagA activated TWIST1 and vimentin expression and inhibited E-cadherin expression by downregulating programmed cell death factor 4 (PDCD4) [78]. CagA also promoted mobility of gastric cancer cells by regulating PDCD4. In addition, *H. pylori* could activate Wnt/β-catenin signaling pathway via the overexpression of β-catenin, and this finally promotes the EMT event [79, 80]. Authors have recently reported that upregulation of Twist, Snail, Slug, vimentin and downregulation of E-cadherin increased ability to migrate [81]. In addition, the upregulation of EMT-promoting miRNAs and downregulation of EMT-repressing miRNAs in *H. pylori*-infected mucosa is revealed [65]. How *H. pylori* acts to these miRNAs deserves further study.

25.4.3.3 Hypermethylation of *CDH1* Promoter

Hypermethylation of *CDH1* in breast cancer cells is a part of the EMT process, and this facilitates the metastasis to other organs, endowing more invasive characteristics [82]. *H. pylori* infection promoted the hypermethylation of *CDH1* promoter [83], and more frequent lymph node metastasis or more invasive invasion to tissue was found in the groups with the hypermethylated gene compared to the other group [84]. This suggested that *H. pylori* infection promoted EMT process through hypermethylation of *CDH1* promoter.

25.4.3.4 Association with Emergence of Cancer Stem Cells

Cancer stem cell theory in which abnormal self-renewing cells were involved in carcinogenesis has been a novel hypothesis. This new concept has remarkable implications for cancer therapy because it suggests that current therapies are successful at eradicating noncancer stem cells rather than cancer stem cells. EMT participates in the carcinogenesis process and is involved in the generation of cancer stem cells. In one study [85], gastric epithelial cells which were cocultured with a cagA-positive *H. pylori* strain were transfected with CagA expression vectors. The expression of epithelial and mesenchymal markers showed that *H. pylori*, via CagA, is responsible for an EMT phenotype associated with an increase in mesenchymal markers as well as CD44 expression, a known gastric cancer stem cell marker [85]. Moreover, infection led to an increased ability to migrate, to invade, and to form tumorspheres. Cell-sorting experiments showed that only the $CD44^{high}$ cells induced by *H. pylori* infection displayed the mesenchymal phenotype and CSC properties in vitro and had higher tumorigenic properties than $CD44^{low}$ cells in xenografted mice. This result suggests that certain association between EMT and cancer and the infection with cagA-positive *H. pylori* induces EMT-like changes and the emergence of $CD44^{high}$ cells with cancer stem cell properties.

25.4.4 Clinical Implications of EMT

25.4.4.1 Markers for EMT

Markers of established or ongoing EMT are quite consistent. Loss or degradation of proteins associated with epithelial homeostasis, cell polarity, and cell adhesion, such as E-cadherin, RhoA, or Plakophilin 2, is frequently observed in EMT [86]. The redistribution to the cytoplasm of β-catenin that is involved in cell-cell adhesion is also a hallmark for EMT. The intermediate filament protein vimentin is frequently overexpressed and contributes to cell migration and is thus regarded as a stable marker of EMT. Dysregulated expression of transcription factors, such as Notch1, Slug, Snail, Twist, or Zeb1, has been described in invasive tumors displaying EMT. Table 25.2 represents dysregulated protein markers that have been frequently used in the assessment of EMT [86].

25.4.4.2 Prognostic Factors

Since the metastasis of malignant cancers accounts for the majority of cancer-related deaths, possible correlations between EMT markers and patient prognosis have been intensively studied in various types of tumors. Therefore, most effort has been put into linking the expression of EMT markers to data on patient

Table 25.2 Frequently used protein markers for epithelial-mesenchymal transition (EMT)

Marker	Original function	Tissue
Downregulated in EMT		
α-Catenin	Cell adhesion molecule	Lung
β-Catenin (membrane)	Cell adhesion molecule	Colon, pancreas (NET)
Claudin	Cytoskeletal filament	Esophagus, breast
Cytokeratins	Cell adhesion molecule	Lung, esophagus
E-cadherin	Cell adhesion molecule	Colon, breast, lung, ovary, esophagus, prostate, cervix
Occludin	Cell adhesion molecule	Ovary
Upregulated in EMT		
Brachyury	Transcription factor	Pancreas, breast, lung
β-Catenin (cytoplasm/nucleus)	Transcription factor	Breast, cervix
EGFR	Tyrosine kinase receptor	Cervix
N-cadherin	Cell adhesion molecule	Ovary, prostate
Notch-1	Transcription factor	Prostate
$p16^{INK4a}$	Cell cycle regulator	Colon, urothelium
Slug	Transcription factor	Breast, ovary
Snail	Transcription factor	Breast, cervix, ovary
TTF-1	Transcription factor	Lung
Twist	Transcription factor	Breast, stomach
Vimentin	Cytoskeletal filament	Breast, esophagus, cervix
ZEB1	Transcription factor	Colon, breast, ovary

Adapted from Steinestel et al. [86]
EMT epithelial-mesenchymal transition, *NET* neuroendocrine tumor, *EGFR* epidermal growth factor receptor, *TTF-1* thyroid transcription factor-1, *ZEB1* zinc finger E-box-binding homeobox 1

survival. With respect to gastric cancer, significant correlations between the expression of Slug and the lymph node metastasis, lymphovascular invasion, and postoperative TNM stage were revealed [52]. In addition, patients with downregulated E-cadherin or upregulated Slug did not show favorable prognosis. That is, patients with upregulation of Slug had a lower 5-year survival rate than patients with upregulation of Slug, when their expression of E-cadherin was not changed [87]. The expression of Twist in gastric cancer tissue also had positive correlations with the size of tumor, depth of invasion, and lymph node metastasis, and the poor prognosis was more prominent in diffuse-type gastric cancer [56]. Positive expression of vimentin is associated with advanced stage and diffuse-type gastric cancer. Expression levels of EMT markers Snail and β-catenin were independent predictors for lymph node metastasis and lymphovascular invasion [88]. Similarly to these results, loss of E-cadherin expression and upregulation of E-cadherin-suppressing factors are likely to be prognostic factors for gastric cancer. However, it may be difficult to yield reliable prognostic information for an individual patient from the expression pattern of EMT markers due to high variability of marker expression patterns in different tumor areas in a heterogeneous sample.

25.4.4.3 Targets for the Cancer Treatments

Since stem cell-like tumor cells bear considerable resistance to conventional therapies and the markers for EMT have been identified in a significant proportion of these cells as described above, much effort has been made to develop antineoplastic therapies that directly target EMT.

Most of therapeutic approaches in this field is aimed for blocking EMT master genes directly or

inhibiting kinase signaling pathways to switch off the EMT master gene expression. They have been developed in hepatic cell cancer, colorectal cancer, and pancreatic cancer with respect to gastroenterological part.

Resveratrol, a dietary polyphenol, downregulated expression of EMT master genes (*Zeb1*, *Snail*, and *Slug*) and impaired cancer stem cell self-renewal capacity, tumor growth, and invasion in a mouse model of pancreatic ductal adenocarcinoma [89]. In the recent years, the role of small, noncoding RNAs in the EMT process has also been further elucidated. Methylation-dependent expression changes in levels of miR-200c regulate invasion and metastasis in cancer via altered miR-200c target gene expression; miR-200 family also activates cyclin D1 leading to loss of mesenchymal characteristics of EMT-progressed epithelium [90–94]. MiR-let-7f is known to be a tumor-suppressing factor targeting MYH9, a metastasis-related gene [95]. MiR-7 is also downregulated in gastric cancer tissue like miR-let-7f, and the expression of miR-7 showed an inverse correlation with metastatic spread or invasion of gastric cancer cells [96]. Taken together, antineoplastic agent using miRNAs could suppress metastasis of gastric tumor.

Conclusions

The EMT is characterized in the morphologic and molecular changes: loss of E-cadherin, change to spindle shape, and gain of the ability to migrate. It is critical for embryonic development, wound healing, and fibrotic disease. Moreover, recent studies have reported that that aberrant EMT activation in the human organ is closely associated with carcinogenesis and tumor progression.

There are a number of proteins that indicate the stage of EMT (i.e., E-cadherin, N-cadherin, and vimentin). The transcriptional proteins such as E-cadherin transcriptional repressors could be also markers for EMT. The EMT process is under tight control of multiple signal pathways including TGF-β-related Smad pathway, one of the important axes.

Therefore, downregulation of E-cadherin function due to mutation, deletion, CpG hypermethylation, and SNAIL-mediated transcriptional repression of the *CDH1* gene leads to EMT. Dysregulation of proteins in EMT pathways and epigenetic mechanisms could be involved in progression of EMT.

Recently, inflammation is regarded to be likely a key inducer of EMT in the pathological settings of cancer progression, and EMT has now been considered to bridge inflammation and cancer. In particular, TGF-β, of which *H. pylori* could induce the production, is the main inducer of Snail, Slug, Twist, or ZEB1. This suggests *H. pylori* infection could involve in gastric carcinogenesis via activation of EMT. However, most studies about EMT have focused on metastatic spread of cancer, so far.

Growing evidence has been recently reported that EMT endows cells not only migratory and invasive characteristics but also stem cell properties. Increased expression of mesenchymal EMT markers was closely connected with the emergence of cancer stem cells. Especially, it has been reported that *H. pylori* infection, one of the major gastric carcinogens, could increase mesenchymal EMT markers and promote migration activity. This implicated that EMT induced by *H. pylori* may be a cause of gastric carcinogenesis by triggering gastric cancer stem cells.

Despite the primitive data about the possible role of EMT in the initiation of cancer, the association between expression of hallmarks of EMT and tumor aggressiveness has been well demonstrated in a variety of malignancies. In addition, antibodies, RNAi compounds, and small molecular inhibitors for EMT signaling could be developed as novel therapeutic agents for cancer.

Consequently, understanding of the role of EMT could be helpful for the early detection and effective treatment of gastric cancer. The association between *H. pylori* and EMT in the field of gastric cancer remains to be elucidated in future studies.

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Gastric Cancer: ABO Blood Type

26

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Abstract

ABO blood type has been known as a genetic factor which affects the development of gastric cancer. However, the mechanism is still uncertain. There are experimental evidences which suggest the role of ABO blood type in the infection of *Helicobacter pylori* (*H. pylori*). Molecular mimicry was shown with Lewis antigens, with which *H. pylori* can evade the host immune system. Blood group antigens were observed to act as an adhesion molecule onto which *H. pylori* attach. Nonsecretors compared with secretors lack "decoys"; therefore, nonsecretors are more vulnerable to intruders. These findings can be clues for future studies.

Keywords

Helicobacter pylori • Blood group antigen • Gastric cancer

26.1 Introduction

Various factors, both environmental and genetic factors, contribute in the development of gastric cancer (GC). ABO blood type is one of such genetic factors. In a textbook of internal medicine by Cecil, blood group A is classified as a risk factor of GC [1]. In the gastric cancer risk index provided by Harvard School of Public Health, blood group A is included as a risk factor and explained that the mechanism how blood group A increases the risk of GC is uncertain but seems related to other genetic factors [2]. The study which provided the foundation of the risk index program proposed the relative risk of blood group A as 1.2 [3].

26.2 The Association of ABO Blood Group and Gastric Cancer

The association of ABO blood group and gastric cancer was first noticed in the 1950s. Later, a research conducted in 15 places in the USA, Europe, and Australia showed that blood type A increases the risk of GC compared with

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blood type O (odds ratio [OR] 1.24; 95 % confidence interval [CI], 1.18–1.30) [4]. In 2010, the result of a prospective study (Scandinavian Donations and Transfusions, SCANDAT) which followed Danish and Swedish blood donors of 1,089,022 during 35 years was published, where blood type A increased the risk of GC compared to blood type O (OR 1.20; 95 % CI, 1.02–1.42) but the risk of peptic ulcer was higher in blood type O [5]. These two studies included only European descendent Caucasians.

Recently, there were studies with Asian population. A study conducted in Japan analyzed the association of ABO blood type and GC by ABO genotype. Genotype OO and BO, compared to genotype AA, had decreased risk of GC by OR 0.70 (95 % CI, 0.50–0.99) and OR 0.53 (95 % CI, 0.36–0.77), respectively. Moreover, addition of A allele was associated with increased risk of GC ($p_{trend} < 0.001$), while addition of B allele was associated with decreased risk of GC ($p_{trend} = 0.023$) [6]. A study conducted in Korea showed that genotype AA and AO, compared to genotype OO, had increased risk of GC by OR 1.56 (95 % CI, 1.08–2.26) and OR 1.57 (95 % CI, 1.21–2.03), respectively. In subgroup analyses, statistically significant association was only observed in females with diffuse-type GC (OR 2.00; 95 % CI, 1.43–2.78) [7].

Researches regarding ABO blood type should take into account that ABO allele frequencies vary widely upon ethnicity and geography. Results of several studies which probed the association of ABO blood type and cancer incidence [8–10] are compared in Table 26.1. The B allele frequency of European descendent Caucasian is half of that of Asian. This difference explains why the role of B allele in the development of GC was prominently noticed in studies done with Asians but not in those with Caucasian.

For such findings to be accepted as a medical fact, there should be an explanation at the level of molecular biology. However, there is no direct evidence yet which connect the ABO blood type and the development of GC. Only recently, ABO antigens were speculated to mediate the pathogenicity of *Helicobacter pylori* (*H. pylori*) and, by doing so, increase the risk of GC.

26.3 Researches Explaining the Interaction Between *H. pylori* and ABO Antigen, the Results, and Hypotheses

ABO antigens are glycolipids which are determined by a gene on chromosome 9q34.2 and expressed on cell membrane. Glycosyltransferases are coded in that gene. For blood type A, *N*-acetylgalactosamine is transferred on the H antigen, and for blood type B, D-galactose is transferred by the blood type-specific transferases. When the gene has a loss of function mutation, such glycosylation cannot occur, and the phenotype becomes blood type O. In case of blood type AB, the person has the two glycosyltransferases and gets A and B antigens altogether (Fig. 26.1). Additive glycosylation onto the already formed ABO antigen can occur by Lewis (Le) enzyme, making Le antigen (Fig. 26.2). These blood antigens are not only expressed on the red blood cells but on various human cells including gastric mucosal cells.

26.3.1 Immune Evasion by Molecular Mimicry

Lipopolysaccharides (LPS) are expressed on the bacterial membrane of *H. pylori*. The O-chain of LPS has polysaccharides with structural similarity to human Le antigen. It is called molecular mimicry, and antigens such as Le^x, Le^y, Le^a, Le^b, Le^c, sialyl-Le^x, and H-1 as well as the related blood groups A and B are described. Le^x and Le^y are common properties expressed in as much as 80–90 % of *H. pylori* strains screened serologically, but Le^a and Le^b are more common in Asian isolates. Moreover, a mosaicism in which Le antigen and blood group antigens are expressed in the same O-chain contributes to antigenic diversity. Genetically modified *H. pylori* strains unable to express Le antigen, compared to the parental strains, failed to induce gastritis in

Table 26.1 ABO genotype and allele frequency observed in cancer researches

	Korean gastric cancer study [7]			Japanese gastric cancer study [6]			Chinese pancreatic cancer study [8]	US Caucasian breast cancer study [9]	North America pancreatic cancer study [10]
	Control ($n=1700$)	Gastric cancer ($n=3245$)	Total ($n=4945$)	Control ($n=1,465$)	Gastric cancer ($n=703$)	Total ($n=2,168$)	Total ($n=1,815$)	Total ($n=3,141$)	Total ($n=6,994$)
ABO genotype frequency, no. of subjects (%)									
AA	154 (9.1)	300 (9.2)	454 (9.2)	117 (8.0)	74 (10.5)	191 (8.8)	117 (6.4)	229 (7.3)	563 (8.0)
AO	494 (29.1)	1,087 (33.5)	1,581 (32.0)	455 (31.1)	245 (34.9)	700 (32.3)	479 (26.4)	1,098 (35.0)	2,492 (35.6)
BB	77 (4.5)	127 (3.9)	204 (4.1)	34 (2.3)	11 (1.6)	45 (2.1)	74 (4.1)	17 (0.5)	67 (1.0)
BO	337 (19.8)	555 (17.1)	892 (18.0)	290 (19.8)	98 (13.9)	388 (17.9)	416 (22.9)	304 (9.7)	751 (10.7)
AB	202 (11.9)	393 (12.1)	595 (12.0)	141 (9.6)	81 (11.5)	222 (10.2)	200 (11.0)	139 (4.4)	336 (4.8)
OO	436 (25.6)	783 (24.1)	1,219 (24.7)	428 (29.2)	194 (27.6)	622 (28.7)	529 (29.1)	1,354 (43.1)	2,785 (39.8)
ABO allele frequency									
A	0.295	0.32	0.312	0.283	0.337	0.301	0.252	0.27	0.283
B	0.204	0.185	0.192	0.17	0.143	0.161	0.21	0.076	0.087
O	0.501	0.494	0.497	0.546	0.52	0.538	0.538	0.654	0.63

Allele frequencies were calculated according to Hardy-Weinberg equilibrium

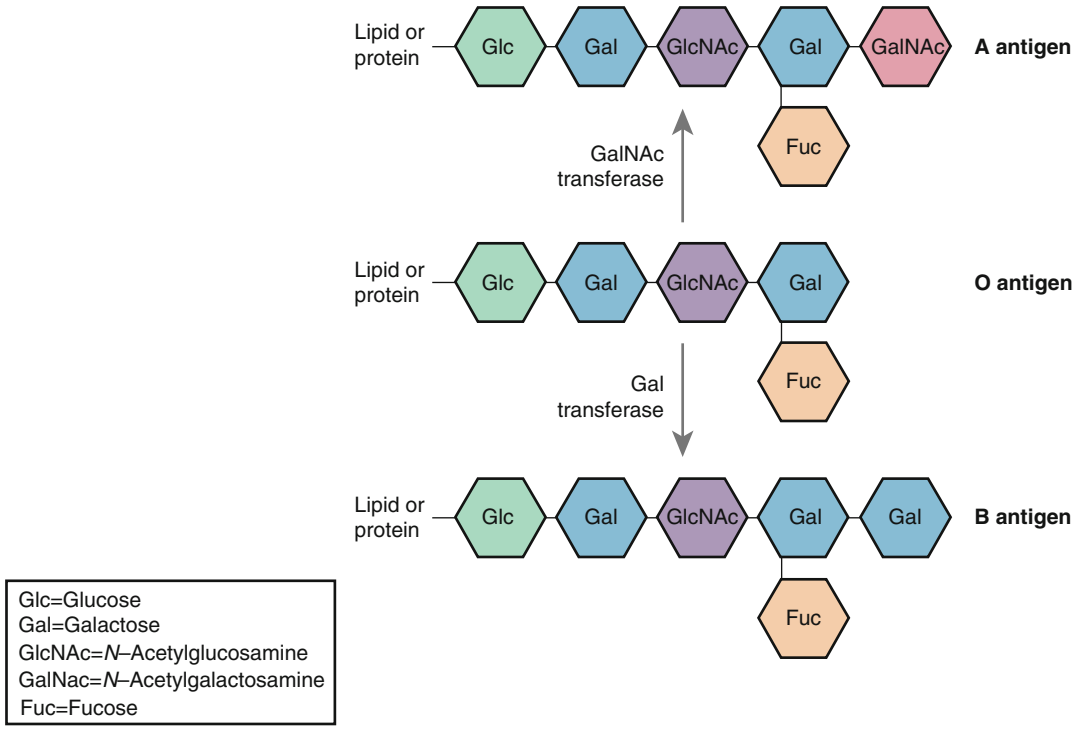

Fig. 26.1 Structures of ABO antigens (Adapted from Lodish et al. [11])

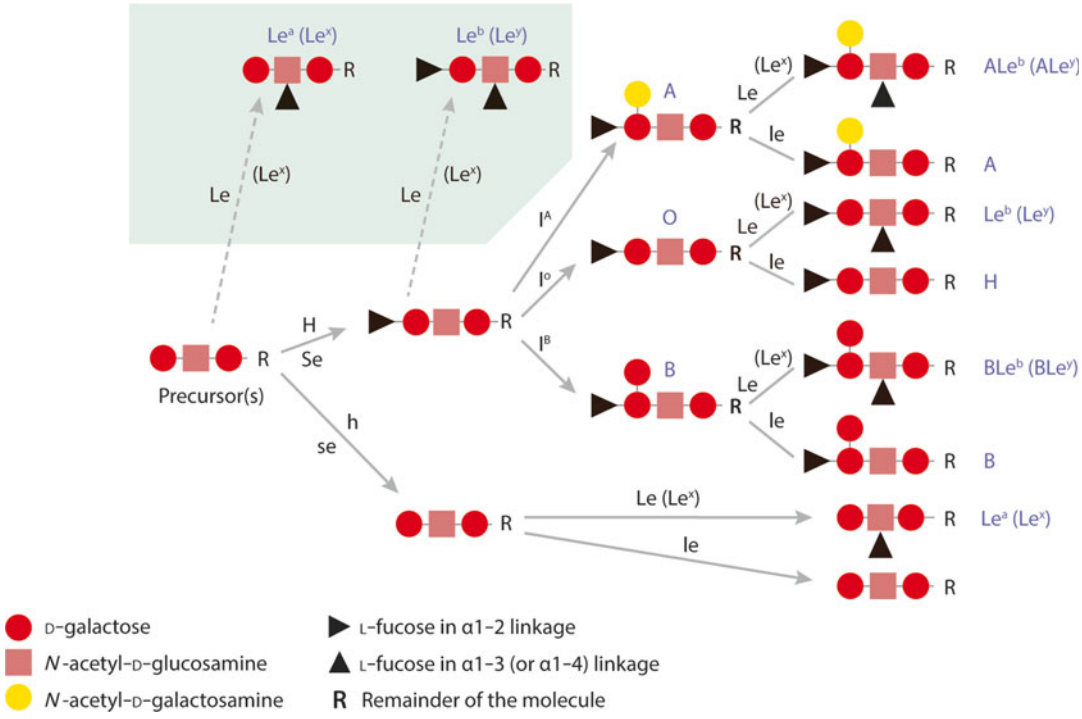

Fig. 26.2 ABO and Lewis antigens (Adapted from Daniels [12])

animal studies, which support that Le antigen has an important role in disease development by *H. pylori*. This phenomenon can be explained that such molecular mimicry helps as a mechanism of immune evasion [13].

26.3.2 Adhesion Molecules onto Which *H. pylori* Can Attach

Another explanation of the role of blood antigen in *H. pylori* infection is that the blood antigens are adhesion molecules onto which *H. pylori* attach. Thus, *H. pylori* can adhere on to the "buttress" molecules on the surface of host mucosal membrane and induce chronic inflammation. For example, a protein BabA expressed on *H. pylori* is known to bind Le^b and related terminal fucose residues found on A, B, and H antigens expressed on gastric mucosal cells. However, not all *H. pylori* strains express BabA since there can be loss of function mutations, and there are strains producing BabA which do not bind Le^b and ABO antigens. The mutated BabA may bind to other unknown oligosaccharides distinct from Le^b and ABO antigens. Functional BabA is more commonly found in patients with duodenal ulcer and gastric adenocarcinoma than in patients with asymptomatic gastritis. Therefore, BabA is considered as an important virulent factor in the pathogenicity of *H. pylori*. This is consistent with the observation that peptic ulcer is more common in blood type O individuals, in whom H and Le^b are predominantly expressed on the gastric epithelium [14].

26.3.3 Decoy Mechanism by Secretors

On the other hand, there is secretor gene (Se) which is inherited independently from ABO blood group. The individuals with functional gene, secretors, have the ability to secrete α1,2-fucosylated glycan produced by ABO gene. However, nonsecretors cannot secrete the glycan but have the glycan expressed on the surface of cellular membrane. Secreted glycans are present in the mucous on the gastric epithelium and competitively bind to adhesive molecules on the bacterial membrane of *H. pylori*. As such, secreted glycans act as "decoy" with which the gastric mucosal cells can evade attachment by *H. pylori*. Therefore, nonsecretors lacking "decoy" seem to be more vulnerable to *H. pylori* infection and chronic inflammation [14].

Conclusions

As discussed, evidences which support that blood group antigens are playing important roles in the pathogenicity of *H. pylori* are observed. Researches up until now were conducted at the level of animal studies and molecular biological experiments that it is not certain whether these findings will be consistently observed in human. Moreover, there hasn't been a study which investigated the direct interaction between ABO blood group antigens and *H. pylori*. Therefore, this topic is an area that future studies are required.

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Gastric Cancer: First Relatives of Gastric Cancer

27

Nayoung Kim and Yoon Jin Choi

Abstract

Family history is an important risk factor of gastric cancer and can be seen from 10 % to 15 % of gastric cancer patients. This factor is partially explained by genetic and environmental factors, and family history is also related to familial clustering of *Helicobacter pylori* (*H. pylori*) infection. This factor is notable because an immediate family history on gastric cancer itself increases gastric cancer genesis by two to three times, and this genesis increases by 5.88 times if *H. pylori* infection is also present. Along with family history as a risk factor, some of gastric cancer types with high genetic dependence can be classified as germline *CDHI* mutation-related hereditary diffuse gastric cancer of which was found to be 0.3–3.1 % in Korea and Japan. Although family history increases gastric cancer genesis, this factor is beneficial for the cancer prognosis. These subjects do frequent health screenings because of their immediate family history and follow good health-related behaviors, and the patients often show microsatellite instability (MSI) and mismatch repair proteins, which are related to good prognosis. *H. pylori* eradication is the most important priority for immediate family members, who are less than 40s, especially in 20s and 30s, of gastric cancer patients. This early *H. pylori* eradication is helpful to avoid future intestinal metaplasia and to restrict synergistic effect of *H. pylori* infection and the immediate family history.

Keywords

Gastric cancer • Family history • *Helicobacter pylori*

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27.1 Introduction

Gastric cancer is currently the fifth most common cancer, with approximately one million new cases diagnosed worldwide each year, and ranks as the third leading cause of cancer-related death [1].

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It represents a particularly important public health issue in East Asian countries, where incidence rates are among the highest observed [2]. The age standardized incidence rate of gastric cancer has been continually decreasing for a few decades in Japan [3] and Korea [4], but the number of new cases still exhibits an increasing pattern that is thought to be mainly attributable to the aging of the population. Gastric cancer is induced by multifactorial interactions that include genetic and environmental aspects [5, 6] and *Helicobacter pylori* (*H. pylori*) infection. Then, the importance of family history has been proposed by many researchers as one of the gastric cancer risk factors [7, 8]. A famous example of gastric cancer pedigree would be Napoleon Bonaparte's family, since Napoleon himself and his family members died with gastric cancer. However, this category was classified as germline *CDHI* mutation-related hereditary diffuse gastric cancer (HDGC), and this rate was found to be 0.3–3.1 % in Korea and Japan [9]. Usually, gastric cancer family history can be seen from 10 % to 15 % of gastric cancer patients, excluding familial gastric cancer patients, and gastric cancer relatives (GCRs) show two to three times higher possibility of getting gastric cancer compared with a normal control group [10–12]. Family history of gastric cancer has been explained along with genetic inheritance and environmental conditions that a family shares, such as dietary habit and smoking, but the relationship of familial clustering of *H. pylori* infection among family members has been added recently [13, 14]. That is, members of the same family tend to have similar environmental factors like socioeconomic status and dietary habit, and there is a possibility that the same strain of *H. pylori* infection is also clustered. Therefore, the results of studies conducted to elucidate whether family history of gastric cancer is/is not an independent risk factor of gastric cancer should be interpreted very cautiously [15]. A population-based, statewide, case-control study from Germany reported that *H. pylori* infection and a family history were strong independent risk factors for gastric cancer, although both were positively related with one another [16]. A Korean study also showed that adjusted odds ratio (OR) for gastric cancer increased threefold for subjects with first-degree GCR (OR 2.85 in comparison with control; 95 % confidential interval [CI], 1.83–4.46) [17]. Those patients had five to eight times higher possibility of getting gastric cancer than a normal control group [16, 17], which suggests that familial aggregation of gastric cancer could be explained at least partly by familial clustering of *H. pylori* infection [18]. Based on these gastric cancer researches, the Maastricht guideline recommends *H. pylori* eradication therapy, if a patient with *H. pylori* infection has family history of gastric cancer [19], and the *H. pylori* treatment guideline of Korea and Asia Pacific also recommended the same procedure [20, 21]. Therefore, this chapter is going to introduce how high is the possibility of getting *H. pylori* infection if a patient has the immediate family history compared with a patient without the family history and the reason of gastric cancer genesis increment when both *H. pylori* infection and immediate family history on gastric cancer are present in a patient. Also, any difference of histological alterations between a *H. pylori*-infected patient with the immediate family history and a *H. pylori*-infected patient without the immediate family history, prognosis after gastric cancer diagnosis, and gastric cancer prevention method for patients with immediate family history on gastric cancer are going to be discussed.

27.2 Difference of *H. pylori* Infection Rate Depending on Family History on Gastric Cancer

The current *H. pylori* infection rate keeps decreasing in the world. For example, *H. pylori* seroprevalence among Koreans over 16 years old in 2005 and in 2011 were 59.6 % and 54.4 %, respectively [22, 23], significantly decreased from 66.9 % in 1998 [24]. More dramatically, the prevalence decreased from 41.7 % [25] to 23.5 % [26] in the Czech Republic between 2001 and 2011. Based on a report from China [27] and German [18], *H. pylori* infection rate was higher in the GCRs than a control group without the family history, and the similar result was also

reported from Korean report [12]. However, a nationwide epidemiological survey based on 10,796 health check-up Koreans in 2011 reported that *H. pylori* antibody-positive rate in the GCRs was 55.1 %, while the rate for the healthy subjects without the family history was 54.4 %, which did not show a difference [23], and this result was similar to the results from the surveys in 1998 [24] and in 2005 [22]. Thus, it can be inferred that simple difference of *H. pylori* infection rate would not influence on gastric cancer rate on first-degree family members of gastric cancer patients. Thus, comprehensive research is needed in the big population from the world.

27.3 Risk Increment of Gastric Cancer in the Presence of *H. pylori* Infection and Family History of Gastric Cancer

The percentage of health check-up subjects who had family history of gastric cancer was found to be 11 % [23] or 11.8 % [28], and family history of gastric cancer showed two to three times higher possibility of getting gastric cancer patients than normal control groups, suggesting that the family history of gastric cancer is an independent risk factor [10–12]. Furthermore, first degree relatives of patients with early-onset gastric carcinoma showed, even at younger ages, a high prevalence of *H. pylori* infection, high risk OLGA (operative link of gastritis assessment), OLGIM (operative link for gastric intestinal metaplasia) stages and dysplasia [29]. This risk factor is partially explained by genetic aspect and environmental aspects (smoking, overconsumption of salt or *N*-nitroso compound, and antioxidant deficiency) [12], and familial clustering of *H. pylori* infection is also related [13, 14]. Especially, there was a synergistic effect if both *H. pylori* infection and family history of gastric cancer exist to five to eight times of getting gastric cancer, higher than a control group [16, 19], and these two factors could be interconnected [16]. First of all, the genetic aspect of gastric cancer genesis has been explained by the susceptibility of genetic polymorphism of inflammation-related genes, such as *interleukin-1β* gene (*IL-1β*), *tumor necrosis factor-α* gene (*TNF-α*), *IL-6*, *IL-8*, *IL-10*, *Toll-like receptor 4* (*TLR-4*), and transforming growth factor (*TGF*)-*β* [30–32]. However, this gene-related explanation has a problem of reproducibility because test results can be different based on patient's ethnicity. For instance, genetic polymorphism of *IL-1β* and *TNF-α* was consistently related to gastric cancer among Western patients [33, 34], while this phenomenon was inconsistent among Eastern patients [35, 36]. In contrast, *IL-8* and *TGF-β* genetic polymorphism and gastric cancer genesis was more consistently related among Eastern patients than Western patients [37–40]. Thus, ethnicity must be considered. These genetic factors were influential when *H. pylori* infection triggered premalignant lesion like intestinal metaplasia (IM), by inflammation of gastric mucosa. Based on a research about risk factors of atrophic gastritis (AG) and IM in the subjects without significant gastroduodenal diseases, AG was related to not only *H. pylori* infection but also *H. pylori* toxic factors like *vacA* m1 and *cagA*. In contrast, risk factors of IM were *H. pylori* infection, smoking, age over 61, spicy food, and *IL-10*-592 C/A [41]. These results suggest that genetic polymorphism is powerful for IM, which increased gastric cancer 10.9 times higher than in the absence of IM [41].

To find out the increasing mechanism of gastric cancer by family history of gastric cancer, comprehensive approach is necessary on the genetic factors, the environmental factors, and *H. pylori* infection and especially on the interaction between *H. pylori* virulence factors and genetic susceptibility. According to a recent comprehensive study regarding 123 gastric cancer patients with gastric cancer history and 639 age and sex-matched control gastric cancer patients without family gastric cancer history, the rate of IM in the body among the GCRs less than 50 years old was higher than that of the control group ($p = 0.018$) [40]. Next, intestinal-type gastric cancer patients with family history of gastric cancer spent their youth in rural area (OR 1.98; 95 % CI, 1.02–3.85), and only a few patients showed *TGFB1*-509 T (OR 0.47; 95 % CI, 0.25–0.86), suggesting that both environmental factors and the genetic polymorphism affect on the genesis of intestinal-type gastric cancer among GCRs [40].

27.4 Risk Factors for Gastric Cancer According to the Number of Affected Relatives and According to the Affected Family Member

Even though it is believed that the gastric cancer risk increases with an increasing number of affected relatives or it could be different depending on affected family member, there have been few reports regarding this factor [7, 17]. For instance, the risk associated with gastric cancer occurrence in any first-degree relative (FDR) tended to be greater for women than for men [7, 17]. In addition, the gastric cancer risk was higher for subjects having an affected mother than an affected father (OR 3.78; 95 % CI, 1.49–9.59 vs. 2.85, 1.46–5.56) [17]. Overall, a parental history of gastric cancer increased the gastric cancer risk (OR 3.30; 95 % CI, 1.89–5.75) [17]. The gastric cancer risk for subjects with a sibling with gastric cancer also increased significantly (OR 2.44; 95 % CI, 1.25–4.74), but the risk was not as strong as that for subject with an affected parent [17]. In addition, the gastric cancer risk increased for those that had one first-degree family member affected with gastric cancer (OR 2.72; 95 % CI, 1.72–4.32), but the risk increased further for subjects with two or more first-degree relatives with gastric cancer (OR 9.60; 95 % CI, 1.18–73.35) [17]. Recently our group further performed the comprehensive analysis in the total of 1,058 gastric cancer patients and 1,268 controls according to the presence or absence of a first-degree relative diagnosed with gastric cancer (GC-relative) [42]. Since the gastric cancer risk increases with an increasing number of affected relatives, the family history-positive group was divided into the two categories, having one GC-relative and having two or more GC-relatives, to evaluate the familial aggregation of gastric cancer [42] (Table 27.1). Residence in rural area in childhood and current or ex-smoking were more likely to increase risk of gastric cancer in the ≥2 GC-relative group (OR 2.14 vs. 7.54; p for interaction = 0.076 and OR 2.23 vs. 6.48; p for interaction = 0.054) [42] (Table 27.1). Moreover, there were an interaction between alcohol consumption and gastric cancer risk in the ≥2 GC-relative group. That is, OR for drinking in this group was sixfold as high as that of one GC-relative group (1.71 vs. 9.58, p for interaction = 0.026). When the amount of alcohol was stratified, heavy drinker (≥144 g of ethanol/week) showed the highest synergistic effect compared to none (24 g of ethanol/week <), light (144 g of ethanol/week <), and ex-alcohol user. Contrary to this, *H. pylori* infection was more closely associated with gastric cancer patients in the single GC-relative group than the at least two GC-relative group [42] (Table 27.2). When multivariable analyses were performed in each group with one GC-relative and two or more GC-relatives respectively, the significant risk factors for gastric cancer in the subjects with single GC-relatives were almost the same as the analysis of the total FH-positive subjects [42] (Table 27.2). Only alcohol consumption was excluded from the risk factors. However, when the subjects were restricted to those with two or more GC-relatives, having *TGFB1*-509 T/T was newly incorporated into the risk factors for gastric cancer along with rural residency in childhood, alcohol consumption, moderate to strong spicy food ingestion, and *cagA* positivity [42] (Table 27.3). When the characteristics of gastric cancer patients were evaluated according to affected relatives, the gastric cancer group with a maternal history had an overall larger number of affected relatives than the group with an affected father or the group with affected siblings or offspring (1.36 ± 0.98 vs. 1.10 ± 0.34 and 1.08 ± 0.27, $p = 0.012$ and $p = 0.034$, respectively) [42] (Table 27.4). When multivariable logistic analysis adjusted for age, gender, *H. pylori* infection, and alcohol consumption was performed in gastric cancer patients with family history, positive maternal history was a risk factor for having two or more GC-relatives (maternal history, OR 4.30, 95 % CI, 1.34–13.79, $p = 0.014$; *H. pylori* infection, OR 0.24, 95 % CI, 0.08–0.82, $p = 0.011$; alcohol consumption, OR 1.89, 95 % CI, 1.14–7.33, $p = 0.025$) [42] (Table 27.4). Moreover, the gastric cancer group with an affected father was younger than the group with an affected mother

Table 27.1 Comparison of clinicopathologic variables with regard to the number of affected relatives of gastric cancer

Variables [reference]	Group with 1 GC-relative			Group with ≥2 GC-relatives			$p_{Interaction}$
	Control ($n=308$)	GC ($n=188$)	OR (95 % CI)	Control ($n=47$)	GC ($n=30$)	OR (95 % CI)	
Age, y, median (IQR)	51 (43–60)	62 (52–69)	**1.07 (1.05–1.09)**	57 (49–65)	63 (55.0–72.3)	**1.08 (1.02–1.13)**	0.807
Male gender [women]	134 (43.5)	122 (64.9)	**2.38 (1.64–3.46)**	14 (30.4)	22 (73.3)	**5.87 (2.13–16.19)**	0.102
Rural residency [urban]	141 (48.1)	120 (66.7)	**2.14 (1.46–3.15)**	22 (52.4)	26 (89.7)	**7.54 (1.98–28.69)**	0.076
Smoker (current/ex) [never]	108 (35.6)	105 (55.9)	**2.23 (1.54–3.23)**	14 (30.4)	22 (73.3)	**6.48 (2.33–18.02)**	0.054
Alcohol [never]	116 (37.7)	95 (50.8)	**1.71 (1.18–2.47)**	12 (25.5)	23 (76.7)	**9.58 (3.29–27.95)**	**0.026**
Income<$5000/month [≥$5000/month]	189 (68.2)	144 (83.7)	**2.42 (1.50–3.90)**	25 (71.4)	22 (78.6)	1.41 (0.44–4.50)	0.584
Spicy food [no]	208 (72.0)	138 (77.1)	1.31 (0.85–2.02)	32 (78.0)	28 (96.6)	7.64 (0.91–64.03)	0.111
Fruit intake ≥3/week [<3/week]	41 (14.0)	44 (24.7)	**1.50 (1.15–1.96)**	13 (31.7)	8 (27.6)	1.26 (0.68–2.33)	0.155
Non-B blood [B]	210 (73.2)	143 (76.9)	1.20 (0.78–1.84)	34 (79.1)	21 (75.0)	0.77 (0.25–2.38)	0.475
Antral IM [none]	91 (29.9)	127 (67.9)	**5.18 (3.49–7.70)**	17(38.6)	18 (62.1)	2.46 (0.94–6.40)	0.158
Corpus IM [none]	67 (22.0)	78 (41.7)	**2.62 (1.76–3.90)**	9 (20.0)	15 (50.0)	**3.60 (1.32–9.80)**	0.562
Current/past HP infection	221 (71.8)	170 (90.4)	**3.70 (2.14–6.38)**	34 (72.3)	22 (73.3)	1.05 (0.37–2.95)	**0.035**
cag A [negative]	71 (29.0)	76 (44.4)	**1.94 (1.28–2.91)**	9 (26.5)	14 (46.7)	2.43 (0.85–6.92)	0.691
TGFB1-509 C/C [reference]	78 (25.7)	57 (31.1)	1 (reference)	16 (35.6)	8 (26.7)	1 (reference)	
TGFB1-509 C/T	150 (49.5)	82 (44.8)	0.74 (0.48–1.14)	22 (48.9)	15 (50.0)	1.36 (0.47–3.99)	0.330
TGFB1-509 T/T	75 (24.8)	44 (24.0)	0.80 (0.48–1.33)	7 (15.6)	7 (23.3)	2.00 (0.50–7.70)	0.214
*IL-1RN*2* carriers [non-carrier]	42 (13.9)	19 (10.2)	0.71 (0.40–1.27)	5 (10.6)	4 (13.3)	1.29 (0.32–5.26)	0.440
IL-1B-511 T allele [C/C]	247 (81.3)	145 (77.9)	0.81 (0.52–1.27)	40 (85.1)	24 (80.0)	0.70 (0.21–2.33)	–
Affected family member							
Mother [no]	98 (31.8)	46 (24.6)	0.70 (0.46–1.05)	22 (46.8)	13 (43.3)	0.77 (0.46–2.89)	0.333
Father [no]	128 (41.6)	72 (38.5)	0.89 (0.62–1.29)	20 (42.6)	11 (36.7)	0.78 (0.31–2.00)	0.797
Sister [no]	36 (11.7)	21 (11.2)	0.96 (0.54–1.69)	16 (34.0)	12 (40.0)	1.29 (0.50–3.33)	0.594
Brother [no]	45 (14.6)	41 (21.9)	**1.64 (1.03–2.62)**	30 (63.8)	19 (63.3)	0.98 (0.38–2.54)	0.340
Offspring [no]	1 (0.3)	7 (3.7)	11.94 (1.46–97.82)	0	0	–	–
Intestinal:diffuse	0	112:70	–	0	19:11	–	0.851
Non-cardia:cardia	0	167:20	–	0	26:4	–	0.753

Adapted from Choi et al. [42]
Data are presented as *n* (%) unless otherwise indicated
Bold style indicates the statistical significance
GC gastric cancer, *GC-relative* first-degree relative diagnosed with gastric cancer, *HP Helicobacter pylori*, *AG* atrophic gastritis, *IM* intestinal metaplasia, *IL* interleukin, *mo* month, *SD* standard deviation, *TGF* transforming growth factor, *OR* odds ratio, *CI* confidence interval

Table 27.2 ORs for the gastric cancer with regard to the amount of alcohol consumption and number of affected relatives of gastric cancer

	Group with 1 GC-relative			Group with ≥2 GC-relatives			
Alcohol[a]	Control ($n=308$)	GC ($n=188$)	OR (95 % CI)	Control ($n=47$)	GC ($n=30$)	OR (95 % CI)	$p_{interaction}$
Never/rare	192 (62.3)	92 (49.2)	1 (reference)	35 (74.5)	7 (23.3)	1 (reference)	
Light	60 (19.5)	35 (18.7)	1.22 (0.75–1.98)	10 (21.3)	9 (30.0)	**4.50 (1.34–15.12)**	0.392
Heavy	31 (10.1)	29 (15.5)	**1.95 (1.11–3.43)**	1 (2.1)	11 (36.7)	**55.0 (6.08–497.42)**	**0.022**
Ex	25 (8.1)	31 (16.6)	**2.59 (1.45–4.63)**	1 (2.1)	3 (10.0)	**15.0 (1.36–166.05)**	0.456

Adapted from Choi et al. [42]
Bold style indicates the statistical significance
GC gastric cancer, *GC-relative* first-degree relative diagnosed with gastric cancer, *OR* odds ratio, *CI* confidence interval
[a]Never/rare drinker refers to a non-drinker or those who drink ≤ 0.5 U/week; 0.5 U/week< light drinker < 12 U/week; heavy drinker ≥12 U/week (1 U = 12 g of ethanol)

Table 27.3 Risk factors for family history-positive gastric cancer according to number of affected first-degree relative by multivariable analyses

	GC-relative = 1[a]			GC-relative ≥2[c]	
Variables	*p* value	aOR[b] (95 % CI)	Variables	*p* value	aOR[b] (95 % CI)
Male gender	**0.002**	2.24 (1.33–3.77)			
Age	**<0.001**	1.06 (1.034–1.09)			
Antral IM	**<0.001**	2.80 (1.65–4.75)			
Current income (<$5,000/month)	**0.017**	2.19 (1.15–4.16)			
HP infection	**0.024**	2.50 (1.13–5.55)			
cagA	**0.006**	2.17 (1.25–3.77)	*cagA*	**0.038**	9.06 (1.12–72.97)
Rural residency in childhood	**0.002**	1.85 (1.10–3.12)	Rural residency in childhood	**0.004**	26.49 (2.90–241.99)
			Alcohol consumption	**0.002**	27.83 (3.37–230.16)
			≥ Moderate spicy food ingestion	**0.004**	65.74 (3.91–105.99)
			TGFB1-509 C/T	0.267	3.42 (0.39–30.05)
			TGFB1-509 T/T	**0.029**	23.74(1.37–410.91)

Adapted from Choi et al. [42]
GC-relative first-degree relative diagnosed with gastric cancer, *aOR* adjusted odds ratio, *CI* confidence interval, *HP Helicobacter pylori*, *IM* intestinal metaplasia, *mo* month, *TGF* transforming growth factor
[a]For 308 control subjects and 188 patients with gastric cancer
[b]Logistic model including terms for age, gender, *H. pylori* infection, *H. pylori* infection by gender, current income, residency during childhood, smoker, alcohol consumption, alcohol consumption by smoker, diet of salty/spicy food, fruit intake, intestinal metaplasia, non-B blood type, *cagA*, *IL-1RN* *2 carriers, *TGFB1*-C509T polymorphism (reference *TGFB1*-509C/C)
[c]For 47 control subjects and 30 patients with gastric cancer

and the group with affected only siblings (57.0 ± 1.3 vs. 62.2 ± 1.2 and 64.5 ± 1.1, $p=0.012$ and $p<0.001$, respectively) [42] (Table 27.4). These results suggest that subjects with family history may be a heterogeneous group in terms of gastric cancer susceptibility. Especially, subjects with ≥2 GC-relatives may need a risk stratification including *TGFB1*-509T/T or amount of alcohol consumption [42]. Taken together, the number of affected gastric cancer family and affected relative might be an interesting topic which could uncover the mechanism behind the GC-relatives, and it needs more research in the future.

Table 27.4 Characteristics of patients with gastric cancer according to different parental history of gastric cancer

Variables	Affected family member				
	Mother ($n=59$)	Father ($n=79$)	Only sibling/offspring ($n=75$)	p value[a]	p value[b]
Male gender, *n* (%)	41 (69.5)	53 (67.1)	47 (62.7)	0.694	0.764
Age at diagnosis, mean (SD)	62.2 (9.2)	57.0 (11.4)	64.5 (9.8)	**<0.001**	**0.012**
GC-relative ≥2, *n* (%)	13 (22.0)	7 (8.9)	6 (8.0)	**0.025**	**0.030**
No of affected family					
member, mean (SE)	1.36 (0.98)	1.10 (0.34)	1.08 (0.27)	**0.012**	**0.034**
HP infection, *n* (%)	64 (85.3)	72 (91.1)	52 (88.1)	0.694	0.563
cagA, *n* (%)	17 (30.9)	33 (46.5)	37 (52.9)	**0.045**	0.076
Rural residency, *n* (%)	39 (68.4)	48 (65.8)	53 (71.6)	0.745	0.748
Alcohol consumption, *n* (%)	29 (49.2)	39 (49.4)	30 (40.5)	0.477	0.980
Spicy food ingestion, *n* (%)	44 (77.2)	60 (83.3)	58 (77.3)	0.592	0.381
Intestinal type, *n* (%)	37 (63.8)	48 (62.3)	46 (63.0)	0.985	0.862
Cardiac cancer, *n* (%)	5 (8.5)	5 (6.3)	14 (18.7)	**0.039**	0.631
TGFB1-509 T allele, *n* (%)	43 (74.1)	55 (71.4)	46 (63.0)	0.340	0.727
*IN-1RN *2* carrier, *n* (%)	7 (12.1)	5 (6.3)	10 (13.5)	0.310	0.240
IL-1B 511 T allele, *n* (%)	45 (77.6)	60 (75.9)	60 (81.1)	0.738	0.823

Adapted from Choi et al. [42]
Bold style indicates the statistical significance
HP Helicobacter pylori, *IL* interleukin, *SD* standard deviation, *SE* standard error, *TGF* transforming growth factor
[a]Compared among the three groups (maternal, paternal, and sibling/offspring)
[b]Compared between maternal and paternal

27.5 Family History of Gastric Cancer as a Risk Factor of Intestinal Metaplasia

There is a high possibility of gastric cancer genesis if a patient has family history of gastric cancer, so it has been predicted that gastric mucosal inflammation due to *H. pylori* infection would be more severe than a patient without the family history. According to a meta-analysis result of six researches that were published until 2009, the rates of AG and IM in the presence of the family history of gastric cancer were 2.2 times and 1.98 times higher than the absence of the family history of gastric cancer, respectively [11]. However, a research from Iran in 2012 reported that family history of gastric cancer only increased IM, but not AG [43]. In addition, Oh et al. had suggested that first-degree relatives had adjusted OR of 2.69 (95 % CI, 1.06–6.80, $p=0.037$) for antral IM in male population in multivariate regression analysis, but there was no increase of AG [44]. Meanwhile, based on analyses of endoscopic observations of 4,023 patients who received health screening in Korea, 11 % of entire patients had family history of gastric cancer, and the endoscopic positive rates of AG or IM were 40.7 % and 12.5 %, respectively [28]. In this research, risk factors of AG were the age from 40 to 59 (OR 2.55), the age over 60 years old (OR 5.00), male (OR 1.38), serologically positive *H. pylori* (OR 1.41), and an academic background less than college education (OR 1.35) [28]. Interestingly the risk factors of IM were very similar to those of AG, but dairy product consumption (OR 1.40) and family history of gastric cancer (OR 1.48) were additionally found [28]. Taken together, it indicates that somehow IM can frequently occur among people with family history of gastric cancer [11, 28, 44], which can increase the risk of gastric cancer in the GCRs. As the most important risk factor of IM is *H. pylori* infection [41] and family history of gastric cancer is also an important risk factor

of gastric cancer, the importance of *H. pylori* eradication in the GCRs before development of IM is suggested.

27.6 Prognosis of Gastric Cancer in the Relatives of Gastric Cancer

The prognosis of a gastric cancer patient who has family history of gastric cancer tends to be better than a patient without family history [45–47]. According to a report from Korea, there were many cases of shallow gastric cancer infiltration in gastric cancer cases of GCRs [45]. In addition, the National Cancer Center in Korea reported that 20.6 % (263 patients) of 1,273 gastric cancer patients had family history of gastric cancer and 71.8 % of these GCRs had intestinal type of gastric cancer, while only 55.1 % of patients without the history had intestinal type ($p = 0.015$) [46]. Also, the patients with family history showed better prognosis, so their disease-free survival rate, recurrence-free survival rate, and overall survival rate were better, and they showed meaningful prognostic differences, especially in the stages of gastric cancer III and IV [46]. Moreover, Italian group reported that 21.9 % of gastric cancer patients had family history of gastric cancer, and 71.8 % of the patients with family history showed intestinal-type gastric cancer, while only 55.1 % of patients without the history had the same type [47, 48]. However, an opposite result was reported from Japan that the prognosis of gastric cancer patients with family history was worse than patients without the history [49]. Also, a report from Taiwan showed that the prognosis was not affected by the family history of gastric cancer [50]. These various results might be caused from the differences in research methods or applicants or the presence of confounding factors. Meanwhile a recent meta-analysis of five reports showed that family history is a beneficial factor for gastric cancer [51]. Perhaps, subjects do frequent health screenings due to their family history of gastric cancer and show good health-related behaviors, such as non-smoking, non-alcoholic drinking, and consistent exercise, and the patients with family history of gastric cancer often show microsatellite instability (MSI) and mismatch repair proteins that are related to the good prognosis [46]. This prediction requires further research, but it is a positive phenomenon that gastric cancer patients with family history show good gastric cancer prognosis.

27.7 Guideline of Gastric Cancer Screening in Case of Family History of Gastric Cancer

Normally, diet therapy is important to prevent gastric cancer. Taking fresh food, minimizing salty and spicy food consumption, and avoiding stimulant food are recommended [52]. Next, *H. pylori* eradication is the most important recommendation among ages in 20s and 30s because it can prevent future IM via early eradication [20, 40]. However, when a survey was conducted among gastroenterology experts in Korea about the statement "*H. pylori* eradication therapy can be helpful to prevent gastric cancer among *H. pylori*-infected patients with immediate family history on gastric cancer," responses were "totally agree" (17.2 %), "generally agree" (58.6 %), "partially agree" (17.2 %), "generally disagree" (3.5 %), "totally disagree" (3.5 %), and "do not know" (0 %). Thus, it can be inferred that the eradication therapy is not much recommended in the clinical field [53].

Once IM occurs, periodical gastroscopy is necessary to find early lesion that is treatable with endoscopy. Nonetheless, the number of gastroscopy can be varied among countries due to a cost-benefit matter. For instance, a cohort study in the United States reported that the possibilities of gastric cancer progression from AG and IM are 0.3 % and 1.0 %, respectively [54]. Also, a cohort study from Netherlands reported that the rates of gastric cancer development from AG and IM within 10 years were 0.8 % and 1.8 %, respectively. Because of these differences, establishing a global consensus is difficult. However, the recent European guideline has selected OLGA, OLGIM, serum pepsinogen level, serological *H. pylori* test, and family

history on gastric cancer, which are helpful to set up a high-risk group for gastric cancer [55]. This guideline recommends having gastroscopy every 3 years, if a patient has broad AG or IM. However, nations with high gastric cancer occurrence, such as Korea, China, and Japan, emphasize patients to have periodic gastroscopy, since it is cost-effective [56–60]. According to a Korea report in 2012, early gastric cancer rates of a group that got gastroscopy annually and a group that had gastroscopy every 2 years were 98.6 % and 80.7 %, respectively, and the rates of treatable cancer with gastroscopy were 56.9 % and 33.3 %, respectively [61]. In comparison, early gastric cancer rate of a group which gastroscopy interval was longer than 2 years was 54.6 % [61]. In addition, the 5-year survival rates of groups that received gastroscopy annually, every 2 years, and longer interval than 2 years were 98.5 %, 92 %, and 86.1 %, respectively [61]. Therefore, it is effective to receive gastroscopy annually in case of high-risk group of gastric cancer, because endoscopically treatable early gastric cancer can be detected [15].

Conclusions

Family history is important as a risk factor of gastric cancer. This factor is partially explained by genetic factor and environmental factors, such as dietary habit, and the factor is also related to familial clustering of *H. pylori* infection. Immediate family history of gastric cancer itself increases gastric cancer genesis by two to three times, and this genesis increases by 5.88 times if *H. pylori* infection exists, too. Thus, *H. pylori* eradication is the most important priority for immediate family members, who are less than 40s, especially in 20s and 30s, of gastric cancer patients. This early *H. pylori* eradication can avoid future IM and restricts synergistic effect of *H. pylori* infection and immediate family history of gastric cancer. Once IM is confirmed, it is important to do gastroscopy periodically to find out early lesion that can be treated with endoscopy. Therefore, annual gastroscopy is recommended in Korea to diagnose early gastric cancer that is treatable with endoscopy.

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H. pylori Infection-Negative Gastric Cancer

28

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Abstract

Helicobacter pylori (*H. pylori*) infection is considered to be the main etiological factor of gastric carcinogenesis. *H. pylori* infection induces superficial gastritis, which progresses to atrophic gastritis with loss of acid secretion and then to dysplasia and cancer. As *H. pylori* are eliminated once they have caused gastric atrophy and intestinal metaplasia after long-time colonization, the possibility of false-negative results of conventional *H. pylori* tests could increase in gastric cancer with severe gastric atrophy. The incidence of *H. pylori* infection-negative gastric cancer (HPIN-GC) varied among studies according to the definition of *H. pylori*-negative infection. When combination of multiple methods (histologic examination, rapid urease test, urea breath test, *H. pylori* culturing, anti-*H. pylori* antibody, and polymerase chain reaction assay for *H. pylori*) are used and past *H. pylori* infection ruled out by the presence of atrophic change, HPIN-GC is compromised about 2–5 % of gastric cancer. The cardia location is common in HPIN-GC. The more advanced pathologic T stage and N stage in HPIN-GC have been reported in some studies. Many previous studies reported that negative *H. pylori* infection was an independent poor prognostic factor, but recent researches found no significant difference in prognosis between *H. pylori*-positive and *H. pylori*-negative cancer.

Keywords

Gastric cancer • *Helicobacter pylori* • Negative infection

28.1 Introduction

Helicobacter pylori (*H. pylori*) is strongly associated with gastric cancer through epidemiologic and clinical studies, and it has been classified as a class I carcinogen by the International Agency for Research on Cancer [1]. A few prospective

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cohort studies suggested that gastric cancer develops only in *H. pylori* infection-positive subjects [2, 3]. However, no evidence of *H. pylori* infection was found in some patients with gastric cancer [4–6], and several studies reported that *H. pylori* infection-negative gastric cancer (HPIN-GC) has distinct clinicopathologic features and prognosis compared with *H. pylori* infection-positive gastric cancer (HPIP-GC). But those studies have not shown the consistent results by various *H. pylori* detection systems that interfered with interpreting the results. This chapter aims to review the research results on the incidence, clinicopathologic characteristics, and prognosis of HPIN-GC.

28.2 Incidence

Results of retrospective studies conducted in Korea have shown that HPIN-GC comprised from 2.3 to 5.4 % of gastric cancers [4, 7–9]. Japanese studies also showed that the incidence of HPIN-GC ranged from 0.42 to 10.6 % [5, 6, 10, 11]. In contrast, the proportions of HPIN-GC in Western countries, such as Italy [12] and Germany [13], ranged from 13.8 to 24.6 %. The results of previous studies on HPIN-GC were summarized in Table 28.1.

The incidence of HPIN-GC varied among studies. Differences in the definition of *H. pylori*-negative infection may partly explain the differences in various studies. In fact, there are many methods provided for detecting *H. pylori* including histologic examination, rapid urease test, urea breath test, *H. pylori* culturing, and anti-*H. pylori* antibody. Polymerase chain reaction (PCR) assay for *H. pylori* may have been more sensitive compared with other diagnostic techniques but is not usually used in the clinical setting. Many studies used a combination of multiple methods for detecting *H. pylori* to minimize the possibility of false-negative *H. pylori* testing results.

However, the diagnostic tests have been challenged because of a natural clearance or an unexpected eradication of *H. pylori* in the gastric mucosa. *H. pylori* are spontaneously disappeared in severely atrophic mucosa and advanced gastric diseases once they have caused gastric atrophy and intestinal metaplasia after long-time colonization [14]. Therefore, it is difficult to determine whether patients who had advanced gastric atrophy and intestinal metaplasia have had previous *H. pylori* infection based on conventional *H. pylori* tests. In addition, the spontaneous seroconversion of anti-*H. pylori* antibodies often occurs [15].

To overcome this problem, some studies from Korea and Japan considered atrophic gastritis and intestinal metaplasia as a result of long-standing *H. pylori* infection and determined the presence of severe gastric atrophy or intestinal metaplasia as having a previous *H. pylori* infection. In these studies, the absence of gastric atrophy confirmed by histologic examination and serum pepsinogen (serum pepsinogen I ≤70 ng/mL and/or pepsinogen I/II ratio ≤3.0) was also added to define HPIN-GC [4, 6, 8–10]; consequently, incidence of HPIN-GC was estimated to 2.3–10.6 % which was much lower than those of the Western studies that did not consider atrophic mucosal changes.

Moreover, in a study in Japan, Matsuo et al. [5] defined the patients with even active gastritis with neutrophil infiltration but without *H. pylori* detection in the histologic examination or with endoscopic atrophy as having a previous *H. pylori* infection and reported that the incidence of HPIN-GC was extremely low (0.66 %). In addition, Ono et al. [10] reported that there was only one (0.42 %) HPIN-GC among 240 early gastric cancers resected by endoscopic submucosal dissection that occurred in the mucosa without histological atrophy, endoscopic atrophy, or serological atrophy confirmed by serum pepsinogen in Japan. Although endoscopic atrophy is commonly used in Japan, there is no worldwide consensus; therefore, the interobserver variability of endoscopic observation of atrophic change could be problematic in the definition of HPIN-GC.

The Western studies did not consider atrophic mucosal changes or intestinal metaplasia. In contrast, studies from Korea and Japan usually consider atrophic change as evidence of previous *H. pylori* infection. Therefore, studies from Eastern Asia report very low incidence of HPIN-GC compared with studies from Western

Table 28.1 Clinicopathologic characteristics of *H. pylori* infection-negative gastric cancer

Author (Publication year)	Duration of study	Country	Number of total subjects	Number of *H. pylori* infection-negative gastric cancer patients (%)	Definition of *H. pylori* infection-negative gastric cancer	Characteristics of *H. pylori* infection-negative gastric cancer
Kim et al. (2015) [9]	2006–2014	Korea	705	28 (4.0)	Negative results of histology, serology, culture, and rapid urease test	–
					Absence of histologic atrophy and serologic atrophy (serum pepsinogen I/II ratio ≤3)	
Kwak et al. (2014) [7]	2007–2012	Korea	1,833	43 (2.3)	Negative results of histology, serology, and rapid urease test	Younger, female, diffuse-type, distant metastasis[a]
					Absence of histologic atrophy	
Kim et al. (2014) [8]	2003–2012	Korea	726	36 (5.0)	Negative results of histology, serology, culture, and rapid urease test	Cardia location
					Absence of histologic atrophy and serologic atrophy (serum pepsinogen I/II ratio ≤3)	
Ono et al. (2012) [10]	2004–2010	Japan	240	1 (0.42)	Negative results of histology, serology, culture, rapid urea test and/or urea breath test	–
					Absence of histologic atrophy, endoscopic atrophy, and serologic atrophy (serum pepsinogen I <70 ng/ml and/or pepsinogen I/ II ratio <3.0)	
Yoon et al. (2011) [4]	2003–2010	Korea	627	34 (5.4)	Negative results of histology, serology, culture, and rapid urease test	Advanced pT stage
					Absence of histologic atrophy and serologic atrophy (serum pepsinogen <30 ng/ml and/or pepsinogen I/II ratio <2.0)	
Matsuo et al. (2011) [5]	1996–2010	Japan	3,161	21 (0.66)	Negative results of histology, serology, rapid urease test and/or urea breath test	Younger, female, diffuse type, macroscopic depressed lesion
					Absence of endoscopic atrophy and histologic gastritis	
Kakinoki et al. (2009) [11]	2004–2007	Japan	386	12 (3.1)	Negative histology	Upper location, poorly differentiated carcinoma
Marrelli et al. (2009) [12]	1988–2004	Italy	297	41 (13.8)	Negative results of serology, PCR assay for *vacA*[b] gene and CagA[c] antibody	Cardia location, advanced pT stage, non-curative surgery, lower 5-year survival rate
Kato et al. (2007) [6]	1993–2004	Japan	748	15 (2–10.6)	Negative serology	–
					Absence of serologic atrophy (serum pepsinogen <30 ng/ml and/or pepsinogen I/II ratio <2.0)	
Meimarakis et al. (2006) [13]	1992–2002	Germany	166	41 (24.6)	Negative results of histology, serology, and culture	Upper location, lower relapse-free and overall survival

[a]Compared to gastric cancer with past *H. pylori* infection
[b]*vacA* vacuolation cytotoxin
[c]*CagA* a product of the cytotoxin-associated gene pathogenicity island

countries. In conclusion, HPIN-GC is compromised about 2–5 % of gastric cancer, when negative *H. pylori* infection was established by the combination of various methods for detecting *H. pylori* and when the absence of atrophic gastritis was confirmed by serum pepsinogen and histologic examination. When endoscopic atrophy was added in the definition of HPIN-GC, HPIN-GC consists of less than 1 % of gastric cancer.

28.3 Clinicopathologic Characteristics

In the literature, the higher distributions of cardia or upper location were frequently reported in HPIN-GC [8, 11–13, 16, 17]. The histologically diffuse type and female predominance in HPIN-GC were also found in some researches [5, 7, 16]. With regard to the pathologic T and N staging, the results were not consistent. Some studies reported the more advanced pathologic T stage [4, 12] and N stage [17] in HPIN-GC compared to HPIP-GC, but some showed the stage of HPIN-GC was not significantly different from that of HPIP-GC [8, 9, 13]. In recent Korean study, HPIN-GC did not have different characteristics compared to HPIP-GC with regard to well-known independent risk factors of gastric cancer such as age, gender, and family history of gastric cancer [9].

28.4 Molecular Characteristics

Recently, Kim et al. compared the p53 overexpression and microsatellite instability (MSI) between HPIN-GC and HPIP-GC in their two researches, and they showed that there was no significant difference between two groups with regard to the p53 overexpression and MSI [8, 9].

28.5 Prognosis

The impact of *H. pylori* infection on survival of gastric cancer is poorly investigated. A few studies reported that the association between negative *H. pylori* infection and clinical outcomes and the results on prognosis of HPIN-GC were not consistent among the studies [12, 13, 17–20].

Meimarakis et al. [13] assessed *H. pylori* infection status of 166 gastric cancer patients who had curative resection using bacterial culture, histological analyses, and serological analyses. In this study conducted in Germany, the overall survival was 19.2 months (range, 7.1–31.3) in 41 HPIN-GC patients and 61.9 months (range, 13.0–110.9) in 125 HPIP-GC patients ($p=0.0009$), and the positive *H. pylori* infection was an independent prognostic factor for overall survival (hazard ratio [HR] 2.16; 95 % confidential interval [CI], 1.33–3.49) in addition to depth of invasion, lymph node metastasis, and age.

In another study conducted in Italy, in which *H. pylori* status was determined by serologic assay of *H. pylori* and CagA antibodies and PCR assay for *vacA* gene in gastric mucosa, 5-year survival rate was 24 % in 41 HPIN-GC patients and 57 % in 256 HPIP-GC patients (log-rank test, $p<0.001$) [12]. *H. pylori* infection, depth of invasion, lymph node metastasis, and tumor location were reported to be independent prognostic factors, and the negative *H. pylori* infection was poor prognostic factor in the early (pT1-pT2) as well as in the more advanced pT classification groups (pT3-pT4). Two Korean studies reported similar results that negative *H. pylori* status was the significant independent poor prognostic factor and determined *H. pylori* infection status of gastric cancer patients who underwent chemotherapy using only histologic examination [18, 19]. Also the meta-analysis involving a total of 12 studies including 2,454 patients with gastric cancer concluded a protective role for *H. pylori* infection in the prognosis of gastric cancer [20].

However, Kim et al. found no significant difference in prognosis between HPIN-GC and HPIP-GC, defining HPIN-GC strictly as a gastric cancer having all negative results for *H. pylori* tests and absence of histologic atrophy and serologic atrophy using pepsinogen [8]. In this study, the 5-year rate of overall survival was 93.8 % in 24 HPIN-GC patients and 96.9 % in 509 HPIP-GC patients ($p=0.685$); the status of *H. pylori* infection was not an independent prognostic factor for overall survival (HR 0.51; 95 % CI, 0.07–3.70). Similarly, the study conducted in China reported

that overall survival and relapse-free survival in HPIN-GC were not significantly different from HPIP-GC patients ($p=0.715$), assessing *H. pylori* infection status using PCR assay for *H. pylori* [17].

Some authors suggest that *H. pylori* infection could improve immune responses against gastric cancer, leading to improved survival of patients with positive *H. pylori* status [13]. However, several investigators doubt the true prognostic significance of *H. pylori* status, suggesting that negative *H. pylori* status may be simply related to more advanced tumor progression such as cardia location and more advanced stage [21]. Further studies will be required to evaluate these hypotheses from the perspective molecular and genetic aspects.

Conclusions

Gastric cancers that are not associated with *H. pylori* infection are rare. HPIN-GC comprised from 2 to 5 % of gastric cancers when past *H. pylori* infection was excluded by the presence of atrophic change. Cardia location is common in HPIN-GC. The prognosis of HPIN-GC has not been fully established. Therefore, further study recruiting a large number of patients for long time and defining negative *H. pylori* infection precisely is needed to understand the exact carcinogenesis of HPIN-GC.

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Gastroesophageal Reflux Disease

29

Jinjoo Kim

Abstract

Helicobacter pylori (*H. pylori*) infection has an influence on the development of gastroesophageal reflux "indirectly" by altering gastric acid secretion. The outcome of *H. pylori* infection and eradication depends on its distribution of inflammation. If the antrum is mainly infected with *H. pylori*, acid secretion would increase, and gastroesophageal reflux disease (GERD) can be developed more easily. On the other hand, if the body is infected, acid secretion would decrease, and the possibility of GERD development gets lower. The outcome of *H. pylori* eradication also depends on the distribution of *H. pylori*-infected area. If *H. pylori* has affected the antrum, GERD symptoms tend to be improved after the eradication. On the other hand, when it has affected the body, GERD symptoms might be getting worse after the eradication. However, *H. pylori* eradication does not need to be hesitated just because of these trends. *H. pylori* eradication itself does not seem to develop GERD. It would be more correct to explain this that *H. pylori* infection has been masking GERD symptoms and *H. pylori* eradication would relieve the inflammation and no longer mask the GERD symptoms.

Keywords

Helicobacter pylori • Gastroesophageal reflux disease • Inflammation • Gastric acid secretion

29.1 Introduction

The relationship between gastroesophageal reflux disease (GERD) and *Helicobacter pylori* (*H. pylori*) has not been established thoroughly yet. Since several epidemiologic studies have shown the increase of GERD and esophageal adenocarcinoma incidence with the decrease of *H. pylori*

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prevalence, studies on the relationship between *H. pylori* and GERD had been started [1]. According to several studies so far, *H. pylori*-infected patients have lower possibility of GERD, Barrett's esophagus, and esophageal adenocarcinoma [2–7]. In this chapter, the relationship between *H. pylori* and GERD would be discussed.

29.2 How *H. pylori* Infections Influence on the GERD

Refluxes of gastric contents into the esophagus happen even in the normal healthy ones, and this does not develop any symptoms usually. If several barriers are weakened, for example, low esophageal sphincter (LES), diaphragm, which is embracing the LES, the location of LES, phrenoesophageal ligament, and the acute angle of His, then these refluxes can develop various symptoms [8–10]. Transient LES relaxation, hiatal hernia, and low pressure of LES are especially important factors, and *H. pylori* itself does not influence on these barriers directly [3, 11–15].

It is speculated that *H. pylori* influences the gastric acid secretion, and this relates to the development of GERD. *H. pylori* alters gastric acid secretion depending on the distribution of *H. pylori*-induced inflammation [16–18]. Indigestion of food into the stomach triggers gastric acid secretion, and the amount of acid secretion depends on the feedback reaction to the gastric pH levels. Usually, owing to proteins within indigested food, food indigestion leads to high pH in the stomach and triggered gastric acid secretion. Gastric secretion stimulates gastric acid secretion of the parietal cells in the body. As a result of this process, pH of the stomach drops and somatostatin secretion is triggered. This somatostatin inhibits gastric secretion, and gastric acid secretion is also inhibited. However, when *H. pylori* influences on the feedback processes, this feedback system no longer works as before [19].

As explained in Fig. 29.1 [20], in the case of astral predominant gastritis, somatostatin secretion

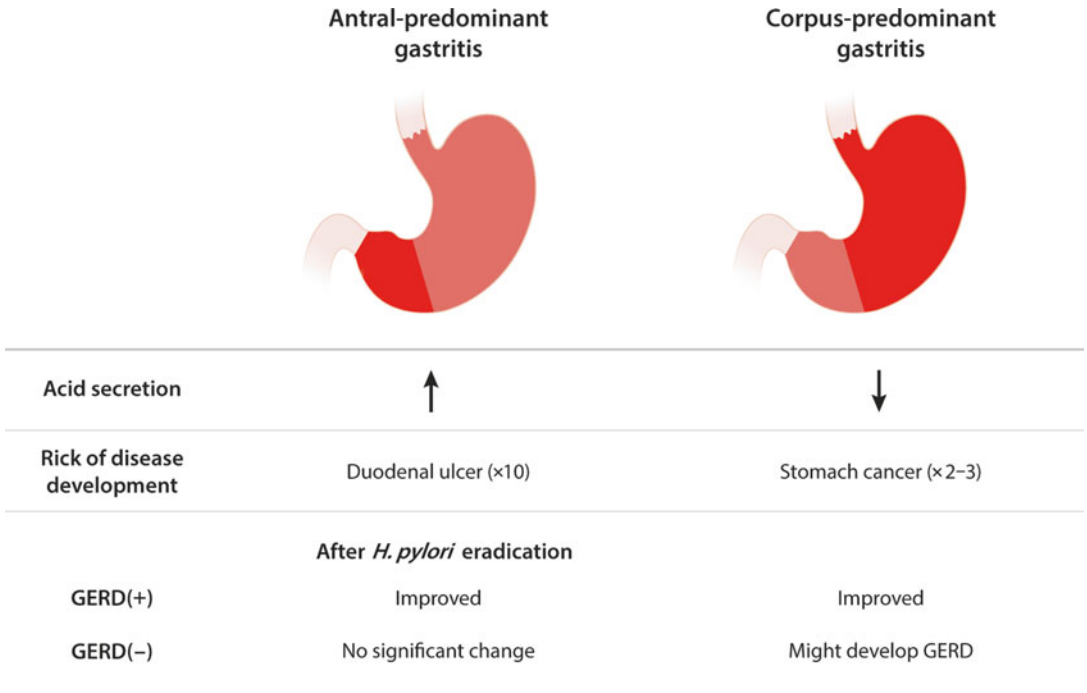

Fig. 29.1 The relationship between gastroesophageal reflux disease (GERD) and *H. pylori* (Modified from Pandolfino and Kahrilas [20])

at the antrum would be inhibited, and gastric acid would not be inhibited enough and gastric acid would be maintained at the higher levels than the normal ones [21]. On the other hand, in the case of corpus predominant gastritis, parietal cells in the corpus are damaged, and acid secretion would decrease. Therefore, *H. pylori* eradication would normalize the levels of decreased gastric acid secretion and might develop GERD symptoms [22–24].

29.3 How *H. pylori* Eradication Influences on GERD

Clinical experience with some patients developing GERD symptoms after *H. pylori* eradication has promote the studies on the effect of *H. pylori* eradication on the development of GERD [25, 26]. The influence of *H. pylori* on the GERD development varies widely from newly developed GERD symptoms to aggravating or alleviating GERD symptoms after *H. pylori* eradication. The results of *H. pylori* eradication can be different depending on the levels of gastric acid secretion and how well the barriers to GERD have worked before the eradication. Also, proton pump inhibitor (PPI) rebound phenomenon might be developed, even though the duration of PPI usage as *H. pylori* eradication is quite short.

According to several studies, when the subjects were followed up after *H. pylori* eradication, for 6 months to 1 year, GERD patients' symptoms and endoscopic findings were not aggravated generally [27, 28]. However, the studies on the actual pH change of the distal esophagus are scant yet, probably because it is not easy to measure the distal esophageal pH [29–33]. There are some discrepancies in the studies about the relation between *H. pylori* eradication and GERD. This might be explained that initial studies could not get the idea that different *H. pylori*-associated inflammation distributions would result in different outcomes. Taking this limitation into account, the results of the studies so far can be summarized as below.

29.3.1 Antral Predominant Gastritis

Antral predominant gastritis leads to increase gastric acid secretion, and this is the high-risk condition to develop GERD. Therefore, *H. pylori* eradication would lead to decrease the risk of GERD development [26, 34–38]. In concordance to this concept, *H. pylori* eradication improved GERD symptoms in the patients with duodenal ulcer and/or GERD. Eight studies with 1,165 duodenal ulcer patients showed that *H. pylori* eradication did not develop any new GERD symptoms and improved pre-existing GERD symptoms [37]. Also, one meta-analysis of 27 studies showed that GERD symptoms were not developed after *H. pylori* eradication of duodenal ulcer patients [38].

29.3.2 Corpus Predominant Gastritis

Corpus predominant gastritis leads to decrease gastric acid secretion, and, in this case, GERD rarely occurs. Therefore, after *H. pylori* eradication, corpus predominant gastritis would be improved and acid secretion would increase leading to the development of GERD. In concordance with this concept, several studies showed that points with severe corpus gastritis rarely had GERD symptoms and improvement of this gastritis led to increase the risk of GERD development [39–42].

It is important to bear in mind that *H. pylori* eradication itself does not develop GERD but just unmasks those symptoms in the patients with weak GERD barriers. Those patients who cannot clear the acid at the esophagus and have hiatal hernias and low LES pressure already have high risk to develop GERD. This has been masked for a while by *H. pylori*-associated inflammation. Figure 29.2 shows this concept as "iceberg of GERD" [16]. This concept can be explained well with the study analyzing the risk factors to develop GERD after *H. pylori* eradication. Patients with hiatal hernia, which is well known as a GERD developing risk factor, showed higher reflux esophagitis after *H. pylori* eradication (26 % vs. 8 %). That is, those with high risks for GERD might develop GERD symptoms and those without them might not.

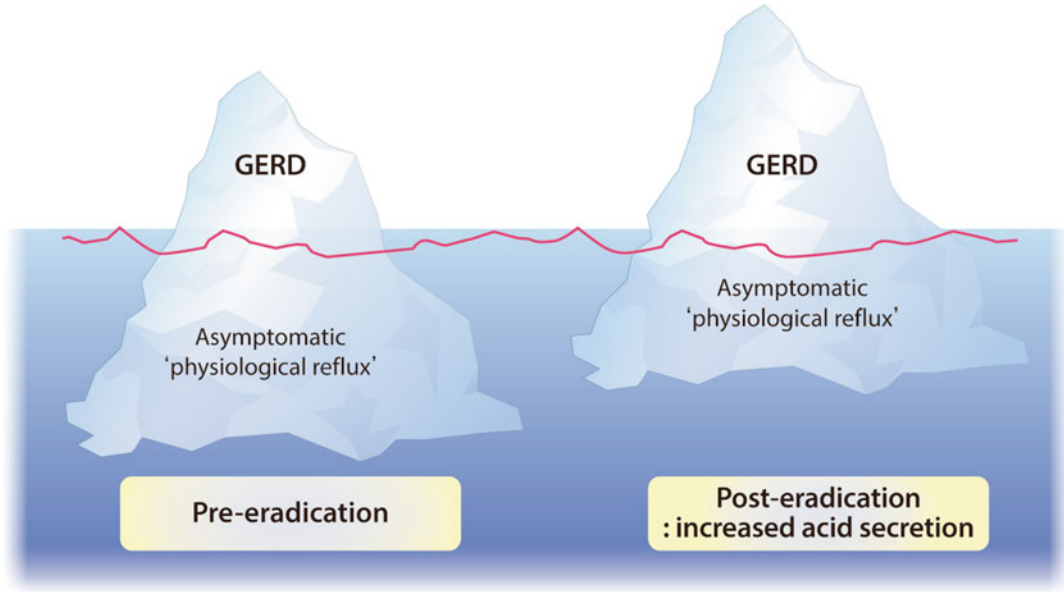

Fig. 29.2 Iceberg of gastroesophageal reflux disease (Modified from Graham [16])

Conclusions

H. pylori influences on the gastric acid secretion and leads to the development of GERD in some patients. However, this risk cannot overweigh the benefit of *H. pylori* eradication. So it is not necessary to hesitate *H. pylori* eradication for concerning this issue.

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NSAID-Induced Gastropathy and *H. pylori* Infection

30

Gwang Ha Kim

Abstract

Gastrointestinal injury by nonsteroidal anti-inflammatory drugs (NSAIDs) or *Helicobacter pylori* (*H. pylori*) occurs through different mechanisms. If these two factors coexist, the risk of gastrointestinal injury increases independently, sometimes synergistically by the two factors. In high-risk patients who require NSAID administration, it is recommended that *H. pylori* infection is examined, and then eradication therapy is done for patients with *H. pylori* infection to prevent peptic ulcer. For initial NSAID (excluding aspirin) users and long-term aspirin users with a history of peptic ulcer complications, tests for *H. pylori* infection and eradication therapy in the presence of *H. pylori* infection are recommended. In countries where the incidence of *H. pylori* infection is high, such as Korea, prevention and treatment strategies for NSAID users with *H. pylori* infection should differ from those in Western countries. Long-term multicenter studies that consider the current clinical situation are necessary in the future.

Keywords

Nonsteroidal anti-inflammatory drugs • *Helicobacter pylori* • Gastropathy • Peptic ulcer

30.1 Introduction

Both *Helicobacter pylori* (*H. pylori*) *i*nfection and nonsteroidal anti-inflammatory drugs (NSAIDs) administration are known major risk factors of gastric and duodenal ulcers. With the effort to reduce the *H. pylori* infection rate and the wide spread of eradication therapy, the incidence of peptic ulcer induced by *H. pylori* infection has decreased. However, with the aging society

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worldwide, the prevalence rates of chronic musculoskeletal, cardiovascular, and cerebrovascular diseases have been increasing. Consequently, the incidence of complications induced by administration of aspirin or NSAIDs, including peptic ulcer, is increasing.

NSAID-related ulcers may be improved by discontinuing the drug administration. However, the drug administration is difficult to discontinue in patients with chronic diseases, where in most cases, continuous treatment for ulcer is necessary. Moreover, in countries such as Korea where the *H. pylori* infection rate is high, many cases of NSAID-related ulcers are accompanied by *H. pylori* infection. When these two factors coexist, the risk of gastrointestinal complications increases. Some reports indicated that the incidence of complications has decreased with *H. pylori* eradication therapy, but other reports indicated no significant difference in the existence of *H. pylori*, making the use of eradication therapy controversial. Although studies of various designs have investigated the effect of *H. pylori* infection on the development of gastritis or ulcer in patients receiving NSAIDs, various controversies remain. In this chapter, I briefly examine gastric damage related to NSAID administration and describe the pathophysiological mechanisms and treatment of ulcer induced by NSAID administration accompanied by *H. pylori* infection.

30.2 NSAID-Induced Gastropathy

30.2.1 Mechanisms

The gastrointestinal mucosa can be damaged via various mechanisms induced by NSAID administration [1–5] (Fig. 30.1). First, NSAIDs can directly act on the gastrointestinal mucosa. When orally administered, NSAIDs bind to the hydrogen ion in the gastric juice and penetrate the cell membrane into the epithelial cell of the gastrointestinal mucosa. This then induces intracellular acidification and inhibits intracellular mitochondria function, inducing cell apoptosis. NSAIDs can also act systematically. The absorbed NSAIDs inhibit cyclooxygenase (COX) activity, reducing endogenous prostaglandin (PG) synthesis and thus inhibiting the gastrointestinal mucosa defensive mechanism induced by PG. Decrease in PG synthesis results in decreased mucus and bicarbonate

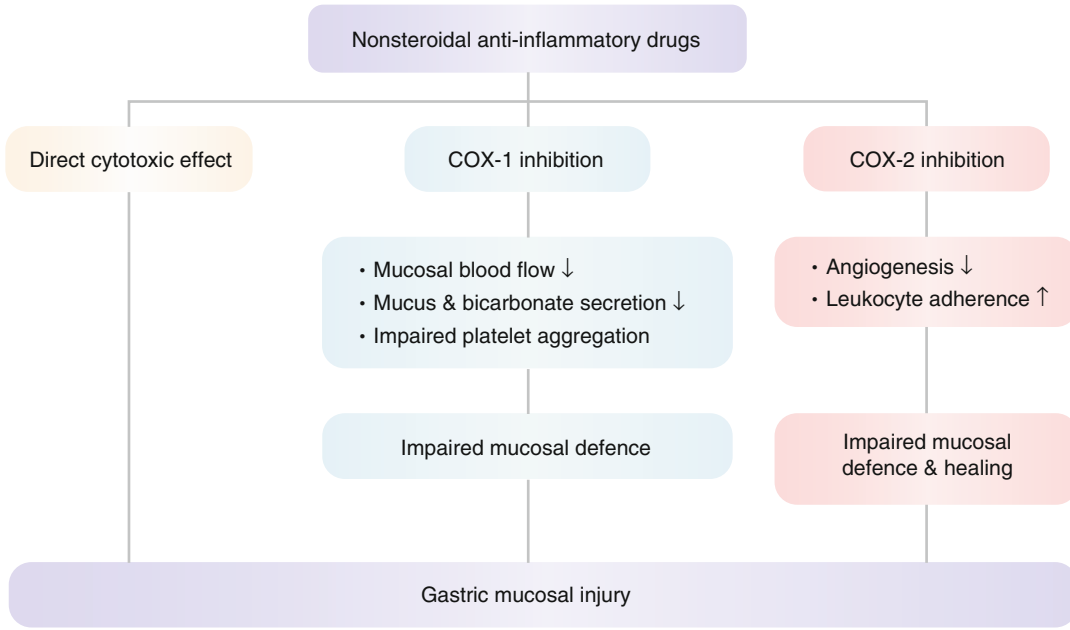

Fig. 30.1 Mechanisms of nonsteroidal anti-inflammatory drug (NSAID)-induced gastric mucosal injury

secretion from the epithelial cells, decreased mucosal blood flow, and decreased proliferation and resistance to injury of epithelial cells. Through these consecutive events, the gastrointestinal mucosa becomes damaged. Moreover, with the relative increase in lipoxygenase activity compared to inhibited COX activity, leukotriene (LT) production is increased. The increased LT production induces spasm to the vascular endothelial cells, leading to ischemia and reperfusion stress, which increases reactive oxygen species production, and thus resulting in inflammation and tissue apoptosis that induce damage to the gastrointestinal mucosa. It also increases the production of inflammatory mediators such as tumor necrosis factor (TNF), inducing damage to the gastrointestinal mucosa. Of the abovementioned mechanisms, the major pathophysiological mechanism of peptic ulcer induced by repetitive NSAID exposure is thought to be the systemic action of NSAIDs, which inhibits COX activity in the gastrointestinal mucosa.

30.2.2 Nonselective and Selective NSAIDs

Nonselective NSAIDs (e.g., indomethacin, naproxen, diclofenac, piroxicam, or ibuprofen) inhibit both COX-1 and COX-2 expressions related to PG synthesis. As an essential constitutive enzyme expressed in normal cells, COX-1 is used to produce PG for normal gastrointestinal mucosa defense. COX-2 is usually induced by inflammation-inducing cytokines such as endotoxin, TNF-α, or interleukin (IL)-1β in pathological status. Although aspirin is classified as a nonselective NSAID, it partially induces a COX-2-mediated response. Once aspirin is administered, the COX-2-mediated response that converts arachidonic acid to 15-hydroxyeicosatetraenoic acid (15-HETE) is induced, and 15-HETE is then converted to 15-epi-lipoxin A4, minimizing gastric damage caused by the 5-lipoxygenase production induced by aspirin administration. Accordingly, compared with other nonselective NSAIDs, it acts as a stronger inhibitor of COX-1 than COX-2. With such mechanism, co-administration of low-dosage aspirin and selective COX-2 inhibitor would cause greater gastrointestinal damage than would a single aspirin administration [5, 6].

Selective COX-2 inhibitors (e.g., celecoxib or etodolac) can conserve the gastrointestinal mucosa defense mechanism induced by PG via COX-1. However, when compared with the placebo control group, the group who received COX-2 inhibitors showed greater damage to the gastrointestinal mucosa. COX-2 expression is increased in peptic ulcer and gastrointestinal inflammation to increase PG production for ulcer healing and to reduce inflammation. Damage to the gastrointestinal mucosa is thought to be induced by blocking these protective effects of COX-2 by COX-2 inhibitors. In addition, it can also inhibit COX-1 expression in the clinically recommended dosage, thus showing the possibility of gastrointestinal mucosal damage even with a single administration of selective COX-2 inhibitor [7, 8].

30.3 Association Between NSAID-Induced Gastropathy and *H. pylori* Infection

30.3.1 Pathophysiology

Gastrointestinal damage by NSAIDs or *H. pylori* occurs via different mechanisms, and the coexistence of these two factors independently increases the risk of gastrointestinal damage [9, 10]. The two factors participate in the life cycle of the gastrointestinal epithelial cells. *H. pylori* infection induces a chronic hyperplastic state of the gastrointestinal mucosa while promoting apoptosis of the epithelial cells. NSAIDs inhibit normal cell life cycles, inhibiting cell growth and promoting apoptosis of the epithelial cells. Moreover, the two factors reduce mucosal viscosity, increasing gastrointestinal damage [11].

The incidence of ulcer occurrence in the *H. pylori* infection group was 4.03 times higher than that in the non-infection group regardless of NSAID administration, and the incidence of ulcer occurrence in the NSAID administration group was 3.10 times higher than that in the non-NSAID

administration group regardless of *H. pylori* infection [12]. Furthermore, NSAID administration and *H. pylori* infection increased the risk of peptic ulcer bleeding by 4.8 and 1.8 times each, and *H. pylori* infection in patients with NSAID administration showed 60 times higher incidence rate of peptic ulcer and had 6 times higher risk of developing bleeding than patients without *H. pylori* infection [9]. In addition, the risk of uncomplicated peptic ulcer was 1.81 times higher in *H. pylori*-positive NSAID users than in *H. pylori*-negative NSAID users [10]. These findings support the fact that the two risk factors represent independent and synergistic risk factors for uncomplicated and bleeding peptic ulcers. Similar results were observed in the patients who were taking aspirin. When aspirin was administered to patients with *H. pylori* infection, the damage to the gastrointestinal mucosa reached the maximum at day 3 of administration and continued until day 14. However, when aspirin was administered after *H. pylori* eradication therapy, the damage to the gastrointestinal mucosa slowly decreased from day 3 [13].

However, a study reported that *H. pylori* infection might be helpful for healing of peptic ulcer in patients who receive NSAID administration [14]. *H. pylori* infection induces excessive COX-2 mRNA expression related to the growth factor, PG synthesis, and epithelial proliferation, thus increasing PG production in the gastrointestinal mucosa, which is helpful for ulcer healing and reduces gastrointestinal complications due to NSAID administration. With such mechanism, when *H. pylori* eradication therapy is administered to patients receiving NSAIDs, the ulcer healing may be delayed [12, 14].

30.3.2 Diagnosis of *H. pylori* Infection in NSAID Users

In Korea, to prevent peptic ulcer in high-risk patients who need NSAID treatment, test for *H. pylori* infection is needed. When *H. pylori* infection is present, eradication therapy is recommended. Test for *H. pylori* infection is recommended when eradication therapy is considered effective, in patients who have received an initial NSAID (excluding aspirin) treatment and in patients receiving long-term aspirin administration who have a history of peptic ulcer complications. However, test for *H. pylori* infection is not recommended in patients receiving long-term NSAID (including aspirin) administration who do not have any history of peptic ulcer complications and in patients receiving long-term NSAID (excluding aspirin) administration who have a history of peptic ulcer complications [15, 16].

Meanwhile, in countries such as the United States or Canada where the incidence rate of *H. pylori* infection is low, test for *H. pylori* infection is recommended in all patients who have been receiving long-term NSAID administration regardless of the existence of risk factors [16, 17]. In countries such as Korea where the incidence rate of *H. pylori* infection is high, test for *H. pylori* infection is deemed necessary only when eradication therapy is expected to be effective. This differs from the guidelines of the West.

30.3.3 Therapeutic Approach

30.3.3.1 *H. pylori* Infection in Initial NSAID Users

NSAIDs (Excluding Aspirin)

In a comparison between *H. pylori* eradication therapy group and non-eradication therapy group before 8-week naproxen administration among patients with *H. pylori* infection, *H. pylori* eradication therapy before NSAID administration recued the occurrence of NSAID-related peptic ulcers [18]. In patients who were NSAID naive, had a positive urea breath test, had dyspepsia or an ulcer history, and required long-term NSAID treatment, *H. pylori* eradication therapy before diclofenac slow release administration for 6 months significantly reduced the risk of ulcers and complications [19]. Therefore, *H. pylori* eradication therapy reduces the incidence rate of peptic ulcer and complications in patients who have received an initial NSAID (excluding aspirin) administration.

Aspirin

Although *H. pylori* eradication therapy is recommended to some patients who have received an

initial aspirin administration, whether the treatment is necessary remains unclear because of the lack of definite evidence. That is, the application of *H. pylori* eradication therapy is limited without systematized studies available.

30.3.3.2 *H. pylori* Infection in Long-Term NSAID Users Without Peptic Ulcer Complications

NSAIDs (Excluding Aspirin)

In a randomized, double blind, multicenter study for *H. pylori*-positive patients requiring NSAID therapy who had no past or current peptic ulcer, there was no difference in the occurrence of peptic ulcers between anti-*H. pylori* treatment followed by proton pump inhibitor (PPI) use and the PPI treatment only [20]. Since there have been no evidences showing that *H. pylori* eradication therapy accelerates the ulcer healing in long-term NSAID (excluding aspirin) users without peptic ulcer complications, *H. pylori* eradication therapy is not recommended, and concomitant PPI administration is deemed necessary during the course of NSAID treatment.

Aspirin

The efficacy of *H. pylori* eradication therapy in long-term aspirin users without peptic ulcer complications has not yet been reported. Thus, whether *H. pylori* eradication therapy is needed in long-term aspirin users without a history of peptic ulcer complications remains unclear.

30.3.3.3 *H. pylori* Infection in Long-Term NSAID Users with Peptic Ulcer Complications

NSAIDs (Excluding Aspirin)

When long-term naproxen users with a history of peptic ulcer complications randomly received PPI administration or 1 week of eradication therapy and then were followed for 6 months, PPI was superior to *H. pylori* eradication therapy in preventing recurrent bleeding [21]. That is, in long-term NSAID (excluding aspirin) users with a history of peptic ulcer complications, continuous PPI administration is more effective than *H. pylori* eradication therapy in inhibiting the development of peptic ulcer complications.

Aspirin

When long-term aspirin users with a history of bleeding peptic ulcer randomly received PPI administration or 1 week of eradication therapy and then were followed for 6 months, *H. pylori* eradication therapy was equivalent to PPI treatment in preventing recurrent bleeding [21]. In another study, patients who had ulcer complications after using low-dose aspirin continuously for more than 1 month and who had *H. pylori* infection were randomly assigned to PPI treatment or placebo after *H. pylori* eradication therapy and then followed up for 12 months [22]. In this study, the rate of recurrence of ulcer complications was significantly lower in the PPI treatment in addition to *H. pylori* eradication therapy. These findings indicate that in long-term aspirin users with a history of ulcer complications, *H. pylori* eradication therapy can reduce the recurrence of ulcer complications.

30.3.3.4 *H. pylori* Infection in COX-2 Inhibitor Users

H. pylori infection often increases the COX-2 mRNA expression related to PG synthesis, epithelial cell proliferation, and growth factor expression. Some animal studies have shown that selective COX-2 inhibitor might aggravate the damage to the gastrointestinal mucosa in the presence of *H. pylori* infection [23, 24]. However, there are limitations, as there are not many studies on this subject.

30.3.4 Guidelines for Management of *H. pylori* Infection in NSAID Users

30.3.4.1 Korea

Guideline of Prevention and Treatment for NSAID-Related Peptic Ulcers (2009) [16]

- *Treatment recommended to patients with NSAID-related dyspepsia and mucosal injury*
  - In high-risk patients requiring NSAID administration, actions need to be taken for diagnosis of *H. pylori* infection to prevent

peptic ulcer. Once the infection is confirmed, eradication therapy should be done (evidence level, moderate; recommendation grade, low).

- *Treatment for NSAID-related peptic ulcers*
  - In patients with NSAID-related peptic ulcers, *H. pylori* infection should be diagnosed and treated (evidence level, moderate; recommendation grade, high).

Guidelines for Diagnosis and Treatment of *H. pylori* Infection in Korea (2013) [15]

- *Is* H. pylori *eradication indicated for preventing the recurrence of disease in a long-term low-dose aspirin user with a history of peptic ulcer?*
  - *H. pylori* eradication is indicated for preventing the recurrence of disease in a long-term low-dose aspirin user with a history of peptic ulcer (evidence level, low; recommendation grade, strong).
- *Does* H. pylori *eradication reduce the risk of peptic ulcers in long-term NSAID users?*
  - *H. pylori* eradication alone does not reduce the risk of peptic ulcers in long-term NSAID users (evidence level, high; recommendation grade, strong).

Guidelines of Treatment for Non-bleeding Peptic Ulcer Disease (2009) [25]

- *NSAID-related peptic ulcers*
  - *H. pylori* eradication therapy can reduce the risk of occurrence of NSAID-induced peptic ulcers (evidence level, high; recommendation grade, strong).
  - In NSAID-related gastric ulcers, *H. pylori* eradication therapy is recommended after healing of gastric ulcers via antiulcer medications (evidence level, moderate; recommendation grade, moderate).

Guidelines of Treatment of Bleeding Peptic Ulcer Disease (2009) [26]

- *H. pylori* eradication therapy reduces the recurrence of peptic ulcer bleeding (evidence level, high; recommendation grade, strong).
- However, continuous NSAID administration can provoke the recurrence of peptic ulcer bleeding even after *H. pylori* eradication therapy. Thus, PPIs should be concomitantly used, or NSAIDs should be substituted to selective COX-2 inhibitors.

30.3.4.2 Overseas Countries

Management of *H. pylori* Infection: The Maastricht IV/Florence Consensus Report [27]

- *H. pylori* infection is associated with an increased risk of uncomplicated and complicated gastroduodenal ulcers in NSAID and low-dose aspirin (acetylsalicylic acid (ASA)) users (evidence level, 2a; grade of recommendation, B).
- Eradication reduces the risk of complicated and uncomplicated gastroduodenal ulcers associated with either NSAID or low-dose ASA use (evidence level, 1b; grade of recommendation, A).
- *H. pylori* eradication is beneficial before starting NSAID treatment. It is mandatory in patients with a peptic ulcer history (evidence level, 1b; grade of recommendation, A).
- However, *H. pylori* eradication alone does not reduce the incidence of gastroduodenal ulcers in patients already receiving long-term NSAID treatment. They require continued PPI treatment as well as eradication treatment (evidence level, 1b; grade of recommendation, A).
- Testing for *H. pylori* should be performed in ASA users with a history of gastroduodenal ulcer. The long-term incidence of peptic ulcer bleeding is low in these patients after receiving eradication even in the absence of gastroprotective treatment (evidence level, 2b; grade of recommendation, B).

Guidelines for Management of *H. pylori* Infection in Japan [28]

- In *H. pylori*-positive patients who are scheduled to start low-dose aspirin therapy, pre-treatment *H. pylori* eradication decreases the risk of peptic ulcer bleeding. However, its effect is weaker than that of PPIs, and eradication therapy alone is insufficient for high-risk patients.

- *H. pylori* eradication therapy can decrease the risk of peptic ulcer or bleeding associated with low-dose aspirin or NSAIDs, but its effect is limited. To minimize the risk of ulcers or bleeding associated with these drugs, it is necessary to take preventive measures such as inhibition of acid secretion by PPI therapy after *H. pylori* eradication, at least in high-risk patients.

Conclusions

With the progression of the elderly society, the number of individuals who take NSAIDs at their own discretion, without physician prescription, has increased. Thus, the incidence of NSAID-related peptic ulcer diseases will continuously increase. In addition, with the high incidence rate of *H. pylori* infection in Korea, the relationship between NSAIDs and *H. pylori* infection and their effects on gastrointestinal injury are predicted to become an important issue in the treatment of peptic ulcer diseases. Although most clinicians worldwide diagnose and treat *H. pylori* infection in patients with a history of peptic ulcer regardless of NSAID administration, there is still controversy in this issue up to now. Thus, long-term multicenter studies that consider the current clinical situation are necessary in the future.

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31 Extraintestinal Manifestations of *H. pylori* Infection: *H. pylori*-Associated Iron-Deficiency Anemia

Yon Ho Choe

Abstract

Adolescents are particularly susceptible to iron deficiency because of their high requirements for iron during the growth spurt and menstrual blood loss in girls. More iron intake and prompt absorption are needed at puberty. *Helicobacter pylori* (*H. pylori*) infection in the gastric mucosa might lead to iron-deficiency anemia (IDA) because it seems to sequester iron and accordingly disturb iron absorption in adolescents whose iron supply is marginal, although the mechanisms by which *H. pylori* infection contributes to IDA remain unclear. When pubescent children or young adults are found to have IDA without evidence of gastrointestinal bleeding, and their anemia is refractory to iron supplementation and unexplained, *H. pylori* infection should be suspected and eradication of the bacteria is necessary.

Keywords

Helicobacter pylori • Iron deficiency • Iron-deficiency anemia

31.1 Introduction

Iron deficiency is the most common cause of anemia. It is caused by low intake of iron, chronic blood loss, malabsorption, and hemolysis. Upper and lower endoscopies are recommended to find out the gastrointestinal causes of iron-deficiency anemia (IDA). However, gastrointestinal lesions contributing to anemia are identified in about 50 % of patients who undergo upper and/or lower endoscopies [1]. Many reports relating *Helicobacter pylori* (*H. pylori*) infection to IDA have been described for the past two decades. Interestingly, the patients have no evidence of gastrointestinal bleeding and their anemia is refractory to iron supplementation and subsided only after *H. pylori* eradication. Currently, according to American College of Gastroenterology Guideline and the Maastricht IV/Florence Consensus Report, *H. pylori* eradication is recommended in otherwise unexplained IDA [2, 3].

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31.2 Clinical Studies

In 1993, Dufour et al. [4] reported a 7-year-old case of *H. pylori*-associated chronic antral gastritis without evidence of hemorrhage or clinical symptoms other than sideropenic anemia, which subsided after *H. pylori* eradication. Since then, there have been similar reports and most of the patients were adolescents [5–16] (Table 31.1). The author, as a pediatrician, also have experienced refractory IDA patients whose stool occult blood tests and endoscopic findings showed negative except for positive *H. pylori* infection. *H. pylori* eradication resulted in cure of IDA. Interestingly, a considerable number of patients with *H. pylori*-associated IDA were adolescent athletes. In 1997, the author conducted a double-blinded, placebo-controlled trial in pubescent children with IDA and coexisting *H. pylori* infection [8]. Of 43 IDA subjects, 22 *H. pylori*-positive patients were assigned to three groups. Group A were given oral iron and a 2-week course of eradication, group B received placebo for iron and eradication treatment, and group C were given oral iron and a 2-week course of placebo. Group A and B subjects, who received eradication therapy, showed a significant increase in hemoglobin level as compared with group C at 8 weeks after therapy ($p=0.0086$). In an open trial conducted in the same center, 11 out of 21 subjects with refractory IDA were confirmed to have *H. pylori* infection without evidence of gastrointestinal bleeding, and they were given oral iron supplementation for 3 months but failed. The levels of hemoglobin and serum ferritin showed a prominent increase after eradication of *H. pylori* ($p=0.0002$) [17]. Since 2005, large-scaled randomized controlled trials have been performed and they revealed strong evidence linking *H. pylori* to the etiology of unexplained IDA [18–24] (Table 31.1).

31.3 Epidemiology

In 1998, a Danish population survey was performed in 2,794 adults, whose serum ferritin, hemoglobin, and IgG antibodies against *H. pylori* were assessed. The study showed that serum ferritin levels are reduced in people with increased IgG antibodies to *H. pylori*, suggesting that *H. pylori* infection affects iron metabolism in humans [25]. In an Australian study, the investigators measured *H. pylori* IgG antibodies, plasma ferritin, and iron intake in 160 women, which revealed that the ferritin concentration of infected women was significantly lower than for noninfected women in spite of the similar daily iron intakes [26]. A cross-sectional national health survey among 1,806 healthy people in Germany showed that *H. pylori* infection was associated with a 17.0 % decrease of the serum ferritin concentration. They also reported that the association between *H. pylori* infection and serum ferritin levels did not vary by gender, age, and iron intake, and it was similar for CagA-positive and CagA-negative *H. pylori* infections [27]. In 2000, the author conducted a population survey for 375 healthy children aged 10–15 years, which showed that the prevalence rate of *H. pylori* infection was 15.5 % in children without IDA and 31.3 % in those affected ($p=0.022$) [9]. In another study for pubescent athletes, prevalence rates of IDA, *H. pylori* infection, and *H. pylori*-associated IDA in female athletes were higher than in the control group. The relative risk of IDA was 2.9 (95 % confidence interval [CI], 1.5–5.6) for those with *H. pylori* infection [12]. In a large-scaled seroprevalence study in 937 pubescent children, the *H. pylori*-positive rates in anemia, hypoferritinemia, and iron-deficiency group were 34.2 %, 29.5 %, and 35.3 %, respectively, compared to 19.6 % in the non-anemia group ($p=0.003$), 19.2 % in the non-hypoferritinemia group ($p=0.005$), and 19.4 % in the non-iron-deficiency group ($p=0.001$) [14]. The *H. pylori*-positive rate in the IDA group was 44.8 % in comparison with 20.0 % in the non-IDA group ($p=0.001$). The associations between iron status and *H. pylori* were largely restricted to girls rather than boys [14]. In another Korean study, the *H. pylori* seropositivity in 753 schoolchildren aged 6–12 years was measured, and serum ferritin levels were significantly lower in *H. pylori*-seropositive children than in *H. pylori*-seronegative controls ($p<0.001$) [28].

Table 31.1 Studies concerning a relationship between *H. pylori* and iron-deficiency anemia

Author	Year	Country	Type of study	Subjects	Cases (*n*)	Results	References
Dufour	1993	Italy	Case report	Adolescent	1	Resolution of IDA after *H. pylori* eradication	[4]
Marignani	1997	Italy	Case report	Adolescent	1	Resolution of IDA after *H. pylori* eradication	[5]
Carnicer	1997	Spain	Case report	Child	1	Resolution of IDA after *H. pylori* eradication	[6]
Barabino	1999	Italy	Case series	Children	4	Resolution of IDA after *H. pylori* eradication	[7]
Choe	1999	Korea	Randomized controlled trial	Adolescents	43	*H. pylori*(+) IDA patients after eradication had increased Hb compared with placebo	[8]
Choe	2000	Korea	Population survey	Adolescents	375	*H. pylori*-associated IDA as a risk factor for subnormal growth at puberty	[9]
Konno	2000	Japan	Case series	Adolescents	6	Resolution of IDA after *H. pylori* eradication	[10]
Ashorn	2001	Finland	Case series	Children	7	Resolution of IDA after *H. pylori* eradication	[11]
Choe	2001	Korea	Controlled trial in IDA/*H. pylori* seropositives	Adolescents	12 athletes, 10 controls	Significant increases in Hb, iron, and ferritin after *H. pylori* eradication c/w controls given iron therapy	[12]
Sugiyama	2002	Japan	Case series	Adult females	2	Resolution of IDA after *H. pylori* eradication	[13]
Choe	2003	Korea	Population survey	Adolescents	937	Prevalence of *H. pylori* infection with refractory IDA much higher than in normal population	[14]
Kostaki	2003	Greece	Case series	Children	3	Resolution of IDA after *H. pylori* eradication	[15]
Hacihanefioglu	2004	Turkey	Case series	Adult females	14	Increase in Hb, iron, transferrin saturation, no change in ferritin	[16]
Mahalanabis	2005	India	Randomized controlled trial	Children	169	Resolution of IDA after *H. pylori* eradication	[18]
Gessner	2006	Alaska	Randomized controlled trial	Children	219	Difficulty in interpretation in endemic area	[19]
Sarker	2008	Bangladesh	Randomized controlled trial	Children	200	Difficulty in interpretation in endemic area	[20]
Fagan	2009	Alaska	Randomized controlled trial	Children	219	Resolution of IDA after *H. pylori* eradication	[21]
Duque	2010	Mexico	Randomized controlled trial	Children	69	Resolution of IDA after *H. pylori* eradication	[22]
Cardenas	2011	Texas	Randomized controlled trial	Children	110	Increase in serum ferritin after *H. pylori* eradication	[23]
Xia	2012	China	Randomized controlled trial	Adolescents	80	Resolution of IDA after *H. pylori* eradication	[24]

IDA iron-deficiency anemia

Adolescents are susceptible to iron deficiency because of their high requirement for iron during the growth spurt at puberty, low dietary intake, and menstrual blood loss in girls. Sports anemia, reported for the first time in 1970, is multifactorial: dilutional pseudoanemia, intravascular hemolysis, starvation anemia, and IDA [29–32]. The most common type of true anemia that affects athletes is IDA. The frequent sources of iron deficiency are blood loss from the gastrointestinal tract and menstruation, poor dietary intake, and iron loss through profuse sweating [33, 34]. The major risk factor for *H. pylori* infection is the socioeconomic status of the family during childhood, as reflected in the number of persons in a household and sharing a bed, absence of a fixed hot water supply, and poor sanitation [35–37]. In Korea, most adolescent athletes live together in training camps for considerable period per year, usually between middle school and college. Therefore, these athletes are susceptible to *H. pylori* infection because of environmental factors. It is consistent with the data from a Korean seroprevalence study performed on 440 students from a regular high school and 220 athletes of a physical education high school; the prevalence of *H. pylori* infection in athletes (43.2 %) was higher than that in the controls (22.7 %, $p = 0.005$) [38]. Taken together, *H. pylori* infection seems to act as "iron stressor," especially in adolescent girls who are vulnerable to iron deficiency, rapidly growing children at puberty, and athletes who are at risk for sports anemia.

However, a couple of studies have reported that there is no association between *H. pylori* and IDA. In a study conducted in 1,060 adults in New Zealand, there were no significant differences in serum ferritin in either males or females between *H. pylori*-seropositive and *H. pylori*-seronegative subjects [39]. A Korean study for 693 children aged 9–12 years showed no significant difference in the seroprevalence of *H. pylori* infection between the IDA group and the nonanemic controls [40]. However, these two reports have limitation that adolescents who are vulnerable to iron deficiency are not included in the subjects.

In conclusion, the literature reveals that *H. pylori*-associated IDA develops mostly at puberty, when the high requirement for iron is necessary [41–43].

31.4 Mechanisms

31.4.1 Chronic Gastrointestinal Blood Loss Induced by *H. pylori* Infection

In 1991, a 15-year-old girl was reported to have syncope leading to the diagnosis of an *H. pylori*-positive chronic active hemorrhagic gastritis. Her hemoglobin values returned to normal and the symptoms disappeared after eradication [44]. According to a study to confirm prevalent iron deficiency among Yupik Eskimos living in Alaska, occult gastrointestinal bleeding appeared to be pervasive in the Yupik population and likely underlay the prevalent iron deficiency. They concluded that an atypical hemorrhagic gastritis associated with *H. pylori* infection was present almost universally and might represent the bleeding source [45].

However, most published reports have found no evidence of bleeding lesions at the time of endoscopy in patients with *H. pylori*-associated IDA.

31.4.2 The Effect of Chronic *H. pylori* Gastritis on Gastric Acid Secretion and Iron Absorption

Dietary iron is available as heme iron (meat), which is readily absorbed, or nonheme iron (vegetable, grain), in which bioavailability is dependent on a variety of factors [41]. Nonheme iron accounts for 80 % of dietary iron in industrialized countries. Crucial to the effective solubility and absorption of nonheme iron is hydrochloric acid in acid secretions. Reduction of the ferric to ferrous form is dependent upon the pH of the gastric juice, and reduction to the ferrous form facilitates membrane transport [46]. Annibale et al. [47] reported high frequency of chronic gastritis in gastric body as

well as antrum in patients with refractory IDA and *H. pylori* infection. They showed that 80 % of their patients had chronic pangastritis. In patients with *H. pylori*-associated corpus gastritis, an inverse relationship was reported between the severity of gastritis and acid secretion, concluding that gastric function can recover to normal or almost normal after cure of *H. pylori* infection [48]. Another study showed that the number of parietal cells in the secretory phase was significantly lower in *H. pylori*-infected patients than in those without *H. pylori* infection, accounting for the higher intragastric pH in patients with *H. pylori*-associated gastritis [49].

An important promotor of iron absorption is ascorbic acid, appearing to act either by promoting reduction to the ferrous form or by forming an absorbable molecular complex with ferric iron, which is insoluble at pH values above 5 [50]. Normally, gastric acid appears to facilitate the chelation in the stomach of ferric salts with ascorbic acid and other substances. Since these chelates then remain soluble in the more alkaline duodenum and jejunum, iron absorption is enhanced [51, 52]. Ascorbic acid is secreted into the stomach at a high concentration, which would support the idea of active secretion. Chronic gastritis seems to interfere with this secretion and reduces the ascorbic acid concentration in the gastric juice [53]. It has been reported that the reduction in gastric ascorbic acid concentration is related to gastric juice pH, the severity and extent of gastritis, and the presence of *H. pylori* [54]. In an Italian study, the vast majority of patients with unexplained IDA and *H. pylori* gastritis were characterized by gastric body involvement, suggesting a plausible explanation for a negative effect of *H. pylori* gastritis on physiological alimentary iron absorption [55].

Annibale et al. [56] evaluated gastric juice pH and gastric juice and plasma ascorbic acid in patients with *H. pylori* infection and unexplained IDA, compared with controls with IDA and a healthy stomach or with controls with *H. pylori* infection and no IDA. They performed the upper and lower endoscopy in patients with unexplained IDA to exclude the possible causes of IDA. Forty-three patients were divided into two groups according to whether they had *H. pylori* gastritis ($n = 30$) or normal gastric histology ($n = 13$). In addition, 11 non-anemic patients with *H. pylori* gastritis were included as another control group. They found that mean intragastric pH concentrations were significantly higher in the patients with *H. pylori*-associated IDA compared with each control group. Ascorbic acid concentrations in gastric juice were significantly lower in *H. pylori*-associated IDA patients compared with IDA patients without *H. pylori* infection and non-anemic patients with *H. pylori* infection. They also reported that 94 % of patients with IDA had pangastritis, whereas most of those without IDA had antral gastritis.

Assuming that *H. pylori* infection affects gastric acid secretion and ascorbic acid concentrations in gastric juice, we can suggest that those parameters be reversible after *H. pylori* eradication. However, only small number of patients received *H. pylori* eradication therapy in the study, and there were no differences in gastric pH and ascorbic concentrations in patients with chronic atrophic corpus gastritis.

31.4.3 Increased Iron Consumption by *H. pylori*

Iron is an essential element for almost all living organisms, as it is a cofactor required for activity of many enzymes [57]. Pathogenic bacteria need to develop specific iron uptake systems in order to thrive in the host environment [50]. In *H. pylori*, not only iron uptake but also iron storage is regulated by ferric uptake regulator (fur), which controls iron transport in bacteria [57]. In iron-replete environments, *H. pylori* expresses the Pfr protein, and the FeoB protein is expressed in iron-restricted conditions.

The Pfr, 19.6 kDa bacterioferritin of *H. pylori*, is known to have a major role in storing iron that the bacterium sequestered, and avoiding toxicity to the cells, under conditions of constant or intermittent iron excess [58]. The author performed sequence comparisons for the *pfr* regions between 16 IDA-positive and 10 IDA-negative groups in

H. pylori gastritis patients, which showed that the mutation in the *pfr* gene did not relate with the clinical phenotype, IDA [59].

FeoB-mediated Fe^{2+} uptake is known to be a major pathway for *H. pylori* iron acquisition. FeoB mutants were unable to colonize the gastric mucosa of mice, indicating that FeoB makes an important contribution to iron acquisition by *H. pylori* in the low-pH, low-O_2 environment of the stomach [60].

The author categorized 14 *H. pylori* gastritis patients into subgroups based on the presence or absence of IDA. Eight patients were diagnosed as having IDA; the other six showed normal hematological findings. According to the sequence analysis of the complete coding region of *feoB* gene, the four polymorphisms of the *feoB* gene appeared to be related to the clinical phenotype of IDA, but the relation was unclear because of the small number of strains studied [61].

Studies regarding the impact of strain differences in iron regulatory genes on IDA are still controversial.

31.4.4 Iron Sequestration in Gastric Mucosa

Is it possible for iron that reached stomach to be sequestered in some space without absorption by *H. pylori* infection? In 1999, Barabino et al. [7] reported that the ^{59}Fe red blood cell use decreased in all their case series, which might suggest that iron was diverted from the bone marrow to some extramedullary focus, possibly the *H. pylori* gastric infection. It was suggested that the iron acquisition system of *H. pylori* by the human lactoferrin receptor system might play a major role in the virulence of *H. pylori* infection, since lactoferrin was found in significant amounts in human stomach resections with gastritis [62]. In that study, investigators were not able to detect any siderophore production in the culture supernatants of *H. pylori* strains under iron-limited conditions. *H. pylori* can, however, use human lactoferrin and heme as an iron source [62]. The lack of siderophore production and the need for heme as a growth factor, which may increase significantly the availability of heme, suggest the existence of a specific heme uptake system in *H. pylori* [63]. Their electrophoretic analysis of outer membrane preparations from *H. pylori* cultured under conditions of iron restriction revealed a couple of proteins to be present at elevated levels, called iron-repressible outer membrane proteins. Dhaenens et al. [64] identified a 70 kDa lactoferrin-binding protein (Lbp) from outer membrane proteins of *H. pylori*. This Lbp was only present when *H. pylori* was cultured in an iron-restricted medium, suggesting its role in iron uptake.

It has been known that lactoferrin concentration is increased in the biopsy specimens of patients with *H. pylori* gastritis and that the levels of lactoferrin correlate significantly with the degree of inflammation of the gastric mucosa [65]. Lactoferrin is an iron-binding glycoprotein that is found in various body fluids such as milk, lacrimal secretions, saliva, and urine [66]. It also has been found in the cardiac and pyloric glands and neutrophils within surface epithelium [67]. Lactoferrin binds free iron with great affinity limiting the amount of ions available for microorganism's metabolism. Therefore, its role in the host defense mechanisms consists in bacteriostatic and bactericidal effects. It is also involved in the modulation of immune system and recent studies show that lactoferrin directly modulates both production and function of neutrophils and monocytes [68].

However, *H. pylori* does not seem to be eradicated by lactoferrin, even though lactoferrin concentration is increased in gastric mucosa with *H. pylori* infection. Rather, it is assumed that *H. pylori* absorbs the iron from lactoferrin via a specific Lbp expressed by *H. pylori* [50]. Husson et al. [62] reported that human lactoferrin supported full growth of the bacteria in media lacking other iron sources, but neither human transferrin, bovine lactoferrin, nor hen ovotransferrin served as a source for iron.

Based on the studies described above, the author investigated the relationship between lactoferrin and *H. pylori* infection coexistent with IDA by determining the lactoferrin levels in gas-

tric biopsy specimens, according to the presence or absence of iron-deficiency anemia [69]. One hundred and one adolescents who underwent gastroduodenoscopy were divided into four groups: controls without *H. pylori* infection (NL; $n=43$), patients with *H. pylori* infection (HP; $n=26$), patients with IDA (IDA; $n=6$), and patients with *H. pylori* gastritis and coexisting IDA (HPIDA; $n=26$). The mucosal level of lactoferrin was highest in HPIDA, followed by in HP, NL, and IDA. When mucosal lactoferrin and blood hemoglobin levels were compared before and after *H. pylori* eradication in 12 HPIDA patients who underwent follow-up endoscopy, lactoferrin levels decreased and hemoglobin levels increased significantly after eradication. These findings support the hypothesis that *H. pylori* gastritis could act as a sequestrating focus for iron [7]. Moreover, this hypothesis also proved to be true by immunohistochemical staining. When the gastric mucosa is infected with *H. pylori*, lactoferrin secretion increased in the glands and neutrophils. Interestingly, the mucosal lactoferrin level in the patient with only IDA was lowest, while it was highest when the patient was affected with both *H. pylori* infection and IDA. The former result seems to reflect that the whole body is short of iron and iron-containing substances such as lactoferrin and the latter to reveal that iron is sequestered in the gastric mucosa by *H. pylori* infection even though the host is suffering from iron deficiency.

Barabino speculated that *H. pylori*-infected antrum could act as a sequestering focus for iron: *H. pylori* infection enhances gastric lactoferrin. The iron bound to lactoferrin is in turn picked up by the bacterium, by means of its outer membrane receptors, for its own growth. As *H. pylori* turnover is very rapid, the bacterial iron stores are rapidly lost in the stools, together with the dead bacteria. This mechanism could explain why an iron supply is no longer available for hemopoiesis, which only enhances *H. pylori* proliferation [70].

Conclusions

Adolescents are particularly susceptible to iron deficiency because of their high requirements for iron during the growth spurt and menstrual blood loss in girls. Although more iron intake and prompt absorption are needed at puberty, *H. pylori* infection in the gastric mucosa might lead to IDA because it sequesters iron and accordingly disturbs iron absorption in adolescents whose iron supply is marginal. It was speculated that even a small negative balance in iron storage at puberty could contribute to IDA because pubescent children are at the risk of iron deficiency [69]. This suggestion has been typically demonstrated in a study conducted in adolescent female athletes group [12] (Fig. 31.1). The above speculation gives us a plausible explanation why only a small proportion of population develops IDA in spite of worldwide *H. pylori* infection. The similar finding has been reported even in an Italian adult study, in which the majority of patients with *H. pylori*-associated IDA were premenopausal women who are vulnerable to iron loss [71].

The biologic mechanisms for *H. pylori*-associated IDA should be assessed in the aspects of two sides (Fig. 31.2). One is bacterial aspect, and the other, host. In the aspect of bacteria, the iron is overconsumed or iron uptake system in the human body is altered by *H. pylori*. Another explanation is possible that there exist *H. pylori* strain differences in contributing to IDA. On the contrary, host reactions to *H. pylori* infection causing IDA include the change in gastric acid secretion, iron sequestration, and accompanying mucosal immune reactions. To date, there has been no clear report that verified the decisive mechanisms by which *H. pylori* infection might lead to IDA. It appears that several pathways are involved separately or in combination [41].

When pubescent children or young adults are found to have IDA, and their anemia is refractory to iron supplementation and unexplained, *H. pylori* infection should be suspected and eradication of the bacteria is necessary. *H. pylori*-associated IDA can be cured by bacterial eradication.

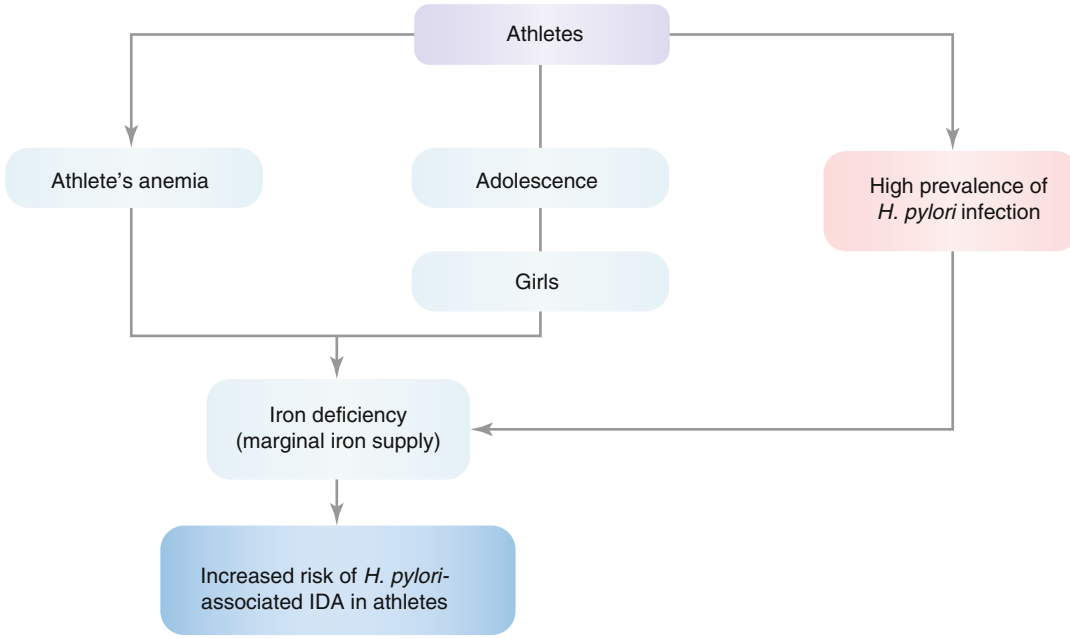

Fig. 31.1 Flowchart of increased risk of *H. pylori*-associated IDA (iron-deficiency anemia) in adolescent female athletes

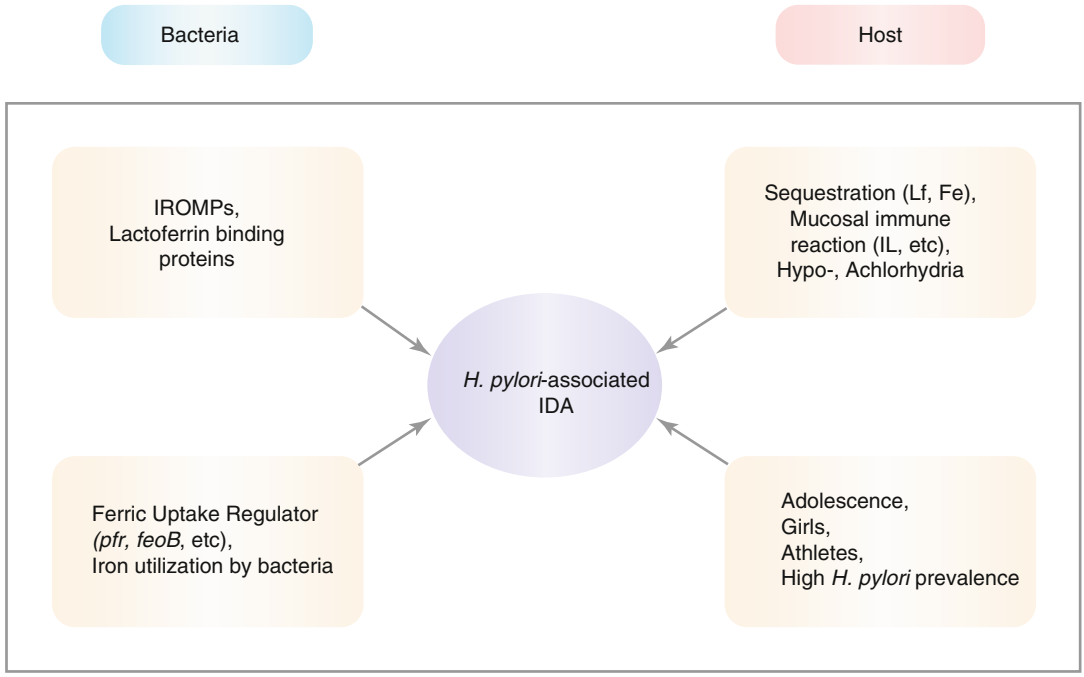

Fig. 31.2 Possible mechanisms by which *H. pylori* infection contributes to IDA (iron-deficiency anemia). *IROMPs* iron-repressible outer membrane proteins, *Lf* lactoferrin, *IL* interleukin

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32 Extraintestinal Manifestations of *H. pylori* Infection: Idiopathic Thrombocytopenic Purpura

Chan Gyoo Kim

Abstract

Epidemiologic studies, randomized controlled studies, and meta-analysis have reported an association between idiopathic thrombocytopenic purpura and *Helicobacter pylori* (*H. pylori*) as well as the improvement of idiopathic thrombocytopenic purpura after *H. pylori* eradication. Because the underlying mechanism has not been determined, there are many hypotheses to explain the causal relationship between ITP and *H. pylori* infection. One hypothesis states that the antibodies associated with *H. pylori* infection, including the CagA protein, cross-react with glycoproteins on the surface of thrombocytes such as glycoprotein IIIa. *H. pylori* eradication is recommended for patients with idiopathic thrombocytopenic purpura and *H. pylori* infection in the guidelines about *H. pylori* in Europe, Japan, Asia-Pacific, and Korea.

Keywords

Helicobacter pylori • Eradication • Idiopathic thrombocytopenic purpura • Immune thrombocytopenic purpura

32.1 Introduction

In addition to idiopathic iron-deficiency anemia, idiopathic thrombocytopenia is one of the hematologic diseases associated with *Helicobacter pylori* (*H. pylori*). For idiopathic thrombocytopenia, *H. pylori* eradication is recommended in the Korean guideline [1] as well as the treatment guidelines from Europe (2012) [2], Japan (2009) [3], and the Asia-Pacific (2009) [4]. In this article, I review the association between *H. pylori* and idiopathic thrombocytopenia as well as the effect of *H. pylori* eradication on idiopathic thrombocytopenia.

32.2 Idiopathic Thrombocytopenic Purpura (Immune Thrombocytopenic Purpura)

Idiopathic thrombocytopenia occurs via an immune mechanism in which the level of thrombocytes decreases below the normal level (150,000/

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uL) due to destruction of thrombocytes by an autoantibody or immune complex. It is referred to as idiopathic when the cause is not clearly determined and as secondary when the immune complex or autoantibody was produced as a result of drug administration, systemic disease, or infection. It was previously known as idiopathic thrombocytopenic purpura but is now known as immune thrombocytopenic purpura (ITP) for both idiopathic and secondary causes [5, 6].

32.3 Association Between Immune Thrombocytopenic Purpura and *H. pylori*

In 1998, Gasbarrini et al. [7] reported that *H. pylori* eradication resulted in an improved number of thrombocytes in 8 of 11 patients with autoimmune thrombocytopenia, and 6 of these 8 patients showed decreased autoantibodies, indicating a potential association between ITP and *H. pylori*. Later, increased thrombocyte levels after *H. pylori* eradication were reported by numerous studies, including a Korean study in which 17 (68 %) of 25 patients with ITP showed complete or partial response [8] and a multicenter, open-label, prospective Korean study with *H. pylori*-positive patients ($n = 20$) with moderate thrombocytopenia (30×10^9/L $\leq$ platelet count $\leq 70 \times 10^9$/L) also showed complete response rate of 50 % 3 months after the eradication treatment [9]; collectively, these findings support a causal relationship between ITP and *H. pylori* infection. A description of the studies reporting increased levels of thrombocytes after *H. pylori* eradication on a regional basis follows. In 29 studies conducted with adults in Asia (23 in Japan, 2 in China, 2 in Iran, and 2 in Korea), 1109 (71.8 %) of 1545 patients with ITP were infected with *H. pylori*. Of the 949 patients who received eradication treatment, it was successful in 827 patients (87.1 %); of these patients, 486 (58.8 %) showed increased thrombocyte levels [9, 10]. In Europe, in ten studies conducted with adults (eight in Italy, one in Serbia, and one in Turkey), 288 (58.2 %) of 495 patients with ITP were infected with *H. pylori*. Eradication treatment administered to 242 patients was successful in 222 patients (91.7 %); of these patients, 108 (48.6 %) showed increased levels of thrombocytes [10] (Table 32.1).

There have been few studies conducted with children. In three studies conducted in Asia (Japan, Iran, and Taiwan), 23.8 % of the 63 patients were infected with *H. pylori*. The eradication rate in the 15 patients who received eradication treatment was 93.3 % (14 patients), with 7 (50 %) patients showing a response in thrombocyte levels. In Europe, 21.1 % of the 332 patients from four studies (two in Italy, one in the Netherlands, and one in Finland) were infected with *H. pylori*. Of these infected patients, 57 patients were administered eradication treatment, and the eradication rate was 93.0 % (53 patients), with 29 patients (54.7 %) showing a response in thrombocyte levels [10] (Table 32.1).

Because spontaneous resolution frequently occurs due to the nature of ITP and *H. pylori* is a common pathogen in the stomach, it is hard to determine the causal relationship between ITP and *H. pylori* infection based solely on the improvement in thrombocyte levels after *H. pylori* eradication. Macrolide-type antibiotics used for *H. pylori* eradication, such as clarithromycin, have an immune regulatory effect and inhibit the production of proinflammatory cytokines, thereby improving the autoactivity of thrombocytes in ITP [11]. Alternatively, this could be the result of the elimination of other thrombocyte autoantibody-inducing bacteria in the body following the administration of antibiotics used to eradicate *H. pylori*. There are also reports claiming that the infection rate of *H. pylori* in patients with ITP is not different from that in the general public [12–16].

However, a systematic review of studies in which *Helicobacter* was eradicated not only in patients with ITP infected with *H. pylori* but also in patients with ITP without *H. pylori* infection reported a significantly higher number of *H. pylori*-positive patients with increased thrombocyte levels than *H. pylori*-negative patients [17]. Only 8.8 % of the patients with ITP who were *H. pylori*-negative had increased thrombocyte

Table 32.1 Association between *H. pylori* and immune thrombocytopenia

	Continent	Country (no. of studies)	Patients with ITP (*n*)	*H. pylori*-infected ITP patients (*n*, %)	*H. pylori*-eradicated patients (*n*)	Patients with platelet response (*n*, %)
Adults	Total		2094	1430 (68.3 %)	1078	618 (56.0 %)
	Asia	Japan (23)	1545	1109 (71.8 %)	827	486 (58.8 %)
		China (2)				
		Iran (2)				
		Korea (2)				
	Europe	Italy (8)	495	288 (58.2 %)	222	108 (48.6 %)
		Serbia (1)				
		Turkey (1)				
	America	Colombia (1)	54	33 (90.6 %)	29	24 (82.8 %)
		Canada (1)				
Children	Total		395	85 (21.5 %)	67	36 (53.7 %)
	Asia	Japan (1)	63	15 (23.8 %)	14	7 (50.0 %)
		Taiwan (1)				
		Iran (1)				
	Europe	Italy (2)	332	70 (21.1 %)	53	29 (54.7 %)
		Finland (1)				
		Netherland (1)				

Modified from Kim et al. [9] and Campuzano-Maya et al. [10]

H. pylori Helicobacter pylori, *ITP* immune thrombocytopenic purpura

levels, compared with 51.2 % of the patients with ITP who were *H. pylori*-positive (odds ratio 14.5, 95 % confidence interval [CI], 4.2–83.2) [17]. Furthermore, in a meta-analysis of only prospective studies, a higher number of thrombocytes were present with *H. pylori* eradication than with failed eradication or without eradication [18]. The weighted mean difference (WMD) of the thrombocyte levels for the group with *H. pylori* eradication was 52.2 (95 % CI, 34.3–70.1, $p<0.0001$) compared with the group with failed eradication and 40.8 (95 % CI, 20.9–60.3, $p<0.0001$) compared with the group without eradication, resulting in significant differences.

Owing to the strong evidence presented by the previously mentioned epidemiologic study of *H. pylori* infection with patients with ITP and the systematic literature review and meta-analysis of the changes in thrombocyte levels after *H. pylori* eradication treatment, Korea, Europe, Japan, and Asia-Pacific guidelines for *H. pylori* are recommending eradication of *H. pylori* for patients with ITP who have *H. pylori* infection [1–4].

32.4 Mechanisms

Because the underlying mechanism has not been determined, there are many hypotheses to explain the causal relationship between ITP and *H. pylori* infection. One hypothesis states that *H. pylori* cause nonspecific phagocytosis of monocytes and develops autoreactivity in B and T lymphocytes by reducing the inhibitory receptor FcγRII B of monocytes and inducing monocyte overexpression. It has been confirmed that this phenomenon is restored to the normal state once *H. pylori* is eradicated [19].

In addition, production of autoantibodies that opsonize thrombocytes for destruction in the spleen might be associated with *H. pylori* infection. This hypothesis states that the antibody against *H. pylori*, especially against the cytotoxin-associated gene A (CagA) protein, causes cross-reaction with glycoproteins on the surfaces of thrombocytes such as glycoprotein IIIa. The evidence for this hypothesis is provided by a study reporting that these antibodies were decreased in some patients who showed

cross-reaction between antibodies and CagA and complete recovery after *H. pylori* eradication. Another evidence is that there were many patients with CagA antibodies in a group that responded to eradication treatment than nonresponded group (83 % vs. 12.5 %, $p = 0.026$) [20, 21]. There are differences in the improvement of thrombocyte levels with *H. pylori* eradication among regional reports, which could explain why *H. pylori* eradication was more effective in Japan, where there is a higher CagA-positive rate than in western countries [22] (Table 32.1).

Conclusions

Although the exact mechanism has not been determined, Epidemiologic studies, randomized controlled studies, and meta-analysis have reported an association between *H. pylori* and ITP as well as improved disease after *H. pylori* eradication. *H. pylori* eradication is highly recommended for patients with ITP who are infected with *H. pylori*.

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Extraintestinal Manifestations of *H. pylori* Infection: Heart Disease

33

Seon Hee Lim

Abstract

Since the discovery that *Helicobacter pylori* (*H. pylori*) is a central player in the pathogenesis of gastric and duodenal ulcers, there have been many studies that demonstrate the role of *H. pylori* infection in extragastric diseases. New research is showing that it may also be linked to hyperlipidemia and heart diseases. However, there is still plenty of skepticism concerning cardiovascular diseases in which this widespread microbe may be implicated. Therefore more studies, especially prospective randomized multicenter studies, are needed to illuminate the role of *H. pylori* infection in heart diseases.

Keywords

Cardiovascular disease • Heart disease • *Helicobacter pylori* • Lipid profile

33.1 Introduction

Epidemiological and clinical studies had shown a possible link between *Helicobacter pylori* (*H. pylori*) infection and the risk for developing heart disease since the mid-1990s, but inhomogeneity in the study population and study methods along with potential confounders have yielded conflicting results.

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The hypotheses of possible association between *H. pylori* infection and coronary heart diseases (CHD) are based on that (1) the infection rate of *H. pylori* being higher in the group of CHD patients; (2) the association between the risk factors, such as blood lipid profile or fibrinogen, and *H. pylori* infection; (3) the positive correlation between C-reactive protein (CRP) which can be associated with CHD and the antibody of *H. pylori*; and (4) the presence of *H. pylori* in the atheroma [1].

Chronic infection of microorganisms accompanying inflammation was considered as one of the major determinants in the pathogenesis of atherosclerosis [2–6]. Infection triggers chronic inflammatory state along with other risk factors,

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such as dyslipidemia, hypercoagulability, and endothelial dysfunction, and contributes in the pathogenesis of atherosclerosis. Since Fabricant et al. [5] mentioned that infection may be related with atherosclerosis, several microbes have been reported as a causative microbe of atherosclerosis, and *H. pylori* is a one of them. Mendall et al. [6] suggested the association of adult CHD with *H. pylori* which was infected at the period of early childhood and kept lifelong seropositivity without eradication. Therefore, many scholars tried to reveal the role of *H. pylori* infection in causation of myocardial infarction (MI) and obtained conflicting results.

The following issues will be discussed: (1) the relationship between *H. pylori* seropositivity and lipid profile; (2) the relationship between *H. pylori* and atherosclerotic disease in the heart, i.e., CHD; and (3) the relationship between *H. pylori* and arrhythmia, i.e., atrial fibrillation (AF).

33.2 *H. pylori* and Lipid Profile

Since Gallin et al. [7] reported that systemic inflammatory response by infection of microbes caused lipid profile change, there have been many studies about the relation of *H. pylori* to lipid metabolism based on epidemiologic studies (Table 33.1) [8–21]. Among patients of CHD who were diagnosed by coronary angiography, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) were not changed, but high-density lipoprotein cholesterol (HDL-C) level was lower in *H. pylori* seropositive group compared to *H. pylori* seronegative group [17]. In another study, *H. pylori* infection was associated with higher triglycerides (TG) and lower HDL-C [22]. On the other hand, there were several studies which couldn't find the association between *H. pylori* infection and lipid profile [9]. Among healthy persons, not among patients, LDL-C level was higher and HDL-C was lower in *H. pylori* seropositive group compared to seronegative group [18]. *H. pylori* infection is associated with lower HDL-C and higher TC, LDL-C, and TG levels. Higher apolipoprotein (apo)-B and lower apo-A levels were also reported [23].

The study results achieved so far about the association between lipid profile and *H. pylori* showed variable study designs, variable study objects, variable analytic method, and then variable study results. Approximate assumption according to the studies about the positive association between *H. pylori* and lipid profile is that *H. pylori* infection may cause elevation of TC, TG, LDL-C, and/or apo-B and decrease of HDL-C and/or apo-AI [8, 10, 12, 13, 16–20, 22]. These positive relations may be due to chronic infection of *H. pylori* that induces the secretion of the cytokines related to chronic inflammation, such as interleukin (IL)-1, IL-6, interferon (IFN)-α, or tumor necrosis factor (TNF)-α, and then these cytokines activate lipoproteinases in the fat tissue and synthesis of fatty acid and degradation of lipid in the liver [13, 24]. Otherwise among the studies so far there were also the studies showing no relation between *H. pylori* infection and lipid profile [9, 11, 14, 15].

The change of lipid profile after eradication also can tell the influence of *H. pylori* infection on lipid profile, but there was no opinions accord about the change after eradication. Majka et al. [10] reported TC and LDL-C decreased in 6 months after *H. pylori* eradication, and Scharnagl et al. [14] also reported the patients who had duodenal ulcer showed HDL-C, apo-AI, and apo-AII increase in 1 year after *H. pylori* eradication. Nam et al. [21] reported successful eradication decreased the risk of high LDL-C and low HDL-C compared to that of the persistent infection, whereas there were several studies which reported TC and TG rather increased after *H. pylori* eradication [25–27]. On this wise, the change of lipid profile by *H. pylori* infection or *H. pylori* eradication couldn't show a constant trend. The association between lipid profile and *H. pylori* has been increasingly discussed but still remains unclear. Therefore the study will need to show concrete evidences that *H. pylori* infection influences lipid profile.

Table 33.1 Overview of investigations concerning the effects of HP infection on lipid profile

References	Study design	Objects	Test for HP infection	Results					Other remarks
				TC	LDL -C	HDL -C	TG	Apo lipoproteins	
Lauria et al. (Finland, 1999) [8]	Single center, cross-sectional	880 Males	HP IgG, IgA	↑	NA	→	↑	NA	
Danesh et al. (UK, 1999) [9]	Case-control	1122 AMI pts, 1122 age-sex-matched controls	HP IgG	→	NA	NA	NA	→	
Majka et al. (Germany, 2002) [10]	Case-control	80 Stroke pts, 80 age-sex-SES-matched controls	UBT	↑	↑	NA	NA	NA	After HP eradication, significant decrease in TC and LDL-C than baseline
Elizalde et al. (Spain, 2002) [11]	Prospective multicenter cross-sectional	830 Pts with GI sx (+) and with EGD, and 466 pts with HP Eradi Tx	CLO and/or UBT and/or histology	→	→	→	→	NA	After HP eradication, minor increase in TC and TG than baseline
Rhee et al. (Korea, 2002) [12]	Single center, cross-sectional	32,998 Healthy persons	HP IgG	↑	→	↓	→	→	
Chimienti et al. (Italy, 2003) [13]	Single center, cross-sectional	211 Healthy males	UBT, CagA IgG	↑	↑	→	→	NA	CagA(−) strains seem to have a major impact in modifying lipid levels than the CagA(+) ones
Scharnagl et al. (Austria, 2004) [14]	Observational, longitudinal	87 Duodenal ulcer pts	CLO, histology, UBT	(↑)	(→)	(↑)	(↑)	AI (↑), AII(↑), B (↑)	():the changes after HP eradication
Montenero et al. (Italy, 2005) [15]	Single center, case-control	59 Atrial fibrillation pts, 45 healthy controls	HP IgG	→	→	NA	→		
Sung et al. (Korea, 2005) [16]	Single center, cross-sectional	58,981 Healthy persons	HP IgG	→	→	↓	↑	AI(↓), B (↑)	

(continued)

Table 33.1 (continued)

References	Study design	Objects	Test for HP infection	Results					Other remarks
				TC	LDL -C	HDL -C	TG	Apo lipoproteins	
Jia et al. (China, 2009) [17]	Single center, cross-sectional	961 CHD pts on coronary angiography	HP IgG	→	→	↓	→	NA	
Satoh et al. (Japan, 2010) [18]	Single center, cross-sectional	6289 Healthy persons	HP IgG	NA	↑	↓	→	NA	In men cf. No association in women
Kim et al. (Korea, 2011) [19]	Single center, cross-sectional	462 Healthy persons	Histology on gastric biopsy	→	↑	→	→	NA	
Lim et al. (Korea, 2013) [20]	Multicenter, cross-sectional	19,272 Healthy persons	HP IgG	↑	NA	NA	→	NA	
Nam et al. (Korea, 2015) [21]	Single center, cross-sectional and longitudinal	4269 Health check-up persons	UBT, CLO, or histology	NA	↑ (↓)	↓ (↑)	→ (→)	NA	():the changes after HP eradication

→ no difference between HP positive and HP negative groups, ↑ higher in HP positive group than in HP negative group, ↓ lower in HP positive group than in HP negative group *HP Helicobacter pylori*, *TC* total cholesterol, *TG* triglyceride, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *NA* not applied, *AMI* acute myocardial infarction, *pts* patients, *SES* socioeconomic status, *UBT* urea breath test, *GI sx* gastrointestinal symptoms, *EGD* esophagogastroduodenoscopy, *Eradi Tx* HP eradication therapy, *CLO* Campylobacter-like organism, *CagA* cytotoxin-associated gene-A, *CHD* coronary heart disease

33.3 *H. pylori* and Coronary Artery Disease

Recent studies suggested a possible link between *H. pylori* infection and CHD, but overall the association between *H. pylori* and coronary artery disease is not strong and is still debated, so well-designed prospective randomized blind study is yet to be needed. Already published studies seem to explore in three aspects: (1) the relation between ischemic heart disease and *H. pylori* infection by serology or histology; (2) the relation between ischemic heart disease and cytotoxin-associated gene-A (CagA) antibody; and (3) the relation between recurrence of ischemic heart disease and eradication of *H. pylori.* The results of the studies about *H. pylori* and coronary artery disease were summarized on Table 33.2 [6, 9, 28–42].

Although some seroepidemiological or clinical studies demonstrated associations between CHD and antibody to *H. pylori* [22, 30, 31, 34, 35, 39, 40, 42], other studies did not [28, 29, 32, 33, 36, 37, 41]. Mach et al. [43] conducted in vivo study about a mouse model of atherosclerosis, but there was no indication the *H. pylori* infection might contribute to the development of atherosclerotic lesion formation. There was a study [44] which reported that endothelial function of vessels was improved after *H. pylori* was eradicated or another study [45] that showed recurrence of CHD was decreased after eradication therapy of *H. pylori*, while there was a study that reported no difference in recurrence of CHD between with and without eradication of *H. pylori* [46].

There is no definite theory yet about mechanisms that *H. pylori* infection causes atherosclerosis, but we can get the presumed pathogenic mechanisms based on the results of many studies; *H. pylori* may secrete cytotoxin and activate inflammatory cytokines, such as TNF-α, IL-6, or fibrinogen, and then induce chronic inflammatory cascades and change lipid metabolism, and these changes may contribute to the development of atherosclerosis [39, 47, 48]. Besides inflammatory reactions by *H. pylori*, circulating antibodies to the heat shock protein (HSP) 60 family in patients with *H. pylori* infection have been reported [49]. This *H. pylori* HSP may contribute to endothelial damage by inducing anti-*H. pylori* HSP antibodies that cross-react with host HSP because of the homology between *H. pylori* HSP with the human HSP and induce immunopathology by molecular mimicry [49–51]. Kountouras et al. [52] presumed monocytes secrete tissue factors with procoagulant activity by *H. pylori* infection, and these factors activate coagulation cascades and then cause CHD. However, this hypothesis is also unclear because there is a contrary result [53].

Chronic infection with *H. pylori* might result in a low-grade acute phase response and increased levels of acute phase coagulation proteins; in particular fibrinogen is a strong predictor of CHD [54, 55]. This may provide a link between infection and acute thrombotic events. Patel et al. [56] supported this suggestion, where elevated fibrinogen levels were independently associated with *H. pylori* infection compared with those free from infection.

There is another presumption that *H. pylori* may induce platelet aggregation and then induce instability of atherosclerosis [57, 58]. Vijayvergiya and Vadivelu [59] summarized the underlying hypothesis of atherogenic capacities of *H. pylori* infection are by chronic low-grade activation of the hemostasis cascade. The possible mechanisms of *H. pylori* related to atherosclerotic change are [59]:

1. Induction of inflammatory response secondary to chronic infectious state
2. Endothelial damage
3. Chronic low-grade activation of coagulation cascade
4. Dysregulation of lipid metabolism resulting in increased TC and TG levels and reduced HDL-C levels
5. Hyperhomocysteinemia

The cytotoxic strains of *H. pylori* bearing the CagA induce a stronger inflammatory and systemic immune response and are associated

Table 33.2 Overview of investigations concerning the effects of HP infection on CHD prevalence

References	Study design	Objects	Test for HP infection	Results	Other remarks
Mendell et al. (UK, 1994) [6]	Single center, case-control	111 CHD pts, 74 controls	HP IgG (ELISA)	*Associated* OR adjusted for confounding factors 2.15 (1.07–4.29)	OR adjusted for confounding factors and childhood environment factors 1.90 (0.91–3.97)
Murray et al. (UK, 1995) [28]	Cross-sectional	1182 men, 1198 women	HP IgG (ELISA)	*Not associated* OR adjusted for confounding factors 1.51 (0.93–2.45)	
Folsom et al. (USA, 1998) [29]	Prospective, case-cohort (F/U 3.3 Y)	498 a cohort samples, 217 incident CHD	HP IgG (ELISA)	*Not associated* Adjusted HR for confounding factors 1.03 (0.68–1.57)	
Pasceri et al. (Italy, 1998) [30]	Single center, case-control	88 IHD pts on the angiography, 88 age-sex-matched controls	HP IgG (ELISA) CagA IgG (Western blot)	*Associated* OR adjusted for confounding factors 2.8 (1.3–7.4)	↑ CAD risk in CagA(+) (OR adjusted for confounding factors 3.8 [1.6–9.1])
Danesh et al. (UK, 1999) [9]	Case-control	1122 AMI pts, 1122 age-sex-matched controls	HP IgG (ELISA)	*Associated* OR adjusted for confounding factors 1.75 (1.29–2.36)	
Pellicano et al. (France, 1999) [31]	Meta-analysis of 24 studies	6603 sample size	HP IgG (ELISA) or UBT	Possible weak association Pooled OR 1.55 (1.38–1.74)	
Whincup et al. (UK, 2000) [32]	Multicenter, prospective, nested case-control (F/U 9.5 Y)	505 CHD pts, 1026 controls	HP IgG (ELISA) CagA IgG (ELISA)	*Not associated* OR adjusted for confounding factors 1.3 (0.88–1.90)	Not ↑ CAD risk in CagA(+) (OR adjusted for confounding factors 1.1 [0.17–1.71])
Zhu et al. (USA, 2002) [33]	Study 1: cross-sectional Study 2: single center, prospective longitudinal (F/U 3 Y)	Study 1: 391 pts who angiography → 63 %, CAD] Study 2: 929 CAD on angiography	HP IgG (ELISA)	*Not associated* Study 1: OR adjusted for confounding factors 1.03 (0.60–1.77) Study 2: HR adjusted for HP IgG(+) 1.12 (0.81–1.54)	
Kinjo et al. (Japan, 2002) [34]	Multicenter, prospective, case-control	618 AMI pts, 967 controls	HP IgG (ELISA)	*Associated in subgroup <55 years* Overall OR adjusted for clinical factors 0.97(0.71–1.32)	In subgroup below 55 years in age (OR adjusted for clinical factors 2.97 [1.37–6.41])

Table 33.2 (continued)

References	Study design	Objects	Test for HP infection	Results	Other remarks
Fraser et al. (New Zealand, 2003) [35]	Case-control	341 AMI pts by registry, 831 community controls	HP IgG (ELISA)	*Associated* OR adjusted for age and sex 1.34 (1.00–1.80) ($p=0.038$)	
Sheehan et al. (Ireland, 2005) [36]	Community-based case-control	227 ACS pts, 277 age-sex-matched controls	HP IgG (ELISA)	*Not associated* OR adjusted for confounding factors 0.9 (0.8–1.0)	
Jin et al. (Korea, 2007) [37]	Single center, prospective, case-control	175 CAD on angiography, 88 controls with normal angiography	Histology	*Not associated* No difference in HP infection rate between CAD pts and controls (40.6% vs. 30.7%, ns), post-PCI re-intervention rate, and formation of new coronary lesions, respectively	
Zhang et al. (China, 2008) [38]	Meta-analysis of 15 case-control studies	2157 CHD pts, 2383 controls	CagA IgG (ELISA or Western blot)	*CagA (+) infection is associated with susceptibility to CHD* OR (random) 2.11 (1.70–2.62)	
Tamer et al. (Turkey, 2009) [39]	Single center, case-control	152 CAD pts (73 ACS, 79 SA), 22 controls	HP IgG (ELISA)	*Associated* ↑prevalence of HP IgG in CAD pts (80.2% vs. 54.4%, $p=0.015$)	No difference in HP IgG (+) rate between ACS and SA
Khodaii et al. (Iran, 2011) [40]	Single center, case-control	500 AMI pts on angiography, 500 controls	HP IgG (ELISA) CagA IgG (ELISA and Western blot))	*Associated* OR (crude) 2.57 (1.89–3.49)	AMI risk in CagA(+) (OR [Crude] 1.67 [1.18–2.36])
Schöttker et al. (Germany, 2012) [41]	Population-based cohort (F/U 5.1 Y)	9953 participants →170 AMI (+)	HP IgG (ELISA), CagA IgG (ELISA)	*Not associated* Adjusted HR for confounding factors 0.70 (0.46–1.08)	CagA(+) (adjusted HR for confounding factors 1.09 [0.76–1.57])
Lai et al. (Taiwan, 2014) [42]	A nationwide retrospective cohort (using the Taiwan National Health Insurance Research database)	17075 HP infected cases, 68,300 age-sex-matched controls	ICD-9-CM 041.86 (HP infection), ICD-9-CM 410, 411.1, 411.8 (ACS)	*Associated* Adjusted HR for confounding factors 1.48 (1.30–1.69)	

HP Helicobacter pylori, *CHD* coronary heart disease, *pts* patients, *ELISA* enzyme-linked immunosorbent assay, *OR* odds ratio, *F/U* follow-up, *Y* years, *HR* hazard ratio, *IHD* ischemic heart disease, *CagA* cytotoxin-associated gene-A, *CAD* coronary artery disease, *AMI* acute myocardial infarction, *PCI* percutaneous coronary intervention, *UBT* urea breath test, *ACS* acute coronary syndrome, *SA* stable angina, *ns* nonsignificant, *ICD-9-CM* International Classification of Diseases, Ninth Revision, Clinical Modification

with increased inflammation in the development of atherothrombosis [60, 61]. The possible importance of CagA in vascular diseases is also supported by evidence of cross-reactivity of anti-CagA antibodies with arterial wall antigens, suggesting a possible mechanism enhancing vascular inflammation and atherosclerosis [62].

Several case-control and cohort studies assessed the association of CagA status with atherosclerotic diseases and reported that the patients with atherothrombotic disease seemed more likely to be infected by the CagA-seropositive strains of *H. pylori* than healthy subjects [9, 38, 40, 63, 64].

A recent meta-analysis showed that infection sustained by those strains may be considered as a marker of vascular damage for both coronary and cerebral vessels [64]. The mechanisms underlying this pro-atherosclerotic effect are probably different. A molecular mimicry between CagA antigen and peptides localized in endothelial cells, smooth muscle cells, and macrophages of both normal and atherosclerotic arteries has already been shown [62].

CagA-positive strains of *H. pylori* have also been found to play a role in the destabilization of coronary plaques, and a meta-analysis has also showed a significant association between infection sustained *by CagA*-positive strains of *H. pylori* and occurrence of acute coronary syndromes [65, 66]. However, there are also the studies that couldn't elicit the association between CagA-positive *H. pylori* and CHD [32, 41].

33.4 *H. pylori* and Arrhythmia

In 1997, Frustaci et al. [67] reported their observation of inflammatory infiltrates, myocyte necrosis, and fibrosis in atrial biopsies of patients with lone AF refractory to antiarrhythmic drug therapy. More and more evidence has shown that inflammatory reaction has been closely related to AF since 1997. The concept that inflammation contributes to at least some types of AF came from the studies that showed that the frequent occurrence (25–40 %) of AF after cardiac surgery and the temporal course of AF occurring after cardiac surgery closely follows the activation of the complement system and release of proinflammatory cytokines [68–70].

The CRP levels were higher in patients with AF than in a control group of patients in normal sinus rhythm, and CRP also predicted patients at increased risk for developing future AF [70–72]. Montenero et al. [15] reported that some patients with *H. pylori* have autoantibodies to the Na^+/K^+-ATPase, the proton pump of gastric parietal cells which is similar to Na^+/K^+-ATPase, the pump of cardiac cells. In fact, cardiac Na^+/K^+-ATPase and Na^+/K^+-ATPase have a similar 35 kDa glycoprotein necessary for their catalytic activity. And they hypothesized that these autoantibodies to Na^+/K^+-ATPase may also bind to Na^+/K^+- ATPase, thus determining atrial damage, and the role of these pumps is to maintain ionic homeostasis by hydrolyzing ATP and therefore loss of this balance may trigger AF by determining abnormal automaticity or triggered activity that causes delay after depolarization inducing very rapid premature atrial contractions.

So far there were some studies shown that chronic *H. pylori* infection was associated in AF and contributed to the occurrence of AF [15, 73–76], but others reported *H. pylori* infection was not related to AF [77–79]. The results of studies about relation of *H. pylori* infection to arrhythmia were summarized in Table 33.3 [15, 76–79].

Table 33.3 Overview of investigations concerning the effects of HP infection on cardiac arrhythmia

References	Study design	Objects	Test for HP infection	Results	Other remarks
Montenero et al. (Italy, 2005) [15]	Single center, case-control	59 Pts with a paroxysmal or persistent AF, 45 healthy controls	HP IgG (ELISA)	HP seropositivity in AF is higher than that in controls (97.2 IU/ml vs. 5.3 IU/ml, $p<0.001$)	HP seropositivity in persistent AF is higher than that in paroxysmal AF ($p=0.027$)
					CRP in AF is higher than that in controls ($p<0.001$)
Platonov et al. (Sweden, 2008) [77]	Single center, case-control	72 Permanent AF, 72 age- and sex-matched controls	HP IgG (EIA)	No difference in HP seropositivity between AF and controls (57 % vs. 55 %, ns)	CRP in AF is higher than that in controls ($p<0.001$)
Bunch et al. (USA, 2008) [76]	Single center, case-control	83 AF pts, 660 controls	HP IgG (ELISA)	HP seropositivity in AF is higher than that in controls (65 % vs. 55 %, $p=0.049$)	
Lunetta et al. (Italy, 2009) [78]	Prospective F/U 7 Y	120 HP(+), 60 HP(-)	HP IgG	No difference in development of AF between HP(+) vs. HP(−) (21 % vs. 18 %, ns)	
Franceschi et al. (Italy, 2013) [79]	Single center case-control	54 Idiopathic dysrhythmia, 50 controls	UBT, CagA IgG (western blot)	No difference in HP IgG(+) between pts vs. controls (42 % vs. 44 %, ns)	CagA IgG(+) in pts is higher than that in controls (65 % vs. 42 %, $p<0.01$)

HP Helicobacter pylori, *pts* patients, *AF* atrial fibrillation, *CRP* C-reactive protein, *ELISA* enzyme-linked immunosorbent assay, *EIA* enzyme immunoassay, *ns* nonsignificant, *F/U* follow-up, *Y* years, *UBT* urea breath test, *CagA* cytotoxin-associated gene-A

Conclusions

So far published studies used various methods for diagnosis of *H. pylori*. The easiest and rapidest, so available for large-scaled test, serologic test for *H. pylori* was used in most of the studies, but there were few studies that used diagnostic method which can evaluate active infection. Moreover there are a lot of confounding factors such as socioeconomic factors, smoking, and genetic backgrounds to consider. Therefore the role of *H. pylori* in CHD is still conflicting.

Investigation about the causal relationship between *H. pylori* and CHD may be very important in the aspect of public health, in the countries where *H. pylori* is more prevalent and cardiovascular diseases are the leading cause of death, such as Korea, China, or India.

Prospective randomized multicenter study may be needed to establish the relation between two diseases and, by extend, causal-and-effect relationship. Especially, prospective population-based randomized interventional study about the influence of *H. pylori* eradication on prevention of primary or secondary occurrence of CHD may be solving clues.

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34 Extraintestinal Manifestations of *H. pylori* Infection: Asthma and Allergic Disorders

Cheol Min Shin

Abstract

Helicobacter pylori (*H. pylori*) prevalence decreases while asthma and allergic disorders rapidly increase mainly in Western developed countries. Numerous epidemiological surveys have reported that there is a meaningful trade-off relationship between *H. pylori* infection and the allergic diseases. Allergic asthma is induced by T cells that secrete Th2 cytokines, such as interleukin (IL)-4 and IL-5. *H. pylori* infection is thought to not only reinforce Th1 immune responses but also suppress Th2 reactions. According to the recent studies using animal models of allergic airway disease, *H. pylori* is expected to reduce airway hypersensitivity by directly inducing regulatory T-cell expressions via dendritic cells. As *H. pylori* prevalence tends to decrease in East Asian countries such as Korea and Japan, mainly among children and adolescents, allergic diseases such as atopic dermatitis, asthma, and allergic rhinitis rapidly increase. Thus, epidemiological surveys on these populations regarding the correlation are warranted in the future.

Keywords

Helicobacter pylori • Asthma • Allergic disorders

34.1 Introduction

Helicobacter pylori (*H. pylori*) infection has been acknowledged as the main cause of upper gastrointestinal tract disorders, such as gastritis, peptic ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer. On the other hand, there have been several lines of studies, which are mainly from the United States and European countries and have reported the association between the reduction of *H. pylori* infection

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and the increment of asthma and allergic disorders. This trade-off relationship between the exposure to the external allergens or microorganisms and asthma is widely acknowledged, which had been explained by hygiene hypothesis that is now called as disappearing microbiota hypothesis. This chapter is going to introduce previous epidemiological researches on the relationship between *H. pylori* and asthma, the pathophysiology of allergic disorders, and the theoretical evidence of the causality on *H. pylori* infection, asthma, and allergic diseases [1].

34.2 *H. pylori* and Allergic Asthma: Epidemiological Evidence

H. pylori has been coexisted with mankind throughout human history. *H. pylori* initially colonizes itself inside the human stomach and then induces chronic immunologic and inflammatory reactions [2, 3]. However, *H. pylori* infection during juvenile period has been rapidly decreased during the process of industrialization, especially among the United States and European countries [4]. The causes of this reduction are thought to be the improvements on hygiene, reduced family-oriented infection due to the decreased number of family members, supplying clean water, and the disappearance of feeding children with chewed foods from their mother or grandmother. Particularly, the broad use of antibiotics among pediatrics is thought to be one important reason of lowering *H. pylori* prevalence in the childhood [5].

The associations between *H. pylori* infection and asthma and allergic disorders have been reported in the cross-sectional studies [6]. According to the study on the adults of Sweden, Iceland, and Estonia, there was a meaningful correlation between positive serum anti-*H. pylori* antibody and the prevalence of asthma and hay fever [7]. In addition, there were meaningful trade-off relationships among the prevalence of *H. pylori*, asthma, and allergy, based on the results from the National Health and Nutrition Examination III (NHANES III) study [8]. Especially, *CagA*-positive strains showed stronger relationship with allergy and asthma. Nonetheless, there have also been numerous studies that showed no or minimal correlations between *H. pylori* infection and asthma [9–13]. Notably, the significant relationship between *H. pylori* infection and asthma is often observed in young and pediatric asthmatics. According to the subgroup analyses that utilized NHANES III cohort, there was a strong relationship on the population less than 43 years old (odds ratio [OR] 0.63; 95 % confidence interval [CI], 0.43–0.93). However, the association in the population over 43 years old was statistically insignificant. Also, there was a statistically significant relationship in those with asthma diagnosed before the age of 15 (OR 0.63; 95 % CI, 0.43–0.93), while the relationship was insignificant among patients who were initially diagnosed with asthma when they became adults. Follow-up studies showed similar results on pediatric asthma [14, 15]. A recent longitudinal study showed a negative correlation between asthma with wheezing sounds and positive serum anti-*H. pylori* antibodies, but there was no significant association between asthma, allergic rhinitis, and atopic dermatitis [16]. Moreover, the recent meta-analyses on previous studies suggested that *H. pylori* infection has weak negative correlation with asthma, especially more on pediatrics than on adults [17, 18].

Asthma diagnosed in the young may be different in terms of etiological aspects with that diagnosed initially during adulthood. Pediatric asthma is often accompanied by atopic dermatitis and allergic rhinitis (allergic march) and is related to primed allergen, IgE mediation, and Th2 inflammation. In comparison, adult asthma originates from various causes, such as smoking, air pollution, and certain jobs, other than allergen. Also, patients defined as "adult asthma" are heterogeneous because they include those with pediatric asthma continued to adulthood and those with recurrent asthma symptoms since childhood, as well as those with asthma initially diagnosed when they were adult. In addition, interpretation of the previous studies requires caution. Several studies with insignificant results have limitations related to the small sample size to obtain statistical significance, and there can be a problem of misclassification because of diagnosing asthma

based on its wheezing sound and the difficulty of infant and pediatric asthma, from the methodological perspective [19]. Moreover, most of epidemiological studies used serum *H. pylori* antigen test to confirm *H. pylori* infection, but the serologic test cannot differentiate past infection from the active one, and some patients with remote past infection are apt to be classified as negative infection (false negatives) due to their decreased serum antibody titer [20].

34.3 *H. pylori* and Allergic Asthma: Proposed Mechanisms Underlying Protective Effects of *H. pylori*

Evidence based on epidemiological studies has the limitation itself, as it shows the association between *H. pylori* infection and allergic asthma only and cannot prove causal relationship between these two factors. Previously there have been only few basic studies on the mechanism of how *H. pylori* infection protects asthma. However, recent studies might suggest several possible mechanisms.

34.3.1 Basic Concept on the Pathophysiology of Allergic Asthma

Symptoms of asthma originate from airway hyperresponsiveness (AHR) and reversible airway obstruction attributed to chronic allergic inflammation on airway, which leads to the recurrent episodes of breathlessness, cough, chest tightness, and wheezing. However, the pathophysiology of these symptoms is complex. For example, cellular assessment of sputum can classify the patients into either eosinophil-predominant or neutrophil-predominant or mixed inflammation. Patients with eosinophilic asthma show a better response to treatment with inhaled corticosteroids compared with those with neutrophil-predominant inflammation. Therefore, although there is no explicit immune-pathological difference based on the clinical symptoms, patients with more eosinophils on their sputum examinations show better responsiveness and prognosis on inhaled corticosteroids than those who have more neutrophils on their sputum [21]. In terms of the genetic susceptibility, "Th2-high" patients, who exhibit increased expression of Th2 cytokine (i.e., interleukin (IL)-4 and IL-13)-response genes such as periostin, show a better response on inhaled corticosteroid therapy than "Th2-low" patients, who do not have those genetic expressions [22].

Recently, animal models of allergic airway disease have contributed to better understanding on the mechanisms underlying allergic asthma. It has been identified that Th2 cells and Th2 cytokines, such as IL-4, IL-5, and IL-13, are important for allergen-induced inflammation and AHR [6], and the contribution of secreted IL-17 by Th17 cells on AHR, via direct stimulation of airway smooth muscles, has been identified [23]. Once the epithelial cells in the respiratory tract are exposed to a specific allergen, they are activated and release epithelial cytokines including IL-25, IL-33, and thymic stromal lymphopoietin that involve the activation of dendritic cells (DCs) and the movement to the regional lymph nodes. DCs are believed to induce the sensitization on certain inhaled allergen and recruiting inflammatory cells [24]. Especially, IL-33 can activate mast cell and basophil, which works with IL-25 to activate innate lymphoid cells (ILCs). Activated ILCs secrete IL-5 and IL-13, which are known as Th2 cytokines, and participate in the induction of bronchial allergic inflammatory reaction. During the sensitization process, IL-4 increases Th2 cell ratio in regional lymph nodes, and the Th2 cells secrete IL-4, IL-5, and IL-13. Among them, IL-5 is important in the survival of eosinophil, IL-13 directly works on the epithelial cells to secrete mucin and chemokines, and IL-13 also works on the activation of smooth muscle macrophages to induce AHR [25, 26].

34.3.2 Hygiene Hypothesis and Disappearing Microbiota Hypothesis

The recent increment of the patients with allergic disorders in developed countries cannot be

explained simply by genetic factors; socioeconomic factors such as nutrition and hygiene, air pollution, and the environmental exposure to tobacco smoking or microbial components are thought to be more important in the development of asthma [6]. Hygiene hypothesis is the most popular one to explain this phenomenon. The hypothesis explains that the reduced frequency and severity of encountering microbial antigens via food, air, soil, or water have led to the increase of allergic disorders [27]. There have been several studies that suggest trade-off relationships between asthma and type A hepatitis, *Toxoplasma gondii* infection, and herpes simplex virus type 1 infection [28], and one study has shown the negative correlation of asthma with residence in rural area among children in southern Germany, because residents in rural area are thought to be exposed more frequently to the various microorganisms [29]. However, hygiene hypothesis makes sense to explain only pediatric asthma and atopic disorders. Therefore, there is a limitation to apply this hypothesis to adult asthma that multiple factors are related as mentioned above.

On the other hand, disappearing microbiota hypothesis explains that a fetus is germ-free *in utero* but becomes exposed postpartum to various microorganisms that inhabit exposed mucosal surfaces such as the skin, nasal and oral cavity, vagina, and gut, in which these microflora constitute a microbiota. The microbiota is considered to play important roles on the immune system after birth [30]. Especially, the gut microbiota could be affected by host factors and environment factors such as labor method and breastfeeding from the earliest life. To date, numerous animal and clinical studies have reported the correlation between the gut microbiota and atopic disorders [31].

It is still unclear how the intestinal microbiota affects the immune system, but the commensal microbiota might regulate the balance of Th1/Th2 immune responses. Th2 reaction is reinforced when there is no microbial colony, whereas microbial antigen in the intestinal tract is expected to stimulate Th1 reaction to generate protective effects on atopic disorders and asthma by Th2 cells [32]. Also, gut microbiota induces T cells to differentiate into the regulatory T cells (Treg), which secrete cytokines like IL-10 and transforming growth factor (TGF)-β to induce anti-inflammatory reactions [30]. DCs on intestinal mucosa deliver antigens to naïve T cells, and they induce T cells to differentiate into the effect T cell or Treg. The DCs may selectively control the action of toll-like receptor (TLR), so unnecessary inflammatory reaction toward indigenous microorganism does not occur to maintain immunologic tolerance. Both DCs and macrophages secrete IL-10, TGF-β, and retinoic acid, which induces the differentiation of the undifferentiated T cells into $CD4^{+}FoxP3^{+}$Treg [33]. According to a recent study, increased sensitivity on inflammatory bowel disease and allergic respiratory disorders was reported in germ-free mice attributed to the loss of variety on natural killer T cells on lung and intestinal mucosal membranes [34]. In addition, this hypothesis was supported by a flora change after antibiotic treatment that induced the increase of serum IgE concentration and the increase of circulating basophils that led to an exaggerated basophil-mediated Th2 cell reaction and allergic inflammatory reaction [35].

34.3.3 Theoretical Hypothesis to Explain Protective Effect by *H. pylori*

Allergic asthma is induced by T cells that secrete Th2 cytokine and suppress Th1 reaction, and *H. pylori* is thought to stimulate Th1 immune responses to protect against allergic disorders. Recent studies reported that *H. pylori* neutrophil-activating protein (HP-NAP) played an important regulatory role while *H. pylori* infection stimulated Th1 mucosal immune responses [36, 37]. According to these studies, HP-NAP repressed the accumulation of eosinophils and the increased of serum IgE proliferation in alveoli when asthma is induced by ovalbumin in allergic asthma animal model [36]. These findings suggest that HP-NAP could be an important substance to elucidate the asthma protection by *H. pylori* [37].

Moreover, airway hypersensitivity was remarkably reduced among *H. pylori*-infected mice; when both newborn and adult mice were

infected with *H. pylori* and then sensitized with ovalbumin in the allergic airway disease mouse model, eosinophil infiltration and IL-5 and IL-13 secretions were substantially reduced. In addition, effector T cells that secrete IL-5 and IL-17A in lungs were also reduced [38]. Interestingly, these effects were dominant on neonatally infected mice, while respiratory tract infection and airway hypersensitivity reduction were minimal among adult infected mice. According to a recent research, *H. pylori* directly works on DCs in the gastric mucosae to induce immunologic tolerance, by which allergy-inducing T-cell activation is suppressed [39]. That is, DCs that are exposed to *H. pylori* move to gut-draining mesenteric lymph nodes and induce TGF-β-dependent FoxP3^{+}Treg differentiation, and Treg inhibits *H. pylori*-specific Th1/Th7 immune response and excessive inflammatory reaction by *H. pylori* infection on gastric mucosa. Also, Treg is transported to the lungs via the bloodstream to induce immunologic tolerance of lung DCs, and Treg secretes cytokines like IL-10 to suppress Th2/Th17 response by certain allergens that are important for the pathophysiology of allergic asthma [40] (Fig. 34.1). These procedures are unrelated to *H. pylori* toxic factors, such as VacA and CagA, and seem to be rather related to cytokines like TGF-β and IL-10 [41].

Conclusions

The trade-off relationship among *H. pylori* infection, asthma, and allergy is based on epidemiological surveys in the United States and European countries, where *H. pylori* prevalence has been rapidly decreased in

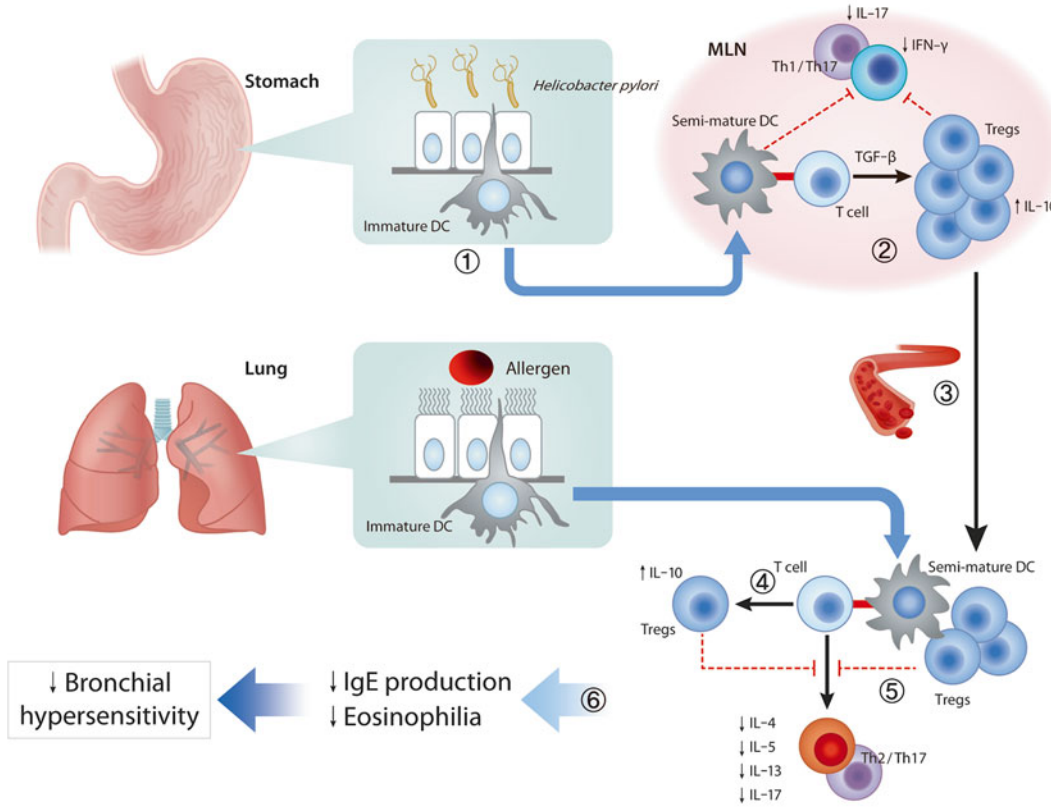

Fig. 34.1 Underlying mechanism of airway hyperresponsiveness suppressed by *H. pylori* infection. (1) Immature dendritic cells (*DC*) can be activated by *H. pylori*. Then the semi-mature DCs move to gut-draining mesenteric lymph nodes (*MLN*), (2) where they induce the expression of regulatory T cells (*Treg*). (3) Tregs move to the lungs via blood stream (4) to induce immunologic tolerance of lung dendritic cells or (5) to directly suppress the Th2 reaction. (6) It leads to the decrease of IgE production/eosinophilia. (Modified from Taube and Müller [6], and Arnold et al. [40])

these regions for the past several decades. The correlation between *H. pylori* infection and asthma is more prominent in pediatric asthma because pediatric asthma and adult asthma are thought to be different in a pathophysiologic aspect. Recently, theoretical evidence suggested that *H. pylori* infection alleviates airway hypersensitivity by allergens in immunological aspect, based on allergic airway disease animal model. *H. pylori* prevalence tends to decrease in the East Asian countries like Korea and Japan, mainly among young children and adolescents, and allergic diseases like atopic dermatitis, asthma, and allergic rhinitis rapidly increase. So epidemiologic surveys on these populations are warranted regarding this issue in the future.

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Part VI

Antibiotic Resistance

Synopsis of Antimicrobial Resistance

35

Francis Mégraud

Abstract

Helicobacter pylori resistance to antibiotics is the main cause of failure of the eradication treatments. The essential mechanism of resistance acquisition is the occurrence of point mutations in genes important for the antimicrobial activity, while efflux pumps have also been described. Resistance to macrolides is steadily increasing around the world and is clinically significant. Besides, the standard methods, it can be detected in a few hours by molecular methods especially real-time PCR followed by a melting curve analysis. Resistance to fluoroquinolones is also important for the same reasons, and some molecular methods can also be applied. Metronidazole resistance is very common, but it can be overcome in vivo. Its in vitro detection lacks reproducibility, and no molecular method is currently available. Resistance to the other antibiotics of potential interest as for amoxicillin, tetracycline, and rifamycin is seldom found.

Keywords

Point mutations • Clarithromycin • Real-time PCR • Prevalence

35.1 Introduction

The discovery of *Helicobacter pylori* (*H. pylori*) was a breakthrough in the field of gastroenterology [1]. Diseases such as peptic ulcer disease for which there was no efficient treatment could suddenly be cured. It is now recognized that most gastroduodenal diseases are indeed infectious diseases and so can be treated with antibiotics. However, a single antibiotic was not sufficient, and in the beginning of the 1990s, a treatment using two antibiotics was proposed, to which an antisecretory drug, as an adjuvant treatment to increase the pH, had to be added [2]. This treatment was successful for almost two decades, but unfortunately *H. pylori* became progressively resistant to some of the antibiotics used, and nowadays, it is no longer a valid option in many regions of the world [3].

In this chapter, we shall review the mechanisms of antibiotic resistance, its clinical consequences,

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the methods that can be used to detect this resistance, and the current prevalence.

35.2 Mechanisms of Resistance to Antibiotics

Bacteria can become resistant to antibiotics by two major mechanisms: (1) acquisition of mobile genetic elements which confer resistance or (2) point mutations which modify the structure of the target [4].

35.2.1 Acquisition of Mobile Genetic Elements

Indeed, this mechanism is the main one in the microbial world. Bacteria can exchange part of their DNA by different mechanisms like conjugation and transduction. By conjugation, the elements are named plasmids and can be transferred from one bacterium (donor) to another (recipient). The plasmid exchange is encoded by the plasmid itself. It is transferred horizontally. It occurs like an outbreak, and most of the bacteria become resistant over a short time lapse. Other mobile genetic elements are the transposons and integrons. Mainly concerning Gram-positive bacteria, the transfer requires a bacteriophage. This is the so-called transduction.

In addition, DNA fragments issued from dead bacteria can also be present in the environment. At a specific moment of the bacterial cycle, these DNA fragments can be integrated into the bacteria. This is the so-called transformation. In all cases, the DNA fragment is able to integrate to the bacterial chromosome and transfer its genes which can concern resistance to antibiotics. Fortunately, this horizontal transmission of resistance is not observed in *H. pylori*.

35.2.2 Resistance Acquired by Point Mutation

Point mutations occur relatively often in bacterial DNA. They correspond to errors in the DNA synthesis. Many point mutations in a gene sequence do not have visible consequences, but others can alter all of the physiological aspects of the bacterium, including antibiotic resistance. Point mutation is the essential mechanism of acquisition of resistance in *H. pylori* and concerns most of the antibiotics used to treat this infection, albeit at very different rates. *H. pylori* shares this particularity with another important bacterium: *Mycobacterium tuberculosis*. Indeed, the point mutation modifies critical sites in target molecules for the antibiotic's activity. The list is presented on Table 35.1. In contrast to the resistance acquired by mobile genetic elements, the resistance acquired by point mutation is transmitted vertically and increases slowly when there is a selective pressure. It is also dependent on the impact of the mutation on the bacterial fitness, especially its ability to replicate. If the mutation has such an impact, then when the selective pressure stops, the bacteria harboring the mutation will progressively disappear from the population, and the wild type without this limitation will only remain.

35.2.3 Efflux Pumps and *H. pylori* Resistance

To maintain cellular homeostasis, some bacteria can actively expel harmful molecules outside of the cell. This mechanism was first described for tetracycline in the 1980s. Hyperproduction of the so-called efflux pumps result in an increase in minimal inhibitory concentration (MIC) values. This mechanism is not common in *H. pylori* but can occur [5, 6].

Table 35.1 Genes concerned by point mutations or other genetic events leading to antibiotic resistance in *H. pylori*

Antibiotic group	Genes concerned
Macrolides	*rrn23S*
Metronidazole	*rdxA*, *frxA*
Quinolones	*gyrA*
Rifamycins	*rpoB*
Amoxicillin	*pbp-1*
Tetracyclines	*rrn16S*

35.3 Resistance Observed in *H. pylori* and Its Consequences

There are six antibiotic groups which can be used to treat *H. pylori* infection, but the problem of resistance is crucial for only two: macrolides (clarithromycin) and fluoroquinolones (levofloxacin) because of their common use and current high prevalence observed. A second group is composed of amoxicillin, tetracycline, and rifabutin because resistance is seldom found or the compound rarely used. Finally, 5-nitroimidazoles (metronidazole) must be considered separately because of their particularity.

35.3.1 Macrolides

The mechanisms of action of macrolides consist of binding to ribosomes at the level of the peptidyl transferase loop of the 23S rRNA gene. Point mutations occurring in this loop may change its conformation and limit the binding possibility [7]. The main positions described as inducing resistance are the transition A2142G, A2143G [7, 8] and the transversion A2142C [9]. There are also rare cases with A2142T. Other mutations have been described, but their impact on the MICs of the bacteria has not been definitely proven. It is interesting to note that all macrolides are concerned by the occurrence of these conformational changes. There is a cross-resistance between all of them. In terms of frequency, transition is by far the most common. The resulting MICs are diverse, and there is no agreement on the mutations which would lead to the highest MIC.

When the standard clarithromycin-based therapy is used, in case of resistance, only the second antibiotic (usually amoxicillin) remains active, but the doses and frequency of administration are not sufficient to obtain *H. pylori* eradication, and there is therefore a limited rate of success. Several meta-analyses have shown that when a strain is clarithromycin susceptible, the rate of success is in the range of 90 % (indeed there are other reasons for failure, e.g., bad compliance, insufficient acid reduction, high bacterial load, etc.), while if the strain is clarithromycin resistant, the rate of success decreases to the range of 20 % and even less if the second antibiotic is metronidazole and resistance to this drug is present.

35.3.2 Fluoroquinolones

The mechanism of action of fluoroquinolones consists of inhibiting the A subunit of the DNA gyrase encoded by the *gyrA* gene. There is a special part of the gene, namely, the quinolone resistance determining region (QRDR), where occurrence of point mutations leads to a lack of binding. There are merely two amino acids concerned in positions 87 and 91 [10, 11]. There is a cross-resistance between the various fluoroquinolones. However, new compounds such as sitafloxacin, used in the Far East, show much lower MICs in case of mutation than it is with levofloxacin and possibly concentrations achievable with the clinical dose used.

When the levofloxacin-amoxicillin-proton pump inhibitor (PPI) regimen is used, generally as a second-line treatment, there is a major impact of levofloxacin resistance, but no meta-analysis has been carried out to evaluate its consequences. Indeed, unfortunately, most of the trials performed did not include susceptibility testing.

35.3.3 Amoxicillin

As the other ß-lactams, amoxicillin acts by interfering with the peptidoglycan synthesis, by blocking transporters named penicillin-binding proteins (PBP). However, amoxicillin resistance is extremely rare in *H. pylori*. It was first described in a patient having received multiple cures of this antibiotic for respiratory tract infections. The mechanism identified is a mutation occurring in the *pbp1A* gene, in particular, a Ser414→Arg mutation leading to a PBP blockage; however, the resistance level was not very high [12]. Another mechanism described is the lack of a specific PBP, PBP-D [13]. By looking at results of prevalence of antimicrobial resistance, one can see in a few articles a relatively high prevalence of amoxicillin resistance. Our opinion is that such results must be considered with

caution if the results were not verified and the mechanisms explored.

35.3.4 Tetracyclines

The action mechanism of tetracyclines is an interference in protein synthesis by binding to the 30S ribosomal subunit. By three adjacent mutations, a nucleotide triplet can be changed (AGA926 to 928TTC) at the 16S rRNA gene level, interfering with binding to the h1 loop, the binding site of tetracyclines [14, 15]. The rarity of tetracycline resistance is due to the low probability to obtain three adjacent mutations. Single or dual mutations are logically more frequent leading to slightly increased MICs.

As stated before, another mechanism may be responsible for resistance in the absence of mutation, i.e., the efflux mechanism. These strains exhibit a decreased accumulation of tetracycline inside the cells. While all tetracyclines may inhibit *H. pylori*, the most active remains tetracycline HCl. It is this drug which is part of the active combination Pylera®, the three-in-one capsule now available to treat *H. pylori* infection.

35.3.5 Rifampin

The mechanism of action of the rifampin is an inhibition of the B subunit of the DNA-dependent RNA polymerase encoded by the *rpoB* gene. As with *M. tuberculosis*, mutations can occur in this gene at specific positions, mainly 524, 525, and 585 [16]. Rifampin resistance is relatively rare, because of the low selective pressure due to these antibiotics. Indeed, they are mainly used to treat tuberculosis, and *H. pylori* resistance has been essentially described in patients having received rifampin to eradicate *M. tuberculosis*. The main rifampin used to treat *H. pylori* infection is rifabutin, but there is a cross-resistance among this group of antibiotics.

35.3.6 5-Nitroimidazoles

5-Nitroimidazoles are mainly used to treat anaerobic infections, but they have shown their efficacy on the microaerophilic bacterium, i.e., *H. pylori*. The compound is a prodrug which must be reduced into a nitrosamine inside the bacterial cell to alter bacterial DNA. To achieve this reduction, there is primarily an oxygen-insensitive nitroreductase encoded by the *rdxA* gene, but a mutation in this gene can render the protein ineffective [17]. Another enzyme, flavodoxin oxidoreductase (FrxA), may also be involved in the reduction process [18]. A further mechanism of resistance, again the efflux system, could also play a role [6].

The most commonly used compound for *H. pylori* eradication is metronidazole, but tinidazole which has a better pharmacokinetic profile has also been used. The particularity of these compounds with regard to resistance is the poor correlation between in vitro and in vivo data, to which the problem of a poor reproducibility of in vitro data must be added [19]. This fact could be explained by the lack of control of the redox potential, which is important in the reduction of 5-nitroimidazoles.

35.4 Methods to Detect *H. pylori* Resistance

The standard method to determine *H. pylori* susceptibility or resistance to antimicrobial compounds is the phenotypic method, i.e., the determination of the MICs against the bacterium. However, given the mechanism involved, it was relatively easy to design molecular methods to look for mutations, and this approach is now more common especially for the main antibiotic used, clarithromycin.

35.4.1 Phenotypic Methods

The current recommendation in Europe (EUCAST) is to perform an agar diffusion method with E-test. E-test is especially adapted to slow-growing bacteria such as *H. pylori*, and a good correlation has been found with the dilution method except for metronidazole as indicated before [19, 20]. The reference method for clarithromycin susceptibility testing remains agar dilution as proposed by the Clinical Laboratory

Standard Institute (CLSI) in the USA. A broth detection method can also be used, but a broth supplementation must be added to allow a good growth of *H. pylori* [21]. Agar diffusion using disks is no longer recommended. Nevertheless, it can be used as a screening method because it is simple and cheap, before using E test in case of doubtful results.

35.4.2 Genotypic Methods

A large number of genotypic methods have been designed especially to detect the mutations associated with clarithromycin resistance, as they are indeed the main reason for failure of the clarithromycin-based triple therapy recommended worldwide for the past 20 years.

These numerous methods have been recently reviewed [22]. The most commonly employed method is a real-time PCR able to detect first *H. pylori* and, if present, second the mutation associated with macrolide resistance.

There are different variants of the method. One is to design *H. pylori*-specific primers on the 23S rRNA gene and then a biprobe (sensor probe and anchor probe) hybridizing close to each other in order to allow a transfer of energy, according to the fluorescence resonance energy transfer (FRET) principle. This method allows the amplification to be followed in real-time, and then a melting curve analysis (MCA) is performed. In the absence of a mutation, there is a perfect match of the nucleotides and the melting temperature is the highest, while if a mutation is present, there is a mismatch and the melting temperature is lower (e.g., 62 °C for the former and 58–53 °C for the latter, according to the type of mutation) [23]. The main advantage of this method is its rapidity, given that a result can be obtained within 2 h after obtention of a gastric biopsy. It also limits the possibility of contamination with amplicons because the reaction is performed in a closed tube. In addition, this method has been used on stool specimens [24]. The main limitation in this environment is the possibility of false-negative results because the amount of *H. pylori* DNA is low in the stools and because inhibitors of the Taq polymerase from the diet can be present [25]. It is important to use a very effective method of DNA isolation. The FRET-MCA method was also applied to detect *H. pylori* resistance to fluoroquinolones using two biprobes [26].

35.5 Prevalence of *H. pylori* Resistance

The relevance of *H. pylori* resistance depends on the prevalence observed. This prevalence is an evolving process which must be followed at successive time points [27].

Numerous studies have been performed. The problem is to test a population representative of the general population, while many studies are issued from referral centers for *H. pylori* treatment with a risk of including patients harboring strains which are not "primary" resistant. It is also important to test an important number of cases, in order to get small confidence intervals of the results. In Europe, we conducted a survey in 2008–2009 including *H. pylori* strains from 2,204 patients in order to compare the prevalence in 18 European countries [28]. The global prevalence of primary *H. pylori* resistance to clarithromycin in adults was 17.5 %, but there were important variations according to the countries. The highest prevalence was in Western/Central and Southern Europe (>20 %), while it was less than 10 % in Northern Europe. The same result was obtained for levofloxacin with a global prevalence of 14.1 %.

Interestingly, for each country, *H. pylori* prevalence was compared to the consumption of the corresponding antibiotic. There was a significant correlation between clarithromycin resistance and the use of long-acting macrolides in the community (Fig. 35.1) and also between levofloxacin resistance and the use of fluoroquinolones, the best fit being for the year 2008. Concerning amoxicillin, tetracycline, and rifabutin, the global rate of resistance obtained by the different laboratories was in the range of 1 %. When the results were verified centrally, it was not possible to confirm amoxicillin resistance. Resistance to metronidazole was globally high (34.9 %), the highest being in Western/Central Europe (43.8 %).

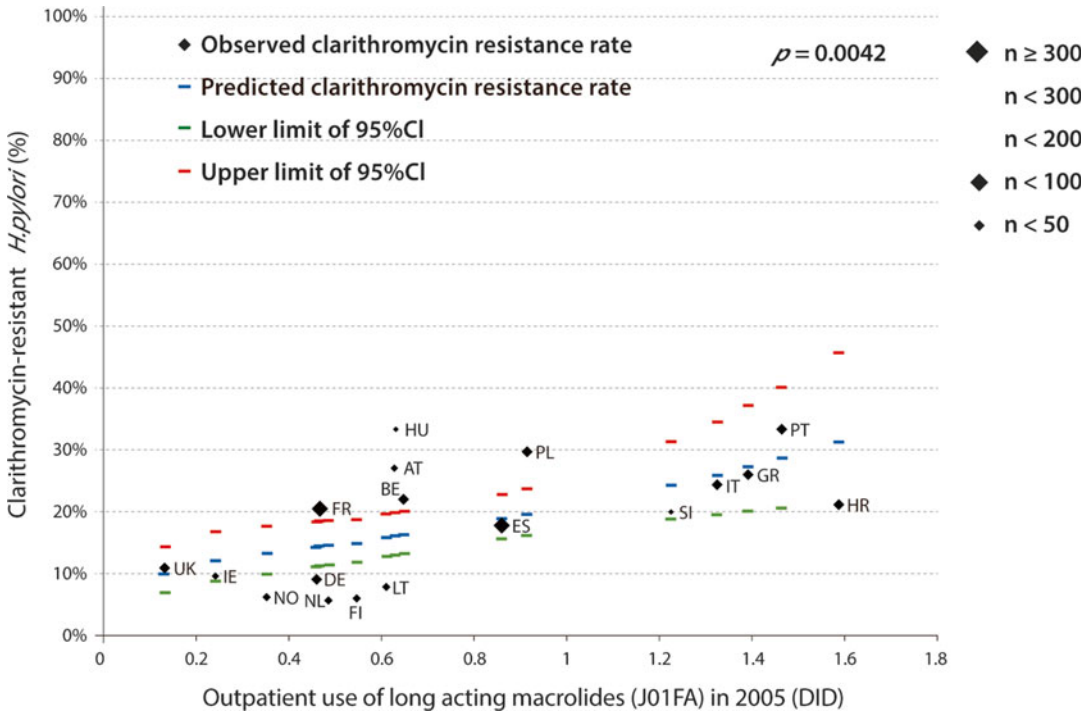

Fig. 35.1 Correlation between long-acting macrolide use in 2008 and *H. pylori* resistance to clarithromycin 2008–2009. *AT* Austria, *BE* Belgium, *HR* Croatia, *FI* Finland, *FR* France, *DE* Germany, *GR* Greece, *HU* Hungary, *IE* Ireland, *LT* Lithuania, *IT* Italy, *NL* The Netherlands, *NO* Norway, *PL* Poland, *PT* Portugal, *SI* Slovenia, *ES* Spain, *UK* England only

A multivariate analysis on the risk factors for resistance indicated the following: for clarithromycin, the region of birth and having another disease than peptic ulcer; for levofloxacin, the region of residence, an age >50 years, and also having a disease other than peptic ulcer; and for metronidazole, the region of birth and being a woman.

In Latin America, a meta-analysis was recently published concerning 12 of the 20 countries [29]. It concerned a long period, from 1988 to 2011; however, they did not find variations according to the year of sampling. There were 56 adult studies included. The global prevalence was 12 % for clarithromycin, 15 % for fluoroquinolones, 53 % for metronidazole, and also 4 % for amoxicillin and 6 % for tetracycline. The highest prevalence of resistance to these last two antibiotics comes from studies carried out in Brazil.

Concerning Asia, one example is a Korean study where they evaluated primary resistance in strains isolated from 347 patients between 2003 and 2012 [30]. When defining three successive periods, they observed an increase in resistance from 17.2 to 23.7 % for clarithromycin, from 4.7 to 50 % for levofloxacin, and from 16.7 to 43.6 % for metronidazole. They also found surprisingly high levels of resistance for amoxicillin and tetracycline.

Conclusions

In summary, the prevalence of *H. pylori* resistance to antibiotics, especially clarithromycin and levofloxacin, is globally increasing in the world, and this is the consequence of the use of antibiotics of these families in the community. Resistance to metronidazole remains high, but this resistance can be overcome. Resistance to amoxicillin and tetracycline, in contrast, is still at a very low level, except probably in rare countries and gives us the possibility to treat this infection.

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Clarithromycin

36

Jung Won Lee

Abstract

Clarithromycin is a macrolide antimicrobial agent that is the main component of *Helicobacter pylori* (*H. pylori*) eradication therapy. Therefore, clarithromycin resistance is the most influential factor to determine either the success or the failure of the antibiotic therapy. Recently, clarithromycin resistance has rapidly increased, which leads to *H. pylori* eradication failure. Clarithromycin resistance mechanisms depend on 23S rRNA gene mutations, and A2143G point mutation is the most critical factor for the determination of resistance. Thus, the pretreatment determination of clarithromycin resistance leads to the increment of successful eradication. Also, genetic epidemiology studies on resistance mutations contribute to the development of rapid molecular diagnostic tests for better *H. pylori* treatment. Therefore, studies to overcome resistance mechanism should be continued.

Keywords

Helicobacter pylori • Antibiotic resistance • Clarithromycin

36.1 Introduction

The combination of proton pump inhibitor (PPI), amoxicillin, and clarithromycin for 7 days has been globally established as the first-line eradication therapy of *Helicobacter pylori* (*H. pylori*) [1]. However, this combination therapy could no longer show the same efficacy as the past due to increased clarithromycin resistance. Thus, some countries no longer consider clarithromycin-containing triple therapy as the first-line treatment. On the other hand, other some nations like the Republic of Korea still maintain clarithromycin-containing triple therapy as the first-line treatment due to the difficulty of establishing an alternative therapy caused by high resistance rate of multiple antimicrobial agents. Then, it is necessary to figure out why clarithromycin resistance is emphasized. If the clarithromycin resistance was not rapidly increased as the present, most of contents in this chapter would not need to be discussed.

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Like this, clarithromycin has been the core of effective and powerful *H. pylori* treatment.

Clarithromycin is a macrolide antimicrobial agent that includes a large tetradecagonal ring inside its molecular structure. Macrolide antibiotics are erythromycin, clarithromycin, and roxithromycin. Clarithromycin is different from erythromycin because the 6-hydroxyl group of clarithromycin is methylated, unlike that of erythromycin (Fig. 36.1). Because of this methylation, clarithromycin is more stable under acidic condition and has better tissue penetration ability than erythromycin, so clarithromycin has been widely used for *H. pylori* treatment. Clarithromycin was introduced to Korea in 1997, and it has been the fundamental antibiotic for *H. pylori* treatment [1].

This chapter is going to introduce the epidemiological studies of the resistance of clarithromycin, resistance mechanisms, and diagnostic methods as a way of overcoming clarithromycin resistance and predicts the directions of future studies based on recent research results.

36.2 The Antimicrobial Mechanism of Clarithromycin

Clarithromycin could be classified into a protein synthesis inhibitor of bacteria. Clarithromycin mainly shows a bacteriostatic characteristic, which means the inhibition of bacterial growth, instead of bactericidal ability. The main mechanism of clarithromycin is to attach to domain V peptidyl transferase ring of ribosomal RNA (rRNA) structure and then inhibits the protein synthesis of bacteria. This inhibition prevents amino acid chain structure formations, and all macrolide antibiotics commonly have this mechanism. In addition, clarithromycin tends to be better absorbed to stomach mucosa and shows better stability in acidic condition [2].

36.3 The Current Status of Clarithromycin Resistance

Clarithromycin was introduced in Korea in 1997. Clarithromycin has been the core of *H. pylori* eradication therapy [3]. After the establishment of antibacterial treatment, clarithromycin-included treatment has become the most remarkable antibacterial treatment and has been used globally, so recently, the resistance rate has increased. The first systematic report on clarithromycin resistance in Korea was introduced by Kim et al. in 2005. The clarithromycin resistance on *H. pylori* in Korea was not reported in 1987, and low incidence (2.8%) of clarithromycin resistance was reported in 1994. However, the resistance was increased by 13.5% in 2003. In addition, according to the study on the changes of clarithromycin resistance prevalence of 347 patients from 2003 to 2012 in Gyeonggi-do,

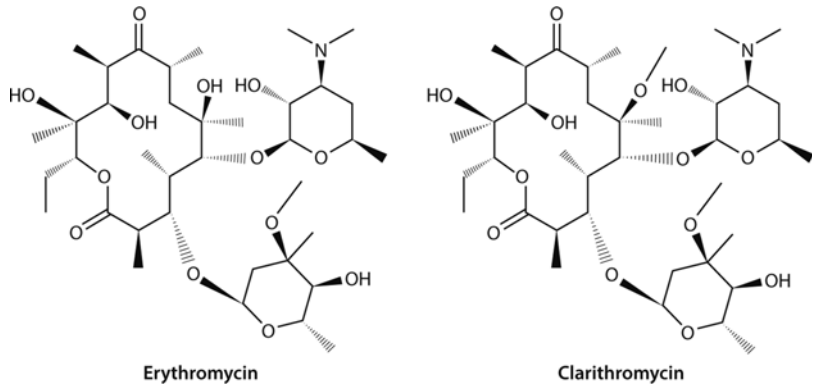

Fig. 36.1 Molecular structures of erythromycin and clarithromycin

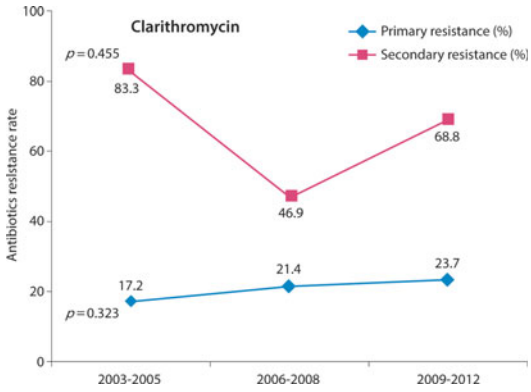

Fig. 36.2 The alteration of primary and secondary clarithromycin resistance rates in Korea (Adapted from Lee et al. [4])

Korea, clarithromycin resistance rates were 22.9 % from 2003 to 2005, 25.5 % from 2006 to 2008, and 37.0 % from 2009 to 2012, which show rapid increments [4] (Fig. 36.2). Nonetheless, the prevalence rates were 17.2 % from 2003 to 2005, 21.4 % from 2006 to 2008, and 23.7 % from 2009 to 2012, which show lower percentages when only patients without past medical history of antibacterial treatments (primary resistance) were counted. Although these rates were obtained from tertiary-referral hospitals, these increased primary and secondary prevalence rates clearly indicate the increased macrolide resistance. In addition, this study identified that the previous medical history of antibiotic treatment is a meaningful risk factor of antibiotic resistance, which indicates that the exposure to antibiotics is related to the development of resistance. Nevertheless, macrolide monotherapy on respiratory disorders is identified to be no longer effective, giving consistent interests that the change of clarithromycin resistance rate is necessary, since macrolide antibiotics are still widely used in community settings. Meanwhile, clarithromycin resistances of three different regions were from 12.5 to 40.0 % based on the multicenter study in Korea by Kim et al. in 2011, so this result suggests that the identification of resistance rates among local communities should be done prior to the clarithromycin-containing eradication treatment [5].

The increase of clarithromycin resistance is a serious issue because this phenomenon is not limited to a single nation. In China, the resistance rate increased from 12.8 % in 2000 to 28.9 % in 2009, and the rate increased from 7.0 % in 2000 to 15.2 % in 2009 in Japan. Similarly, the United States showed around 10.0 % of prevalence during 1990s, and the rate has increased to 15 % nowadays. The prevalence rates were significantly different among European nations (Fig. 36.3); Austria showed 36.6 % of prevalence rate during similar years, while northern European nations showed half of that of Austria [6–8]. These differences are caused by different antibiotic prescription patterns among the nations, and nations with especially high macrolide antibiotic uses on respiratory diseases show higher resistances [7, 9].

36.4 Diagnosing Clarithromycin Resistance

The method of diagnosing clarithromycin resistance is relatively well established. The most popular tests that are used by current researchers are agar-dilution test and E-test. The criteria for the antibiotic resistance of bacteria follow the criteria of internationally certified antibiotic resistance test standards, which are the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) in the United States and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) in Europe. CLSI recommends agar-dilution test as a gold standard for clarithromycin resistance, and the test determines a resistant-positive result if minimal inhibitory concentration (MIC) is >1 μg/mL. However, many researchers prefer E-test due to financial and time issues, and they often set their standards of determining resistance with minimum MIC values higher than 1 μg/mL. E-test is cheaper and easier to perform compared to agar-dilution test, but the MIC determination is less accurate and CLSI does not recommend this test. This is a problem that needs consensus among researchers based on precise evidences.

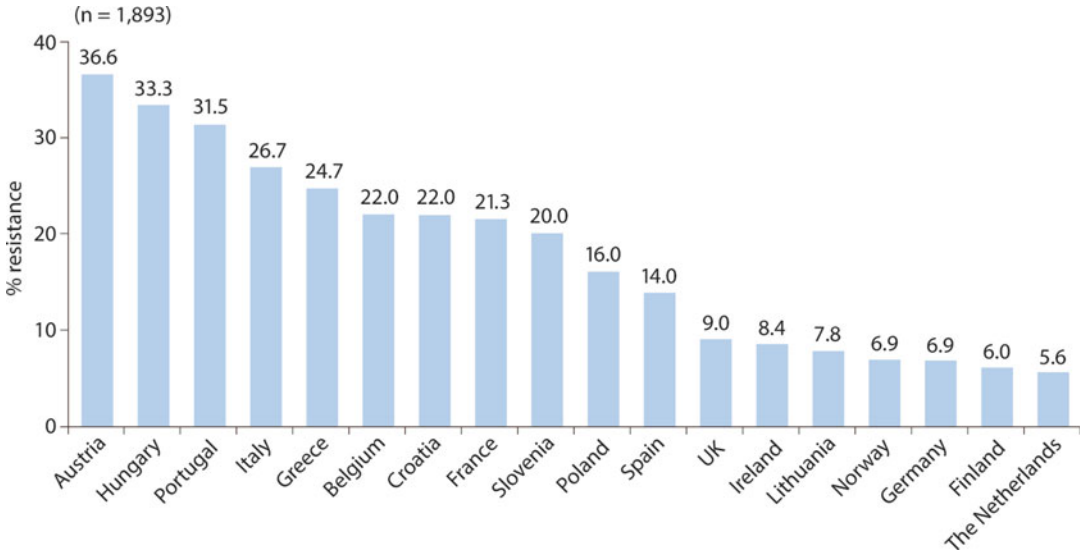

Fig. 36.3 The current clarithromycin resistance rates in Europe (Adapted from Megraud et al. [7])

36.5 Mechanisms of Clarithromycin Resistance

Clarithromycin resistance of *H. pylori* is mainly about the target alteration for antibiotics, like other Gram-negative bacteria. The target alteration occurs in 23S ribosome, which is the component of 50S ribosome, and the 23S ribosome is crucial for *H. pylori* protein synthesis. This alteration is caused by either a posttranscriptional methylation or the alteration of 23S rRNA sequences. Also, the activation of efflux pump is an important mechanism [10].

Clarithromycin target alteration takes the most important role for clarithromycin resistance, among all of the known macrolide antibiotic resistance mechanisms. Especially, the alterations of several 23S rRNA sequences induce the resistance of *H. pylori*. This alteration reduces the adhesion of macrolide antibiotics on their targets, so the resistance occurs. Studies about the target alteration mechanism have been reported since mid-1990s. In 1996, frequent transformations that the replacements of adenine of 2142 and 2143 among 23S rRNA sequences into cytosine or guanine were reported at first. Particularly, A2142C, A2142G, or A2143G mutation is identified to be highly related with high resistance on

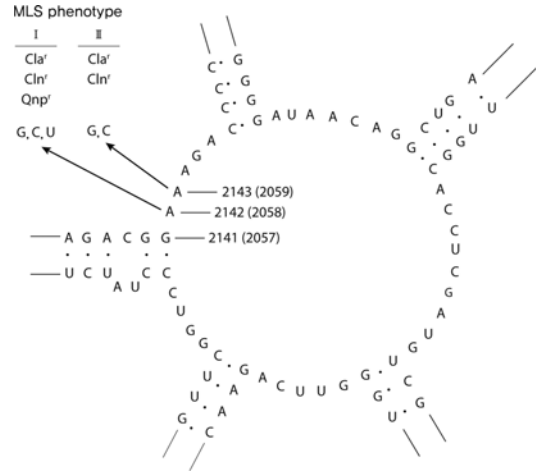

Fig. 36.4 Domain V (peptidyltransferase loop) structure of 23S rRNA gene (Adapted from Wang and Taylor [11])

macrolide antibiotics [11] (Fig. 36.4). Also, T2182C mutations in macrolide-resistant *H. pylori* were frequently reported. Other mutations, such as A2136G, G2141A, and A2144T, were also reported, but these are considered to be less influential, since they are not consistently reported by later studies.

Another important resistance mechanism is the activation of efflux pump. There are five well-known efflux pump systems so far, which are multidrug and toxic compound extrusion

proteins, major facilitator superfamily, ATP-binding cassette superfamily, and resistance-nodulation cell division (RND) [12–14]. Especially, *H. pylori* is known to have RND-type multidrug efflux pump. In 2005, *hp0605-hp0607*, *hp0971-hp0969*, *hp1327-hp1329*, and *hp1489-hp1487* are reported to be the candidates of RND-type multidrug efflux pump resistance of *H. pylori*. Also, *hp0605-hp0607* (*hefA*) overexpression and its direct interference on the expression of *H. pylori* multidrug resistance (MDR) efflux pump have been identified. The *hefA* gene-expressed MDR efflux pump is initially known to exist in bacteria that show multidrug resistance due to external stimulations, like repetitive exposure to antibiotics. However, there is no study in Korea regarding this issue so far, and the same studies for explaining the resistance mechanism are only limited to laboratory levels outside Korea.

36.6 Current Clarithromycin Resistance Studies and Its Prospection

36.6.1 Current Clarithromycin Resistance Studies

The target alteration is the most contributing mechanism of clarithromycin resistance on *H. pylori*. Thus, finding the distributions of mutated genes that cause antibiotic target alterations is the priority.

In 2008, Kim et al. confirmed 23S rRNA resistance gene mutations among clarithromycin-resistant *H. pylori* colonies of Korean patients from 2003 to 2006 via DNA sequencing [15]. As a result, 71.4 % of patients who previously received *H. pylori* eradication therapy showed A2143G transformation, and the rest 28.6 % of patients showed T2182C transformation. Also, both A2143G and T2182C mutations coexisted in 70.3 % of secondary colonies with previous *H. pylori* antibacterial treatments. In conclusion, it is confirmed that a past antibiotic exposure contributes to high resistance as well as complicated mutation, and the most important genetic mutation of resistance is identified to be A2143G (Table 36.1).

Table 36.1 The current distributions of 23S rRNA resistance genes in Korea

Genotypes of 23S rRNA mutation	No. of isolates (%)	
	Primary isolates	Secondary isolates
A2143G	15 (71.4)	5 (7.8)
T2182C	6 (28.6)	5 (7.8)
A2143G + T2182C		45 (70.3)
A2143G + T2182C + T2190C		4 (6.3)
A2143G + T2182C + C2195T		3 (4.7)
A2143G + T2182C + A2223G		2 (3.1)
Total	21 (100)	64 (100)

Adapted from Kim et al. [15]

Along with those resistance mechanisms, Hwang et al. reported about the role of A2143G genetic mutation in 2010. This study directly analyzed how point mutation contributes to resistance expression and the success of antibacterial treatment [16]. Among six mutated A2143G *H. pylori* colonies in the study, none of those was susceptible to clarithromycin-included standard triple therapy. Thus, it could be concluded that A2143G transformation is the important transformation to determine the success of eradication therapy. In 2013, there was a report on the relationship between eradication results and 23S rRNA resistance mutated genes, based on 110 patients who had *H. pylori* eradication therapy in Jeju Island, Korea. Among those 110 patients, only 23 patients (18.1 %) had A2143G mutation. However, 87.5 % of unsuccessful antibacterial therapy results could be seen among patients with A2143G transformed colonies, which confirms that A2143G mutation status significantly affects on the successful eradication therapy.

As mentioned in previous sections, clarithromycin resistance is the nationwide problem. Some countries try new medications or new treatment plans, such as the sequential treatment, and they get some good results from those. However, it is difficult to find and to apply an effective alternate treatment due to prevalent resistance

phenomenon in Korea. On the one hand, clarithromycin itself can be used as a very effective antibiotic, as long as there is no resistance. Some researchers investigating antibiotic resistance commonly argue that the clarithromycin-included triple therapy can be still used as an effective treatment with minimal adverse effects, if there is no clarithromycin resistance. Thus, numerous researchers have focused on developing new tests to easily evaluate clarithromycin resistance.

Molecular diagnostic methods about the genes responsible for resistance have been studied since the late 1990s. Most of the diagnoses were the modifications of polymerase chain reaction (PCR) [17]. However, these modifications did not show any clinically superiority to previous MIC tests in terms of time and financial conditions. This is because patients who failed their first eradication therapies usually get successful eradications after the second or third treatment, and performing antibiotic susceptibility tests after treatment failure was not easily done all the time.

GenoType HelicoDR® test was a remarkable diagnostic test published in 2009 because it is a mixture of PCR and DNA strip method, and the test could diagnose A2142C and A2143G transformations from a stomach mucosal biopsy tissue sample within 6 h [18]. Also, this test can diagnose *gyrA* gene mutation, which is a quinolone resistance gene, so the test was considered to be effective. A GenoType HelicoDR® test study was conducted to identify its clinical efficacy, and the test predicted resistance gene expression with meaningful selectivity and specificity. This study showed that the test can be used to predict an eradication failure due to clarithromycin resistance, but further studies are no longer held because of numerous false-positive results and the difference in the distribution of resistance genes, compared to Europe that this test was developed [19]. Another promising test would be dual-priming oligonucleotide (DPO)-based multiplex PCR of Seegene Inc. that was reported by Megraud et al. of France [20]. Megraud et al. utilized 127 gastric mucosal tissues to compare the difference with E-test in this report, and there was 95.3 % conformity between the two tests, which can be accepted as effective. However, further studies on DPO-based multiplex PCR are not actively reported in nowadays. Recently, a simplified molecular test has been launched to confirm the presence of *H. pylori* clarithromycin-resistant mutant genes via DNA sequencing by a company in Korea. This test takes approximately 3 days, but it uses DNA sequencing that allows various mutant identifications regardless of their types, so this test is currently covered by national health care insurance of Korea after the notification from the Ministry of Health and Welfare. This method is expected to be a better way to easily avoid clarithromycin resistance in actual clinical settings.

These molecular genetic studies pursue a quick and easy diagnosis of resistance. Increasing antibiotic resistance keeps lowering the possibility of successful experimental antibiotic treatments without prior resistance diagnoses. Although a new test is developed, it takes long time to prove better efficacy and financial benefits compared to previous tests. Therefore, constant interests by researchers are necessary, and resistance mechanisms should be studied more.

36.6.2 The Prospect About Future Clarithromycin Resistance Studies

Most of researchers have mainly focused on the antibiotic target alteration mechanism, while they have given less interest on other mechanisms. Thus, studies on new resistance mechanisms are performed by using whole-genome sequencing or next-genome sequencing methods. In 2014, Yoshino Yamaoka research team used next-genome sequencing to analyze low-level resistance-related genes, except for A2143G mutation that is related to high-level resistance. This study reported the existence of new resistance gene by confirming that *hp1048* (*infB*) and *hp1314* (*rp122*) sequence mutations induce resistance on ATCC 26695 *H. pylori* colonies via transformation experiment [21]. Particularly, a very high resistance, which is represented as MIC >256 μg/mL, could be seen, if resistant on both *infB* and *rp122* genes.

In 2014, Toshihito Tanahashi research team reported a new genetic mutation other than efflux pump-related mutation, by using whole-genome sequencing. Especially among RND-type efflux family genes, single-nucleotide variants of *hp0605-hp0607*, *hp0971-hp0969*, *hp1327-hp1329*, and *hp1489-hp487* were identified and their sequences were reported [22]. Along with these discoveries, a turning point to overcome resistance based on efflux pump mechanism is expected. Also, there is a report that a biofilm formation is significantly related to a resistance expression, so new research results should be focused as well [23].

In terms of quinolone, new medications are continuously developed, and most of these quinolones insist they could overcome resistance gene mutations [24]. In comparison, it is unfortunate that macrolide antibiotics still could not demonstrate new medication developments.

In 2010, Graham et al. [25] have set the maximum limitation of local community prevalence that clarithromycin can be applied during first-line eradication therapy, as 15 %. In other words, the study advised to minimize clarithromycin use during first-line eradication therapy, if the prevalence of a community setting is higher than 15 %. However, this recommendation is difficult to be accepted in Korea because of high resistance rate of multiple antimicrobial agents. Especially, clarithromycin can be still effectively used for antibacterial treatments, if there is no clarithromycin resistance. Therefore, continuous study on diagnosis of resistances prior to eradication is necessary.

Conclusions

Clarithromycin is the core of *H. pylori* eradication therapy due to its minimal adverse effects and outstanding antibacterial activity, by taking two oral tablets per day. However, there are many limitations on using clarithromycin due to the recent increase of resistance. The clarithromycin-resistant mechanisms depend on 23S rRNA gene mutations and efflux pump activation. The most significant gene mutation would be A2143G mutation, and this mutation is closely involved in the failure of eradication therapy. Epidemiological studies for mutations which were responsible for antimicrobial resistance should be continued because these studies could provide effective information which could be useful for the development of novel molecular diagnostic tool or antimicrobial agent.

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Amoxicillin

37

Yong Hwan Kwon

Abstract

Treatment of *Helicobacter pylori* (*H. pylori*) is warranted and normally done with combination of antibiotics (such as amoxicillin, clarithromycin, or others) and a proton pump inhibitor. However, the eradication rate of legacy triple therapy has been decreasing because of the increase in resistance to clarithromycin. Likewise, treatment failure due to amoxicillin, a primary antimicrobial agent for *H. pylori* eradication, resistance has been noted since 20 years ago. Increasing levels of resistance to amoxicillin contribute to treatment failures, and higher levels of resistance are believed to be due to multiple genetic mutations. However, the exact mechanism of amoxicillin resistance and its contribution to eradication failure has not been fully elucidated.

Keywords

Amoxicillin • Antibiotic resistance • Eradication • *Helicobacter pylori*

37.1 Introduction

Helicobacter pylori (*H. pylori*) is a major cause of peptic ulcer disease and potentially stomach cancer [1–3]. Recent decades have seen the standardization of treatment for *H. pylori* eradication, and all of the worldwide consensus conferences have recommended the use of triple therapy consisting of a proton pump inhibitor (double dose) and two antibiotics, mainly amoxicillin (1 g b.i.d. [twice a day]) and clarithromycin (500 mg b.i.d.) or metronidazole (500 mg b.i.d.), as first-line therapy [4, 5]. However, this conventional triple therapy have been showing decreased eradication rate with geographic difference [6, 7]. It is important that treatment failures of *H. pylori* occur principally because of poor patient compliance or bacterial resistance [8]. Antibiotic resistance of *H. pylori* has been regarded to be mainly related with metronidazole and macrolide such as clarithromycin [9–16]. Amoxicillin is a β-lactam antibiotic and constitute the primary *H. pylori* eradication regimen [17–19]. In the past, even

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until the last decade, it was generally considered that amoxicillin resistance of *H. pylori* was absent or very rare. But currently, reports on the isolation of amoxicillin-resistant *H. pylori* are increasing worldwide [7, 14, 20–23]. The objective of the present chapter is to review the prevalence of *H. pylori* resistance to amoxicillin, clinical importance, and the resistance mechanism of amoxicillin in *H. pylori* infection.

37.2 Prevalence of *H. pylori* Resistance to Amoxicillin

Until the end of the twentieth century, resistance to penicillins (e.g., amoxicillin) was very rare in *H. pylori*. By contrast, many other bacteria exhibit widespread resistance to these antibiotics (clarithromycin and metronidazole) [21, 24–26]. The prevalence of antibiotic resistance in various regions is correlated with the general use of antibiotics in the region and *H. pylori* strains have been divided into five major groups (East Asian type, south/central Asian type, Iberian/African type, and European type) according to geographical associations [6, 7, 27]. The most important factor resulting in antibiotic resistance is poor patient adherence to the drug regimen. However, the incidence of amoxicillin (minimal inhibitory concentration [MIC] ≥0.5 mg/L, respectively) in *H. pylori* seems to increase in geographic regions where these antibiotics can be obtained without prescription. The first indications of an increased incidence of amoxicillin resistance in *H. pylori* came from amoxicillin-resistant isolates obtained from dyspeptic patients in Italy and the USA [28]. Amoxicillin resistance of these isolates is not stable, as it is lost upon freezing at −80 °C. Thus, these isolates are referred to as amoxicillin tolerant rather than amoxicillin resistant [28]. Later, stable amoxicillin-resistant *H. pylori* isolates were obtained from dyspeptic patients living in different geographic regions [29, 30].

However, most of the surveys previously reported that resistance to amoxicillin is either null or less than 1 %, indicating that it is not yet a problem [7]. In Europe, available data from two studies enrolling 599 patients found a prevalence rate <1 % (3/599, 0.5 %; 95 % confidence interval [CI], 0.06–1.06). Recent study reported the rate of amoxicillin resistance was 0.7 % in 1,893 European adults [31]. According to systemic review, the primary amoxicillin resistance was detected in 184 out of 1,640 tested patients (11.2 %, 95 % CI, 9.6–12.7) [21]. In Europe, available data from two studies enrolling 599 patients found a prevalence rate <1 % (3/599, 0.5 %; 95 % CI, 0.06–1.06) [21]. On the contrary, conflicting data were reported in two African studies [21]. Indeed, amoxicillin resistance was absent in a study from Senegal enrolling 40 patients, while an astonishingly high prevalence was reported in Cameroon (113/132, 85.6 %; 95 % CI, 76.9–91.5) [32, 33]. Similarly, the prevalence of amoxicillin resistance widely varies in Asian countries, ranging from 0 % in 61 patients in Japan, 8.8 % (10/113, 95 % CI, 3.6–14.0) in Korea, and 36.1 % (48/133, 95 % CI, 27.9–44.2) in Taiwan, although another study performed in Taiwan found a prevalence as low as 0.9 % (2/210, 95 % CI, 0.3–2.2) [14, 21, 34–36]. According to another Japanese study, no amoxicillin resistance strains were detected in the strains isolated between 1985 and 1996, while the rate of resistance was determined to be 1.1 %, 2.1 %, 5.4 %, 5.6 %, 0 %, 8.8 %, and 1.5 % each year, respectively, from 1997 to 2003 [37]. The percentage of resistance to amoxicillin strains increased from 2000 to 2003. The total eradication rate of *H. pylori* in the patients who showed amoxicillin-resistant strains was 71.0 % (44/62) [37]. In Korea, the resistance to clarithromycin, tetracycline, ciprofloxacin, and levofloxacin increased during the period of 2007–2009 compared with 2003–2005 [14, 38]. However, amoxicillin resistance slightly decreased from 6.1 to 4.8 % [38].

37.3 The Role and Resistance Mechanism of Amoxicillin in Eradication of *H. pylori*

37.3.1 Target of ß-Lactam Antibiotics in *H. pylori*

The cell wall of Gram-negative bacteria consists of a number of layers, differing greatly in their chemical composition. The inner most membrane

is the cytoplasmic membrane followed by a thin peptidoglycan layer, different in composition and thickness from the Gram-positive peptidoglycan layer (Fig. 37.1). Outside of the peptidoglycan layer is the outer membrane which contains lipopolysaccharide, consisting of lipid A, core polysaccharide, and O antigen. The peptidoglycan layer consists of *N*-acetyl glucosamine and *N*-acetyl muramic acid with a pentapeptide side chain. These units are cross-linked, forming a peptide bond between D-alanine on one chain and diaminopimelic acid on the other chain in Gram-negative bacteria. This cross-linking function is performed by transpeptidase enzymes known as penicillin-binding proteins (PBPs). The transpeptidase domains of these enzymes utilize an active site serine to perform the catalytic reaction [39]. PBPs also have transglycosylase activity responsible for peptidoglycan polymerization and insertion into pre-existing cell wall especially during new cell growth [40, 41]. β-lactam antibiotics are bacteriocidal and inhibit the synthesis of new peptidoglycan layer in actively dividing cells. Transpeptidase enzymes utilize an active site ser-

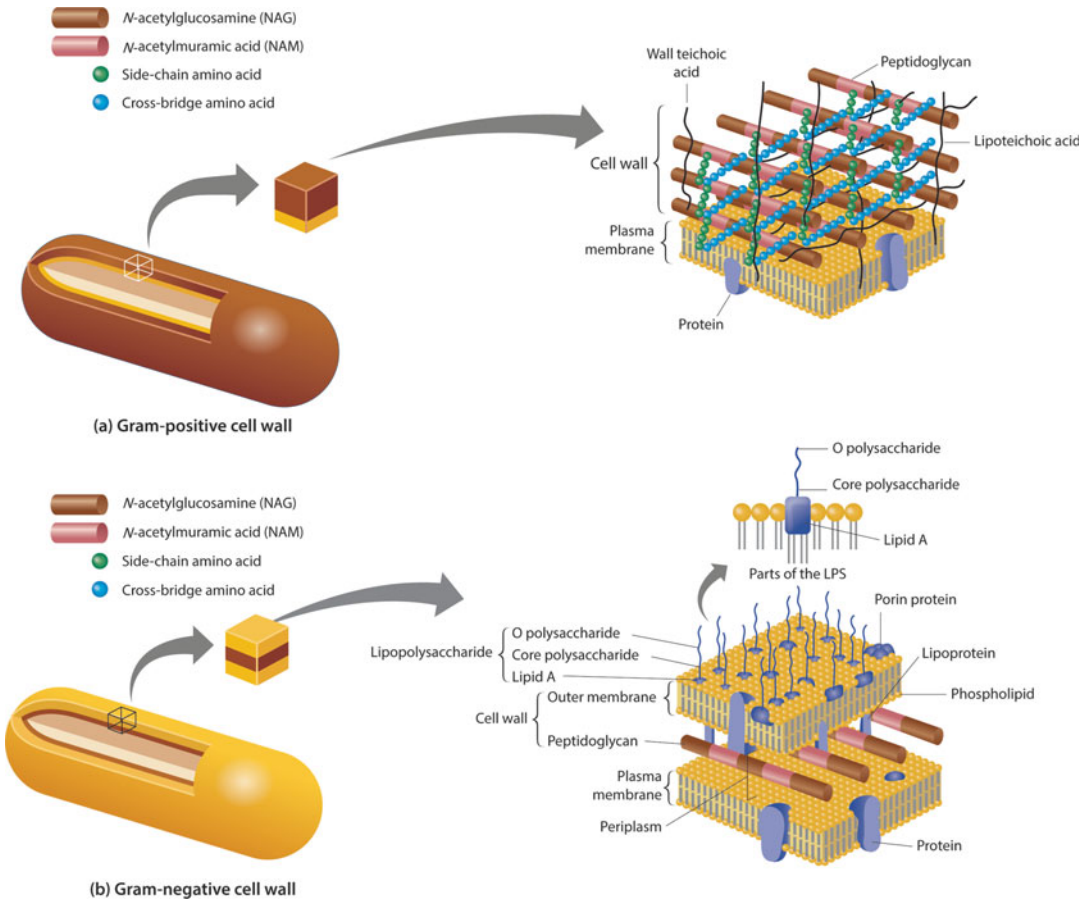

Fig. 37.1 The structures of Gram-positive and Gram-negative cell wall. (**a**) In the Gram-positive bacteria (those that retain the purple crystal violet dye when subjected to the Gram-staining procedure), the cell wall consists of several layers of peptidoglycan. Running perpendicular to the peptidoglycan sheets is a group of molecules called teichoic acids which are unique to the Gram-positive cell wall. (**b**) In the Gram-negative bacteria (which do not retain the crystal violet), the cell wall is composed of a single layer of peptidoglycan surrounded by a membranous structure called the outer membrane. The outer membrane of Gram-negative bacteria invariably contains a unique component, lipopolysaccharide (LPS or endotoxin), which is toxic to animals. In Gram-negative bacteria, the outer membrane is usually thought of as part of the cell wall (Adapted from Gerard J. Tortora, Berdell R. Funke, Christine L. Case. Microbiology: an introduction, 12th ed. New York: Pearson Education, Inc. 2016. p82 by permission of Pearson Education, Inc.)

ine and perform their catalytic cycle by way of an acylation/deacylation pathway. β-lactam antibiotics efficiently inhibit the bacterial transpeptidases; therefore, these enzymes are often termed penicillin-binding proteins or PBPs. They are able to do this owing to the similarity of the β-lactam moiety with the $_{D}$-alanine–$_{D}$-alanine substrate. In the presence of the antibiotic, the transpeptidases form a lethal covalent penicilloyl–enzyme complex that serves to block the normal transpeptidation reaction. This results in weakly cross-linked peptidoglycan, which makes the growing bacteria highly susceptible to cell lysis and death [39]. Amoxicillin is extended spectrum penicillin, and clavulanate potassium is a β-lactamase inhibitor [42]. The molecular formula is C16H19N3O5S and the molecular weight is 419.45 (Fig. 37.2). Amoxicillin also acts on cell-wall PBPs and may have a role as an important stress factor for the morphologic changes by expression induction of some peptidase enzyme genes that results in modification of the cell-wall compositions in coccoid forms. Amoxicillin had the greatest in vitro bactericidal effect on bacillary forms of *H. pylori*; however, in 2× MIC, it had no bactericidal effect on coccoids [43]. In addition to the potent in vitro effect of amoxicillin against *H. pylori*, this antibiotic has a little in vivo bactericidal effect [44]. Transition to coccoid forms correlates with accumulation of the *N*-acetyl-$_{D}$-glucosaminyl-β(1,4)-*N*-acetylmuramyl-$_{L}$-Ala-$_{D}$-Glu (GM-dipeptide) motif. This modification may have a role in resistance to bactericidal agents such as amoxicillin that act on the cell wall such as amoxicillin [45].

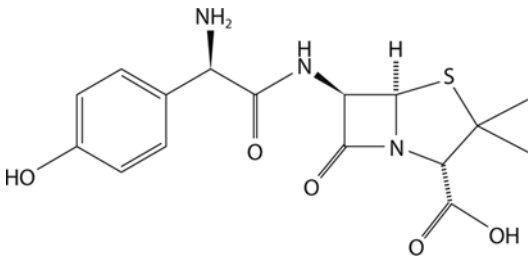

Fig. 37.2 The molecular formula of amoxicillin (2*S*,5*R*,6*R*)-6-{[(2*R*)-2-amino-2-(4-hydroxyphenyl)-acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-24-carboxylic acid

37.3.2 The Resistance Mechanism of Amoxicillin

β-lactams are rendered inactive against bacteria by way of three primary mechanisms of resistance (Fig. 37.3). The most common mechanism is the production of enzymes that degrade or modify the antibiotic before it can reach the appropriate target site. In this case, the β-lactamase family of enzymes degrade β-lactam antibiotics and are found widely disseminated among Gram-positive and Gram-negative bacteria. The second mechanism is the alteration of the antibiotic target site. In this case, the β-lactam-resistant cell-wall transpeptidases perform this role; this is now a major cause of resistance in several pathogens including the problematic Gram-positive staphylococcal and streptococcal species. The final mechanism is the inhibition of access of the antibiotic to the target by way of altered permeability or forced efflux. For example, this can be performed by the MexA, B–OprM antibiotic efflux pump, which is a major cause of resistance in Pseudomonas and in other pathogenic Gram-negative species [39]. In *H. pylori*, however, β-lactamase activity has not been established, and resistance seems to be mainly mediated by alterations to PBPs. The PBPs are a set of enzymes involved in the synthesis of the peptidoglycan layer of the bacterial cell wall and include transpeptidases, transglycosylases, endopeptidases, and carboxypeptidases [46, 47]. All conventional PBPs contain characteristic penicillin-binding protein motifs (PBP motifs): SXXK, SXN, and KTG (X is a variable amino acid residue) [48]. In *H. pylori*, nine putative PBPs have been identified; four PBPs have been identified using labeled antibiotics. DelLoney et al. [20] reported the following molecular masses of four major PBPs in *H. pylori* ATCC 43579: 66, 63, 60, and 47 kDa. The molecular mass of a small PBP was reported in the range of 30–32 kDa by Dore et al. [28] (named PBPD) and Krishnamurthy et al. [49] (named PBP4). They also identified three high-molecular-mass PBPs (PBPs 1, 2, and 3) from *H. pylori* 84–183 in the range of 66–55 kDa and indicated that these PBPs corresponded to PBPs A, B, and C [49], previously described by Ikeda et al. [50]. Other PBPs

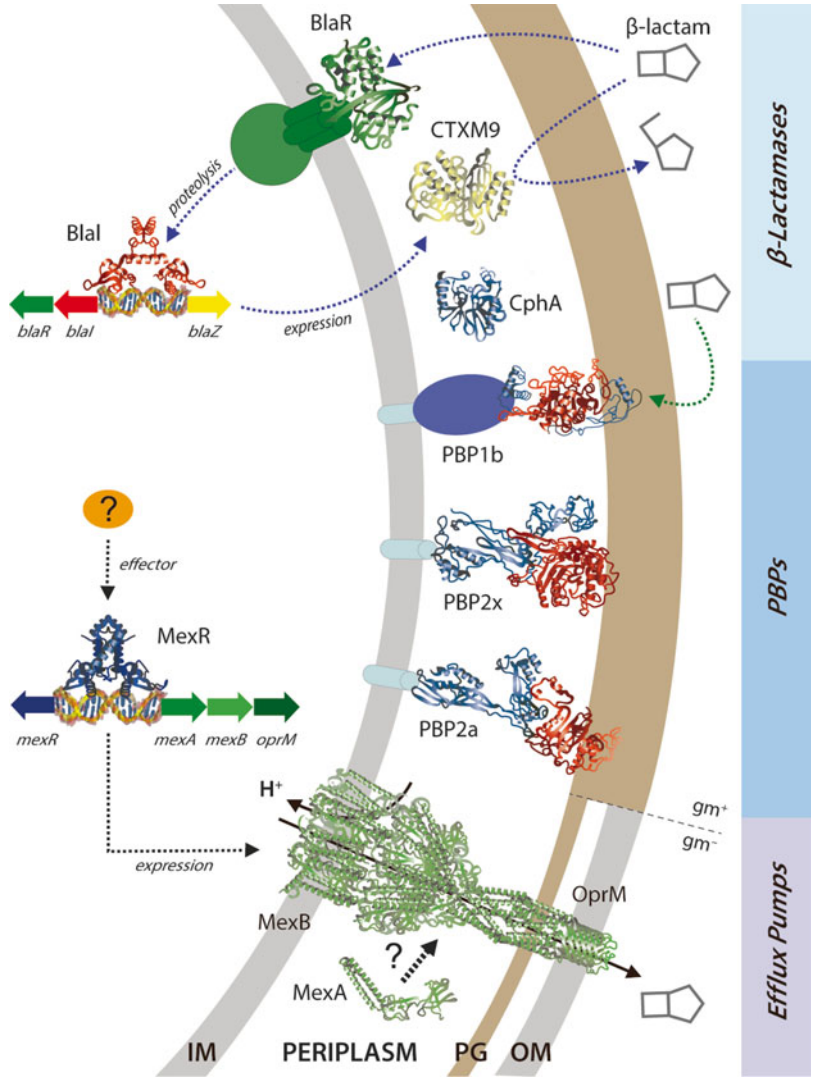

Fig. 37.3 The mechanism of β-lactam antibiotic (Adapted from Wilke et al. [39])

in *H. pylori* were recently identified by Harris et al. [48] at 72, 62, 54, 50, 44, 33.5, 30.5, and 28 kDa. Gram-negative bacteria have porin proteins in their outer membrane which facilitate the transport of hydrophilic solutes. In previously obtained clinically resistant strains, changes in PBP1 alone do not account for all of the resistance observed. In addition to changes in PBPs, reduced membrane permeability and active efflux have been suggested as mechanisms contributing to higher resistance to β-lactam drugs. A change in the property of PBP, either a decreased affinity for amoxicillin [51] or point mutation in the *pbp1* gene [52], is the main mechanism that affects the amoxicillin resistance of *H. pylori* [53]. PBP1 has been studied most extensively and has been shown to play a significant role in conferring β-lactam resistance to *H. pylori* [54]. A careful review of the amino acid changes in *pbp1* in amoxicillin-resistant strains from previous publications has led to our hypothesis that only a few of amino acid substitutions can alter amoxicillin-binding Ser414Arg [55–59], Thr438Met [54], Phe473Leu [60], Ser543Arg [53, 60], Thr556Tyr [53, 56, 61, 62], and Asn562Tyr [53, 56, 58, 59, 61] (Table 37.1). These amino acid substitutions have

Table 37.1 Review of previously identified mutations in *PBP1* from amoxicillin-resistant isolate

	V	V	P	S	E	S	S	S	T	Y	E	M	S	D	T	S	T	N	A	T	G	P
	16	45	372	402	406	414	414	417	438	484	506	515	517	535	541	543	556	562	562	593	595	600
Author	I	I	S	G	A	R	A	T	M	C	A	I	T	N	I	R	S	Y	T	A	S	T
Co and Schiller [54]									O													
Gerrits et al. [22]				O	O	O		O														
Kwon et al. [53]											O	O	O	O		O	O	O				
Matteo et al. [63]																	O					
Okamoto et al. [61]																	O	O		O		
Paul et al. [57]						O				O					O		O	O				O
Rimbara et al. [58]						O												O				
Qureshi et al. [60, 65]			O						O							O	O	O				
Kim and Kim [66]	O	O					O												O	O	O	

PBP penicillin-binding protein

appeared either as a single change with corresponding increased amoxicillin resistance [54, 55, 63] or as parts of clusters of changes [53, 56, 58, 60–62]. In all cases, the authors obtained transformants with increased MICs but not higher than 0.5–2 mg/L. However, sequencing of *pbp1A* in these different experiments showed different mutations associated with resistance. Dore et al. [28] reported that the 30–32 kDa PBP (PBP D) band observed in amoxicillin-susceptible strains was not detected in resistant strains from various sources and proposed that PBP D may play a role in the amoxicillin-resistant phenotype of *H. pylori*. They did not discuss whether the disappearance of the band is due to a lack of the PBP D protein or to reduced affinity for [3H] benzylpenicillin because of a change in PBP D. However, the contributions of these individual mutations to amoxicillin resistance are not clear, since several other mutations are also present in *pbp1*. By culturing an amoxicillin-susceptible strain with increasingly higher concentrations of amoxicillin, it was also possible to obtain strains with MICs of 4–8 mg/L which exhibited a decreased affinity for this drug [64]. Amoxicillin-resistant *H. pylori* strains accumulated less [^{14}C]-penicillin G compared with amoxicillin-susceptible strains, both in the presence and in the absence of CCCP, thus excluding the role of an active efflux mechanism [53, 64]. This suggests that at least in some strains, β-lactam resistance in *H. pylori* is partly due to an increased diffusional barrier, an effect that could be explained by changes in outer membrane proteins. By comparing an in vitro-selected amoxicillin-resistant strain with its amoxicillin-sensitive parent strain, it has been determined that both changes in PBP1 and Hop Proteins B and C also seemed to contribute to resistance [54] and the combined effects of amino acid changes occurring in PBP1, HopC, HefC, HofH, and possibly PBP2, recently [65].

However, most of these individual mutations that contributed to amoxicillin resistance were evaluated by comparing the amoxicillin-resistant *H. pylori* strains with reference strains or using site-directed mutagenesis to create mutations individually in *pbp1* and transformed each altered *pbp1* into amoxicillin-sensitive strain to monitor its effect on the MIC of *H. pylori* amoxicillin, in vitro. Thus, the clinical significance of these mutations to affect amoxicillin resistance and failure of eradication was doubtful.

Conclusions

Reports on the incidence of amoxicillin resistance are still conflicting and not investigated, yet. If amoxicillin resistance in *H. pylori* would further increase, this would pose the serious problem of reduced efficacy of amoxicillin-containing regimens. Based on previous studies, the complexity of several mechanisms (*pbp* gene mutations, membrane permeability alterations, efflux pumps, etc.) was related with amoxicillin resistance in *H. pylori* infection. Moreover, these mutations would affect the failure of *H. pylori* eradication with amoxicillin-containing therapy, which is another problem. Many clinical and experimental data would be needed to understand the effect and resistance of amoxicillin in *H. pylori*.

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Fluoroquinolone

38

Jung Won Lee

Abstract

Fluoroquinolone antibiotics demonstrate excellent antimicrobial efficacy in various clinical field. Especially, levofloxacin and moxifloxacin could be used for *Helicobacter pylori* (*H. pylori*) eradication. It could be used effectively for *H. pylori* eradication with fewer side effects. However, it shows rapid increase of resistance recently. The increase of resistance has become a significant limitation for providing effective eradication treatment result. The main antibacterial mechanism of fluoroquinolone is the interruption of DNA replication by interfering with DNA gyrase and topoisomerase IV. Resistance of fluoroquinolone antibiotics was derived from the mutation of *gyrA* which encodes DNA gyrase A, especially point mutation of Asn-87. Novel fluoroquinolones show improved efficacy as to overcome the resistance caused by *gyrA* mutation. Epidemiologic study for point mutation of resistance gene might be helpful for the development of molecular diagnostic tool and novel fluoroquinolone agent. And further constitutional efforts are needed especially to devise more easier and effective fluoroquinolone-containing treatment regimen.

Keywords

Helicobacter pylori • Antibiotics • Resistance • Fluoroquinolone

38.1 Introduction

Triple therapy containing a high-dose proton pump inhibitor (PPI), amoxicillin with clarithromycin, the so-called legacy triple therapy, has been used for first-line eradication treatment for *Helicobacter pylori* (*H. pylori*) infection. Because of the high prevalence of metronidazole resistance, clarithromycin-containing therapy has been the mainstream of triple therapy. The eradication

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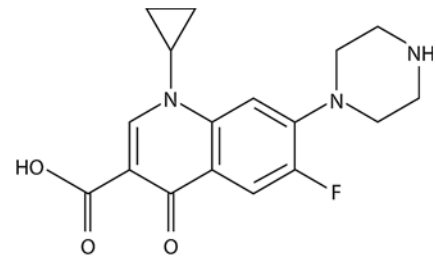
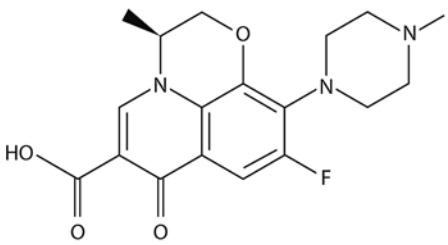
rate of legacy triple therapy has been decreasing progressively because of the increase in resistance to clarithromycin. There has been urgent need to find alternative antimicrobial agent [1, 2].

Quinolone antibiotics have become a good alternative agent with considerable efficacy at the present status. The quinolones are a family of synthetic antibiotics developed at 1960s. The initial quinolone agents merely have limited indications due to their limited efficacy. At 1980s, quinolone antibiotics evolved to fluoroquinolones by attached fluorine bond. This structural modification provided significantly improved potency to quinolone antibiotics. Representative second-generation quinolone is ciprofloxacin. Ciprofloxacin show excellent antimicrobial efficacy especially to Gram-negative bacteria. After ciprofloxacin, next-generation fluoroquinolones such as levofloxacin and moxifloxacin were developed with more extended efficacy even to Gram-positive bacteria (Fig. 38.1). These next-generation fluoroquinolones have excellent bioavailability – about ≥90 % – and their desired effect could be easily achieved by oral route. They also have minor side effects. Levofloxacin and moxifloxacin provided considerable antimicrobial efficacy compared with ciprofloxacin, especially for *H. pylori*. For this reason, many researchers have investigated *H. pylori* eradication treatments containing fluoroquinolone, competitively. However, resistance of fluoroquinolone to *H. pylori* has increased in association with the rapid increase in fluoroquinolone use for other diseases and has become considerable limitation for maintaining effective *H. pylori* eradication [2, 3].

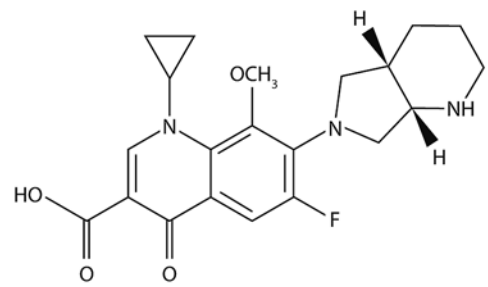
From this background, we describe mechanism of action, epidemiology, diagnosis, and resistance mechanism to fluoroquinolone in this chapter to provide updated clinical implication and perspectives for more effective *H. pylori* eradication therapy using fluoroquinolones.



Ciprofloxacin



Levofloxacin



Moxifloxacin

Fig. 38.1 Molecular structure of fluoroquinolone antibiotics

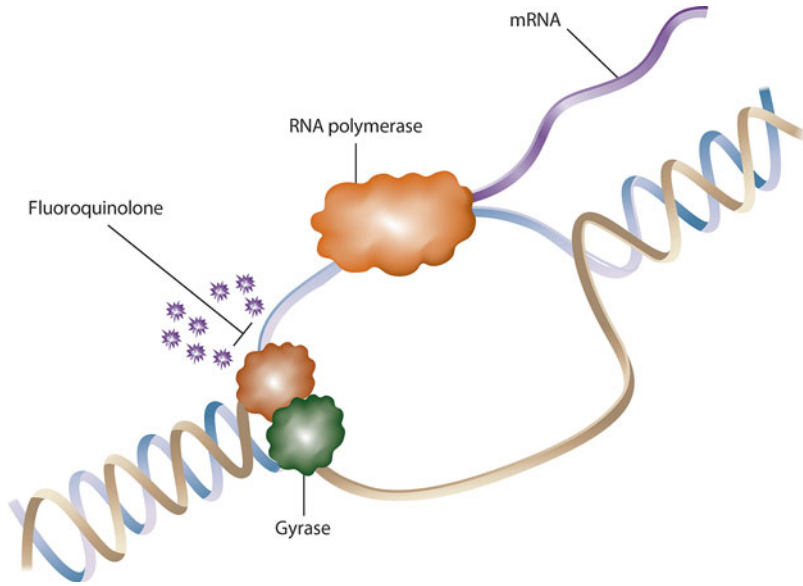


Fig. 38.2 Mechanism of action of fluoroquinolones

38.2 Mechanism of Action of Fluoroquinolone

The main role of gyrase and topoisomerase was negative supercoiling by cutting DNA double helix during the initial phase of DNA replication. The mechanism of fluoroquinolone is the interruption of DNA replication by interfering with DNA gyrase and topoisomerase (Fig. 38.2). The first of these targets is DNA gyrase, a type II topoisomerase composed of two *gyrA* subunits and two *gyrB* subunits [4]. The second target for the fluoroquinolones is topoisomerase IV, made up of two *parC* subunits and two *parE* subunits. The protein subunits coded by *parC* and *parE* are homologous to the *gyrA* and *gyrB* subunits, respectively. These DNA gyrase and topoisomerase were coded by *gyrA*, *gyrB*, *parC*, and *parE* in the quinolone resistance-determining region (QRDR). Quinolone antibiotics bind to DNA gyrase complex and prevent DNA gyrase dissociation. It could lead to DNA fragmentation, eventually replication inhibition.

38.3 Epidemiology of Fluoroquinolone Resistance

Because of the difficulty in culture and antimicrobial sensitivity testing, there have been a few reports on the antibiotic resistance of *H. pylori*. Fluoroquinolone resistance of *H. pylori* was not reported until 2005. Kim et al. demonstrated that ciprofloxacin, levofloxacin, and moxifloxacin minimal inhibitory concentrations (MICs) tend to shift more higher during the period of 1987–2003 [5]. The resistance rates of fluoroquinolones were steadily increased initially from 13.9 % (1994) to 33.8 % (2003).

The isolates obtained from patients without previous *H. pylori* eradication treatment were defined as primary. However, for patients with previous unsuccessful eradication, the isolates were defined as secondary strains. Trends for the prevalence of fluoroquinolone resistance rates in primary and secondary clinically isolated *H. pylori* were published at 2013 [6]. Trends of MICs in *H. pylori* isolates during three periods of time (2003–2005, 2006–2008, and 2009–2012) were investigated. Increase trends regardless of the primary and secondary isolates were founded in both of levofloxacin and moxifloxacin (5.7–34.6 %, $p<0.001$) during 2003–2012 [6] (Fig. 38.3). But this study has a limitation for the relatively low number (374 strains) of strains. Moreover, there could be a possible selection bias by investigating from a referral center. Regarding higher prescription rate in clinical field especially otorhinolaryngological and respiratory causes, it is inferred that increasing trends

of fluoroquinolones would be sustained in the future. Another report also demonstrates that the problems with high resistance rates of fluoroquinolone were prevalent between three hospitals with different location in Korea, all above 20 % [7] (Fig. 38.4).

Increased fluoroquinolone resistance is also a worldwide problem, especially for Asian countries including Taiwan, China, and Japan even more European countries. The study performed in Taiwan reported about 10 % fluoroquinolone resistance rate at 2004. For Japan 2007, about 15 % of fluoroquinolone resistance rate was reported [8]. Differences of fluoroquinolone resistance rate between countries were more enlarged especially for European countries. It has been well known that the resistance rate of fluoroquinolone in Italy was low. However, recent published articles reported increased resistance rate up to 22.1 %. The study performed in Greece over 10 years (2000–2010) demonstrates only small increase of fluoroquinolone resistance, 0–5.7 % [9]. For United Kingdom, 17 % of resistance rate was reported at 2012 [10]. But research performed in Portugal reported high prevalence of resistance up to 33.9 % [11, 12]. The study performed in Brazzaville, Congo, demonstrates 50 % rate of resistance [13]. Consequently, the recent worldwide increase in resistance has become a significant limitation for providing effective eradication treatment using fluoroquinolone [14]. These results are in agreement with the previous studies in European countries and in Japan. Commonly, resistance rate of secondary *H. pylori* isolates tends to be higher compared with primary isolates. From this background, Megraud et al. [15] also suggested that increasing trends of fluoroquinolone resistance rate might correlate with overall fluoroquinolone consumption. The present authors also agreed this statement when considering the rapidly increasing trends reported in Korea [15].

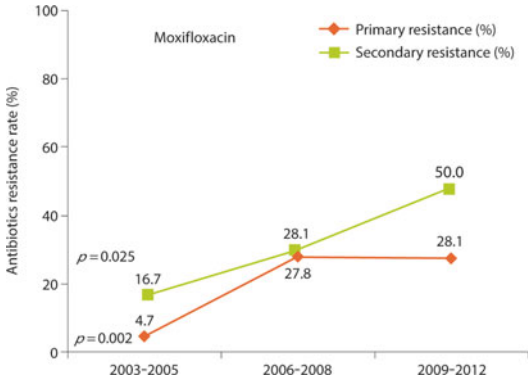


Fig. 38.3 Change of moxifloxacin resistance rate (Adapted from Lee et al. [6])

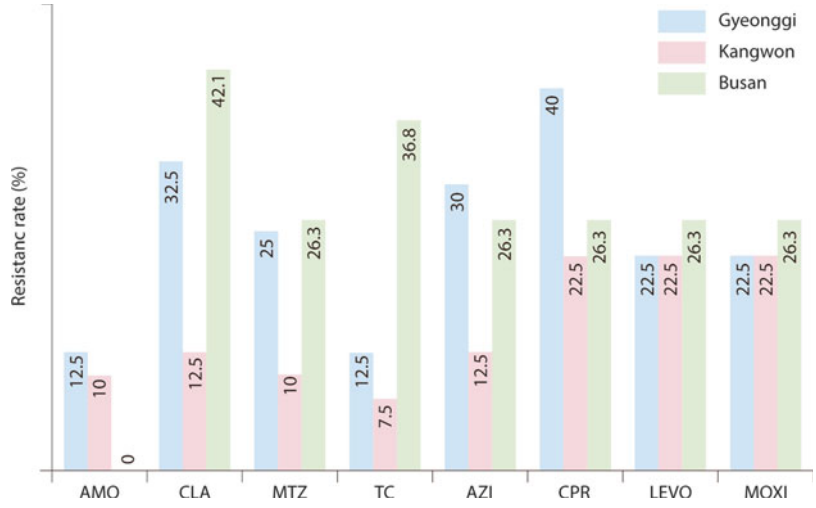


Fig. 38.4 Regional difference of antimicrobial resistance rate. *AMO* amoxicillin, *CLA* clarithromycin, *MTZ* metronidazole, *TC* tetracycline, *AZI* azithromycin, *CPR* ciprofloxacin, *LEVO* levofloxacin, *MOXI* moxifloxacin (Adapted from Kim et al. [7])

38.4 Diagnosis of Fluoroquinolone Resistance

The most reliable diagnostic method for the determination of fluoroquinolone susceptibility is agar-dilution method. But E-test also has been widely used for their simplicity and low cost. The Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical Laboratory Standards) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommended diagnostic procedures and cutoff values at their guidelines. However, the cutoff value of fluoroquinolone was only provided by EUCAST, MIC above 1.0 μg/mL. Hence, majority of research articles about fluoroquinolone for *H. pylori* followed breakpoints of >1.0 μg/mL as published elsewhere provisionally not as a guideline. In my opinion, breakpoints of fluoroquinolone for *H. pylori* should be updated upon substantially accumulated evidences.

38.5 Mechanism of Fluoroquinolone Resistance

38.5.1 Mechanism of Fluoroquinolone Resistance of *H. pylori*

There are four different mechanisms of fluoroquinolone resistance discovered by the study of other bacteria. These mechanisms include the alteration of drug targets such as DNA gyrase or topoisomerase IV, the activation of a drug efflux pump system, a decrease of drug permeability in the membrane, and the plasmid-mediated resistance (Fig. 38.5).

The most important resistance mechanism is target alteration. Fluoroquinolones usually inhibit DNA gyrase for Gram-negative and topoisomerase IV for Gram-positive bacteria. This mode of action could be achieved by binding of fluoroquinolone antibiotics to bacterial gene which encoded gyrase or topoisomerase

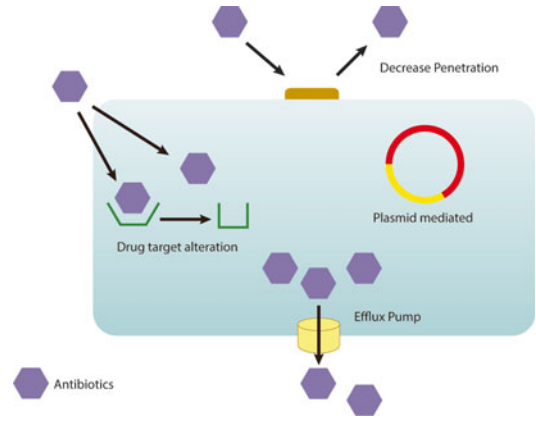


Fig. 38.5 Resistance mechanism of fluoroquinolone

IV. However, if the bacterial gene experiences critical point mutation which could cause three-dimensional conformational changes, binding of fluoroquinolone could not happen, and it can cause the development of resistance. In addition, active efflux mechanism of *H. pylori* could be responsible for the development of multidrug resistance. Efflux pump can eliminate intracellular toxic compound to extracellular and is known to have an important role in the development of multidrug resistance. Efflux pump of *H. pylori* was encoded for *hefA* gene of *H. pylori* genome [16]. Usually intrinsic resistance might be related with the presence of efflux pump. But acquired resistance could be developed by efflux pump overexpression. It was assumed that altered drug permeability and plasmid-mediated resistance might be present in *H. pylori*. But the impact of altered drug permeability and plasmid-mediated resistance were still not investigated. Additionally, any material which can directly inactivate antibiotics like β-lactamase was not reported in *H. pylori*.

38.5.2 Distinct Feature of Genes Responsible for Fluoroquinolone Resistance

Until present, the most effective and decisive mechanism of resistance for fluoroquinolone is the target alteration of binding site. However,

H. pylori do not possess *parC* or *parE* genes that encode the topoisomerase IV unlike other species [17]. Therefore, mutation of DNA gyrase genes, especially *gyrA*, is known to play an important role in the determination of antimicrobial susceptibility. And it has been also suggested that *gyrB* mutations did not play an important role in fluoroquinolone resistance in *H. pylori* [18]. Therefore, this difference of genome must be the critical reason why *H. pylori* strains with single-point mutations of *gyrA* appear to be more difficult to overcome than other species of microorganisms which inhibition of topoisomerase IV still negatively affects the growth of pathogens. In conclusion, it could be explained that fluoroquinolones have limited target to *H. pylori*, approximately half, compared with other common bacterial species.

38.6 Present Status of the Studies About Fluoroquinolone Resistance

The cornerstone research about resistance mechanism of fluoroquinolone for *H. pylori* was published at 2003 by Tankovic et al. [18]. They performed determination of MICs by the agar-dilution method for nine ciprofloxacin-susceptible and two ciprofloxacin-resistant isolates. They found that the presence of a *gyrA* mutation was associated with higher MIC values. So, they concluded that the presence of a *gyrA* mutation might have decisive role in the determination of susceptibility. And it is inferred that the impact of *gyrB* might not be decisive at least for *H. pylori*.

The first systematic report regarding fluoroquinolone resistance gene in Korea was published in 2005 by Kim et al. [5]. The authors assessed the prevalence rate of primary fluoroquinolone resistance over the 16 years (1987–2003). Moreover, authors demonstrate distribution of point mutation of the *gyrA* gene at A272G and G271A by sequence analysis in 14 fluoroquinolone-resistant strains at first in Korea.

The most notable and frequently cited report was published at 2006 by Nobuo Aoyama in Kobe, Japan [8]. The authors performed levofloxacin susceptibility test by E-test in clinically isolated *H. pylori*. Pattern of mutations in the quinolone resistance-determining regions of the *gyrA* and *gyrB* genes was evaluated by direct sequencing of 68 levofloxacin-resistant and 50 susceptible strains. There was a significant difference in the presence of *gyrA* mutations between levofloxacin-resistant (83.8 %) and levofloxacin-susceptible (14.0 %) strains. A notable point in this study is the analysis regarding the difference of impact which is affected by the location of *gyrA* point mutation, Asn-87 or Asp-91. So the authors concluded that *gyrA* mutations at Asp-91 tended to be associated with low-level resistance, but those at Asn-87, with high-level resistance.

At 2011, the study about the distribution of *gyrA* point mutations and overall resistance rate of fluoroquinolone in Korea was published [19]. This research performed *H. pylori* isolation from the patients. DNA sequencing of the QRDR of *gyrA* was performed in 89 fluoroquinolone-resistant and 27 fluoroquinolone-susceptible isolates. The most common mutations were Asp-91 (36.0 %) and Asn-87 (33.7 %). The most common substitution of *gyrA* in Asn-87 was found to be Asn-87-Lys. In the case of Asp-91, Asp-91-Asn was the most common. Additionally, the most important point of the study was the results about the outcomes of fluoroquinolone-containing treatment. Eradication failure was only noted for two cases with the Asn-87-Lys mutation. But, no treatment failure was observed among the three patients with Asp-91-Asn mutated strains. Moreover, Rimbara et al. [20] also had similar opinion. They performed a series of experiments consisted of MIC, sequencing, and transformation. The authors propose that mutation from Asn-87-Lys at *gyrA* confers higher resistance to levofloxacin and gatifloxacin compared with the mutation dose from Asp-91-Asn. In conclusion, Asn-87 point mutation might be a more important determinant for failure of fluoroquinolone-containing eradication compared with Asp-91 mutation.

38.7 Perspective and Limitation of Studies About Fluoroquinolone Resistance Mechanism

The studies about the mechanism of fluoroquinolone resistance could be applicable for two aspects. First, it could be the basis for the development of diagnostic tool for antimicrobial resistance. Second, it could be helpful to find appropriate therapeutic target for overcoming resistance.

As described earlier, the major mechanism of fluoroquinolone resistance, especially for *H. pylori*, is the point mutation of the Asn-87, Asp-91 of *gyrA*. This inspired the development of the molecular diagnostic method that combines polymerase chain reaction (PCR) and DNA strip hybridization. GenoType® HelicoDR (Hain Lifescience, Germany) could be directly applicable to gastric biopsy specimens and could draw result within 6 h. GenoType® HelicoDR test could identify majority of point mutation of 23S rRNA and *gyrA*.

Researches about the evaluation of clinical usefulness of GenoType® HelicoDR test was performed and published at 2014 in Korea [21]. The sensitivity and specificity of GenoType® HelicoDR test in determination of 23S rRNA mutation were 94.9% and 87.1%, and those of *gyrA* 98.2% and 80.0%, respectively. But this research could not be extended because of the different prevalence of fluoroquinolone resistance compared with those of other countries. Relatively low sensitivity and false-positive rate were additional problems. But with the presence of several shortcomings, it showed high negative predictive value for detecting 23S rRNA (96.4%) or *gyrA* (97.4%) mutations. It is inferred that GenoType® HelicoDR test could be effectively use in predicting treatment failure due to resistance of clarithromycin or fluoroquinolone. Additionally, dual-priming oligonucleotide (DPO)-based multiplex PCR method used for the detection of 23S rRNA mutation (clarithromycin resistance) could be applicable for *gyrA* mutation. But it also needs further research.

Novel fluoroquinolones such as sitafloxacin, gemifloxacin, and finafloxacin have been developed and studied [22–24]. Studies about these novel fluoroquinolones generally demonstrated positive favorable outcomes. These fluoroquinolones commonly presented the proofs that these materials even could overcome resistance caused by *gyrA* mutations. However, these novel fluoroquinolones should undergo sufficient preclinical stage and should find suitable indications. Moreover, the most fundamental unsolved problems about resistance mechanism were the presence of resistance caused by mutations other than *gyrA* mutations. Nevertheless, it is reasonably explained by mutations of 23S rRNA were present at clarithromycin resistance *H. pylori* > 90% [25]. But for fluoroquinolone, only 70–80% of resistance strains have the point mutation of *gyrA* [8, 19, 20]. Above all things, the authors concluded that further efforts are needed to elucidate the complete mechanism of fluoroquinolone resistance.

Megraud et al. [15] recommended in 2013 that fluoroquinolone antibiotics should be prescribed only for patients without prior history of fluoroquinolone treatment for any cause. The authors also agree to this statement. Considering the updated results of fluoroquinolone antibiotics, fluoroquinolones have sufficient efficacy and bioavailability even with a few side effects. These antibiotics deserve further dedication of efforts.

Conclusions

Fluoroquinolone antibiotics show excellent antimicrobial efficacy to *H. pylori* with fewer side effects. However, the recent increase of fluoroquinolone resistance has become a significant limitation for providing effective eradication treatment result. Unlike common bacteria, *H. pylori* do not have the *parC* or *parE* genes which encode topoisomerase IV. Therefore, mutation of DNA gyrase genes, especially Asn-87 point mutation of *gyrA*, is known to play a decisive role in the determination of susceptibility. Epidemiologic study for mutations of resistance gene might be helpful for the development of molecular diagnostic tool and novel fluoroquinolone agent. In conclusion, further efforts are needed especially to devise new therapeutic agent, diagnostic tool, and more effective fluoroquinolone-containing regimen.

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Metronidazole

39

Sun Min Lee

Abstract

Metronidazole is one of the major antibiotics for the eradication of *Helicobacter pylori* (*H. pylori*). The metronidazole resistance rate of *H. pylori* is relatively high compared to other antibiotics. It appears to be different among nations and areas with a tendency of increase. Gene mutations in the genes encoding nitroreductases have been majorly studied as the mechanism of metronidazole resistance. Representative genes related to metronidazole resistance are *rdxA* and *frxA*. The gene mutation patterns appear differently in *H. pylori* strains isolated from patients. The effect of the gene mutations are proven by confirming the induction of resistance with transformation of mutated *rdxA*. Efflux pump system is also involved in the occurrence of resistance. In resistant strains, *hefA*, the gene encoding the efflux pump is highly expressed, and this status results in the inhibition of drug influx. Overall, *hefA* is overexpressed in the early stage of the resistance to inhibit the influx of the medicine; then the mutations in *rdxA* and *frxA* cause the abnormal activity of nitroreductases resulting in reinforced resistance.

Keywords

Antibiotic resistance • *Helicobacter pylori* • Metronidazole

39.1 Introduction

Metronidazole, a nitroheterocyclic compound, is one of the major drugs used for the eradication of *Helicobacter pylori* [1]. This antibiotic is effective in diverse bacterial or protozoal infections including *H. pylori* infections, pelvic inflammatory disease, endocarditis, bacterial vaginosis, dracunculiasis, giardiasis, trichomoniasis, and amebiasis. It is activated through

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reduction by anaerobic microorganisms, so it is majorly used for eradication of anaerobic bacteria or protozoa. The resistance rate of metronidazole which has increased worldwide is higher than those of other drugs and affects the eradication outcome [2–5]. Therefore, it is important to prevent the occurrence of resistance and provide solutions for the resistant bacteria by understanding the mechanisms.

In this chapter, we will discuss the mechanism of action, the present conditions, and mechanisms of metronidazole resistance.

39.2 Epidemiological Aspects of Metronidazole Resistance

39.2.1 The Resistance Rate

Metronidazole-resistant *H. pylori* are found worldwide, and the resistance rate differs according to nations. It is reported that the resistance was relatively low in the USA, Europe, and Japan [6–8]. Some areas in Asia and Middle East have high resistance rates [9–11] (Table 39.1). The metronidazole resistance rate of *H. pylori* is also different in several regions of Korea. As investigated in 2008, the resistance rate was the lowest at Gangwon (10 %, $n=40$), compared with Gyeonggi (25 %, $n=40$) and Busan (19.2 %, $n=19$) (Table 39.2). Two hospitals in Seoul were shown to have high resistance rate of 50–66 %, according to research in 1989, 1994, and 2003 [3].

Studying the changing aspect by year, the resistance rates appeared to increase overall. The tendency was shown in several hospitals in Korea; at Hanyang University Hospital, it increased from 52.9 % to 66.2 % as investigated in 1987 and 2003, and at Samsung Medical Center, it was 33.3–47.7 % examined in 1994 and 1998–1999 [5]. At Seoul National University Bundang Hospital which locates in Gyeonggi province, it was 34.8 % in 2003–2005, 23.8 % in 2006–2008, and 35.8 % in 2009–2013, which were relatively lower [16].

39.2.2 Correlations Between the Resistance and the Eradication Failures

The *H. pylori* eradication rates tend to decrease with high resistance rates [17]. The gap appeared differently according to the eradication therapy; eradication rate declined from 93 % to 19 %, and it retained 94 % in some cases [18, 19]. The difference of eradication rates between resistant strains and susceptible ones was analyzed in 1996–1997, and regimens with eradication rates higher than 90 % were BAM, BTeM, OAM, LCM, OCM, and BTML (A, amoxicillin; B, bismuth citrate; C, clarithromycin; L, lansoprazole; M, metronidazole; O, omeprazole; Te, tetracycline) (Table 39.3). Among them, the eradication rate of resistant strains with the triple therapy OCM was the same with susceptible strains (94 % vs. 94 %) [23]. Also in the case of BTMO, there was no significant difference between susceptible and resistant strains: 81 % and 82 % each [29]. This indicates that metronidazole resistance would be overcome in some cases [18,

Table 39.1 Metronidazole resistance rates of *H. pylori* according to nations (%)

Regions	USA [7]	Europe			Middle East	Asia		
		Northern [8]	Central/ Western [8]	Southern [8]	Israel [13]	Kolkata (India) [10]	Beijing (China) [14]	Japan [6]
Year	1998–2002	2008–2009	2008–2009	2008–2009	2000–2001	2000–2001	2009–2010	2002–2005
Resistance rate (%)	25.1	28.6	43.8	29.7	60.7[a], 38.2[b]	85	61.6	3.3–5.3

Adapted from Lee and Kim [12]

Studied nations, patient enrollment periods, and metronidazole resistance rate (%) (metronidazole resistant if MIC ≥8 μg/mL)

[a]Resistance rate in patients treated with metronidazole

[b]Resistance rate in patients not treated with metronidazole

Table 39.2 Metronidazole resistance rates of *H. pylori* according to areas in Korea (%)

Regions	Seoul [5]					Seoul [3]			Gyeonggi [15]	Gangwon [15]	Busan [15]
Year	1994	1995	1996	1997	1998–1999	1987	1994	2003	2008	2008	2008
Resistance rate (%)	33.3	38.5	42.6	40.6	47.7	52.9	61.1	66.2	25.0	10.0	19.2

Adapted from Lee and Kim [12]
Resistance rate (%) means the rate of patients with resistant *H. pylori* in total enrolled patients

Table 39.3 Difference of eradication rates according to metronidazole resistance

Treatment	Reference	Metronidazole susceptible	Metronidazole resistant
BAM	Logan et al. [20]	93	19
	Burette et al. [21]	90	63
	Rautelin et al. [22]	91	63
BTeM	Noach et al. [19]	96	38
	Lerang et al. [18]	92	79
OAM	Lerang et al. [23]	96	77
	Thijs et al. [24]	95	69
LCM	Harris et al. [25]	92	75
OCM	Lerang et al. [23]	94	94
OCTi	Moayyedi et al. [26]	70	30
BM	DeCross et al. [27]	81	16
BTMO	Van der Hulst et al. [28]	81	82
BTML	Graham et al. [29]	100	41

Adapted from Alarcón et al. [17]
Eradication rate (%) of *H. pylori* isolated from patients
A amoxicillin, *B* bismuth subcitrate, *C* clarithromycin, *L* lansoprazole, *M* metronidazole, *O* omeprazole, *Ti* tinidazole, *Te* tetracycline

22, 24], whereas with BAM or BTeM, the eradication rate fell from 90–96% to 19–79% [18–22]. Also there were great differences in the cases treated with BM (81% vs. 16%) and BTML (100% vs. 41%) [30, 31]. Moreover, a lower eradication rate is associated with sequential treatment which is being suggested as an alternative regimen of the standard triple therapy [32].

The relation with metronidazole resistance and *H. pylori* eradication rate was also confirmed in animal studies. ICR mice were infected with either metronidazole-resistant or metronidazole-susceptible *H. pylori* strains. As a result, the eradication rate was significantly lower in the mice infected with resistant bacteria (Table 39.4). Therefore, the correlation between resistance and eradication failure was confirmed in vivo. However, in this case, only metronidazole was used, not applying the triple therapy or other combinations, so it cannot be considered as the practical therapy conditions. The significant difference in eradication rates according to resistance can be determined with further animal studies with other therapies.

Table 39.4 Change of eradication rate after induction of metronidazole resistance in ICR model

Metronidazole (mg/kg)	Eradication rate (%)[a]	
	Metronidazole susceptible	Metronidazole resistant
100	5/5 (100)	3/5 (60)
32	4/5 (80)	0/5 (0)
10	2/5 (40)	0/5 (0)

Adapted from Matsumoto et al. [30]
Eradication rate (%) of treatment with metronidazole (10, 32, 100 mg/kg) in ICR mice infected with metronidazole-susceptible or metronidazole-resistant *H. pylori*
[a]Eradication rate (%) = (number of mice eradicated)/(total number of mice) × 100

39.3 Principles of Metronidazole Resistance

39.3.1 Mode of Action

Metronidazole is one of the 5-nitroimidazole drugs which are activated as antibiotic metabolites through intracellular reduction. In the reduction process, the nitro group (RNO_2) is reduced to anionic radical ($RNO_2^{\bullet -}$), RNO, and RNHOH (chemical formula 1):

Chemical formula 1.

$$RNO_2 \rightarrow RNO_2^{\bullet -} \rightarrow RNO \rightarrow RNHOH$$

The metabolites disrupt the structure of DNA, by diminishing the helical structure and breaking the strands, and cause apoptosis by inhibiting nucleic acid synthesis [33–35] (Fig. 39.1). Because metronidazole is in low redox potential (−486 mV), it is only reduced in cells with electron donor with lower redox. This makes the medication specifically toxic in anaerobic bacteria. Metronidazole is diffused through cellular membranes, and the inflow is facilitated by decrease of intracellular concentration by the metabolic reactions.

39.3.2 Mechanism of Resistance

The most reliable hypothesis for the resistance mechanism is the obstruction in the reduction process. In this hypothesis, electron transport proteins in *H. pylori* are not expressed because of the mutations encoding the proteins. The reductases are ferredoxin (FdxA), flavodoxin (FldA), ferredoxin-like proteins (FdxB, OorDABC, PorCDAB), NAD(P)H flavin oxidoreductase (FrxA), and oxygen-insensitive NAD(P)H nitroreductase (RdxA). Particularly, *rdxA* and *frxA*, genes encoding RdxA and FrxA, have been considered as causative factors of resistance. As described above, many researches have been performed to understand the mechanism of metronidazole resistance. In this section, studies up to date would be discussed.

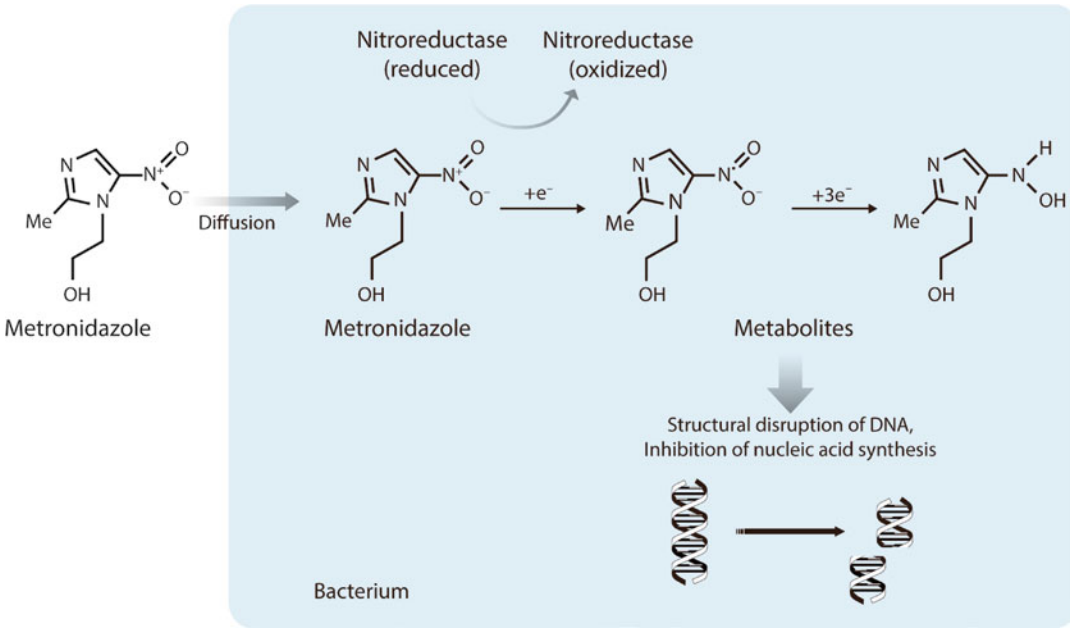

Fig. 39.1 Mode of action of metronidazole (Modified from Mann [35])

39.3.2.1 Mutations in *rdxA* and *frxA*

The pattern of point mutations in *rdxA* appears different in each metronidazole-resistant strain. Thirty strains isolated from patients were compared of *rdxA* sequences, and 122 different point mutations were found [36]. Missense mutations (frameshifts, nucleotide substitution), nonsense mutations (change to stop codon) that prevents normal protein expression, and nucleotide insertions that prevent normal protein expression were all observed. *rdxA* mutations comprised amino acid substitutions compared with other housekeeping genes because *rdxA* frequently has mutations in the first two codons not the third codon (the wobble position), which affects the amino acid identity [36, 37]. Another study analyzed the upstream and whole sequences of *rdxA* and *frxA* in 19 isolated strains comparing according to the metronidazole resistance, to find the random pattern of point mutations. Resistant strains had nonsense mutations or missense mutations. The gene mutations were shown to be different in all strains, and two types of mutations were commonly found in two isolated strains: G to A at position 401 of *rdxA* and G to T at position 624 of *frxA* [37] (Table 39.5). In summary, gene mutations of *rdxA* and *frxA* occur distinctly in different strains and certain genotypes that induce metronidazole resistance are not determined at present.

To prove that *rdxA* and *frxA* mutations cause the metronidazole resistance, it should be demonstrated that the resistance occurs by the inactivation of *rdxA* and *frxA*. Two independent studies confirmed that the metronidazole-susceptible *H. pylori* acquire resistance through transformation experiments [38, 39]. The results proved the role of *rdxA* mutations in the antibiotic resistance. Metronidazole-resistant strains were rendered susceptible by transformation with a functional

Table 39.5 Mutations of *rdxA* and *frxA* genes and RdxA and FrxA proteins in metronidazole-resistant *H. pylori*

		Mutation			
H. pylori strain	MIC (μg/mL)	*rdxA* gene mutation[a]	RdxA amino acid mutation[c]	*frxA* gene mutation[a]	FrxA amino acid mutation[c]
0844	8	G169T	V57F	A104C	E35A
1247	8	G184A	V62I	G624T	K208N
0937	12	G56A	C19Y	G391T	E131stop
0888	16	G223T	E75stop	C394T	E131stop
0871	32	N[b]	N[b]	TT160-1 insertion; A163T	L54F; K55stop
1021	48	G103T	E35stop	T540A	Y180stop
1221	128	C148T	Q50stop	G482T	C161F
				G624T	K208N
1202		T162 deletion	F54L	N[b]	N[b]
		G163T	V55 stop		
1122	128	G401A	S134N	A54 deletion	K18N
				T116A	L39stop
1199	256	A187T	K63stop	T271 deletion	Y91M
				G295T	V99stop
0940	>256	G401A	S134N	C215T	L71F
				A217G	S72G

Adapted from Han et al. [37]

RdxA oxygen-insensitive NAD(P)H nitroreductase, *FrxA* NAD(P)H flavin oxidoreductase, *MIC* minimum inhibitory concentration

[a]Nucleotide A from the first codon (ATG) for FrxA was counted as +1

[b]No specific changes as compared with genes or proteins from metronidazole-susceptible *H. pylori* isolates

[c]The initiation amino acid (methionine) was counted as +1

rdxA gene; conversely, susceptible strains were rendered resistant by *rdxA* inactivation [38]. Expression in *Escherichia coli* of *frxA* made this naturally resistant species metronidazole susceptible, and *frxA* inactivation reinforced metronidazole resistance in cells with no *rdxA* but had little impact on the metronidazole susceptibility of *rdxA*+ bacteria [39]. Overall, it could be concluded that some *rdxA* mutations induce metronidazole resistance, and *frxA* mutations enhance the resistance.

39.3.2.2 Structural Alterations in RdxA

RdxA is composed of two functional homodimers and the RdxA dimer contains two flavo mononucleotide (FMN) cofactors. Each monomer is comprised of 210 amino acids that form eight α-helices and five β-strands [40] (Fig. 39.2). Martínez-Júlvez et al. analyzed the protein structure of RdxA and classified them into five types. The first one is the mutation type that decreases the affinity of apoprotein to FMN cofactors. RdxA normally combines with FMN cofactors by hydrogen bonding, static electricity, and hydrophobic interactions; however mutations in the side chain that combine to FMN may cause weak bonding of RdxA and FMN. This type includes mutations in R16, S18, K20, N73, I142, G162, and K200. The second type is associated with the instability of the RdxA dimer structure. Mutations in the positions of dimer boundaries L42, S43, R41, Q50, V55, M56, I142, G145, K202, and L209 are expected to induce the structural instability. The third type indicates the mutations in the positions around the redox center of FMN. These are suggested to affect the redox function of the enzyme through induction of certain chemical characteristics. Mutations in C19, Y47, and C159 can be included in this type. The fourth type including mutations in G149 and H17 destabilize other protein structures but dimer structures. Lastly, mutations that occurred in A143 and V192 affect the resistance but the mechanism cannot be explained currently. These understandings help us how *rdxA* mutations affect the structure of RdxA and induce the antibiotic resistance [40].

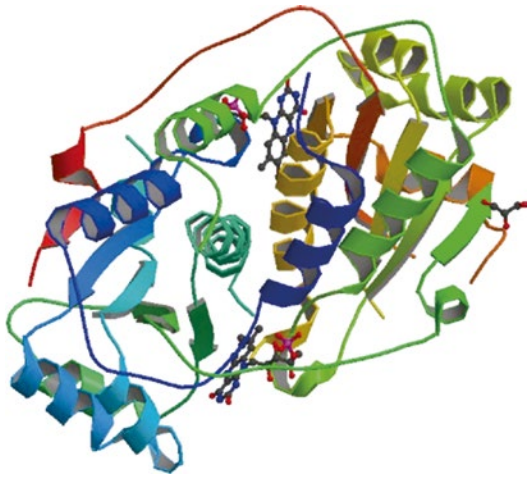

Fig. 39.2 Structure of *H. pylori* RdxA (Image from the RCSB PDB (www.rcsb.org) of PDB ID 3QDL. Adapted from Martínez-Júlvez et al. [40] and Berman et al. [41]). RCSB PDB is a member of worldwide PDB (wwPDB) (www.wwpdb.org) (Adapted from Berman et al. [42])

On the other hand, the process of metronidazole reduction can be expected analyzing structure of RdxA. Distinguishing point of RdxA structure is the cysteine residue; RdxA have six cysteine residues in one subunit, while other nitroreductases contain one cysteine residue in average. These cysteine residues are supposed to be associated with the enzyme-substrate binding. In RdxA, there is a gap called isoalloxazine ring between the Y47 and isoalloxazine of FMN, which is adequate for the enzyme-substrate binding. C184 and C159 locate near the isoalloxazine ring; however, because only C159 targets the isoalloxazine ring, C159 alone is expected to affect the enzyme reaction. It can be predicted that C159 take part in the reduction process of metronidazole by providing a proton to the N1 atom of FMN resulting in the decrease of redox potential of FMN [40] (Fig. 39.3).

39.3.2.3 Other Mechanisms of Resistance

As discussed above, gene mutations have been suggested as the major mechanism of metronidazole resistance. However, possibilities of other

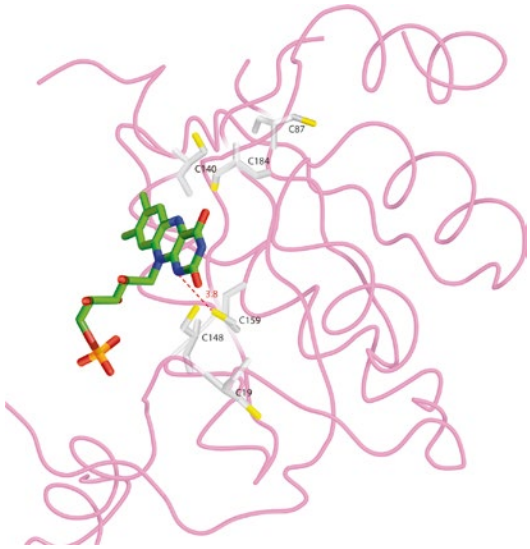

Fig. 39.3 Relative positions of the FMN and six cysteine residues in the RdxA monomer. The cysteine residues (S atoms in *yellow*) and FMN cofactor are described with a stick model. The short distance (*red dotted line*) between the C159 and the isoalloxazine ring is also shown (Modified from Martínez-Júlvez et al. [40])

mechanisms have emerged since resistance strains without specific gene mutations were reported. In the experimental results regarding this issue, 11 among 15 metronidazole-resistant-isolated strains had identical *rdxA* sequence with susceptible ones [43]. Another study reported three among ten isolated strains were found to have metronidazole resistance although they were determined as *rdxA+frxA+* by real-time PCR [44]. These results prove the existence of other mechanisms.

A novel mechanism is the efflux of metronidazole through overexpression of *hefA*, the gene encoding TolC efflux pump. In a research, the transcriptional level of RND efflux pump system was investigated in the early stage of resistance. *H. pylori* were induced to acquire metronidazole resistance through exposure to metronidazole with lower concentration than the minimum inhibitory concentration (MIC). As a result, in the resistant strains, the expression level of TolC efflux pump increased, while there was no significant change in the reduction level of metronidazole. Therefore, TolC efflux pump is expected to be associated with the incidence of metronidazole resistance in *H. pylori* [45].

Conclusions

The metronidazole resistance rate of *H. pylori* is increasing, and sometimes it affects the failure of eradication; therefore it is necessary to control the resistance. Compliance of appropriate treatment is critical to control the resistance because the bacteria acquire metronidazole resistance when they are consistently exposed to the low concentration of the antibiotic.

Through many experimental reports, the overall mechanism of metronidazole resistance can be suggested. At early stage of resistance, overexpression of *hefA* inhibits the influx of metronidazole and then mutations in *rdxA* and *frxA* intensify the resistance. Mutations in *rdxA* and *frxA* are the major factors in the acquisition of metronidazole resistance, so there have been relatively many studies about them. The pattern of *rdxA* mutation appears to be diverse in each resistant strain. Structural analysis suggests that transition of C159 to alanine or serine is one of the important mutations that trigger structural changes. Most studies have expected that there are unknown mechanisms related to metronidazole resistance. Therefore, it is required to find novel pathways of resistance to understand the whole mechanism of metronidazole resistance.

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Part VII

Treatment

Synopsis of Antibiotic Treatment

40

David Y. Graham and Marjan Mohammadi

Abstract

Helicobacter pylori (*H. pylori*) is a common infection responsible for considerable morbidity and mortality worldwide. It is now recommended that all *H. pylori* infections be cured unless there are compelling reasons not to. Like other infectious diseases, the most reliable method to achieve high cure rates is to base therapy on the results of patient-specific susceptibility testing. Empiric therapy must be based on knowledge of the local susceptibility patterns tempered by data obtained from the patient concerning their antibiotic exposure. Data from clinical trials without susceptibility testing cannot be reliably used in other locations. Because of increasing resistance, triple therapies based on clarithromycin, metronidazole, or levofloxacin should only be used for susceptibility-based therapy. If there are no options other than to give empiric therapy, only those regimens proven to be effective locally should be used. Special efforts should be made to enhance patient adherence. In most regions 14-day four-drug combinations are required for successful empiric therapy. Testing to confirm cure should be routinely done.

Keywords

Helicobacter pylori • Therapy • Susceptibility-based therapy • Patient compliance

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40.1 Introduction

Although *Helicobacter pylori* (*H. pylori*) infections can result in peptic ulcer disease or gastric cancer and are one of the most common chronic bacterial infections of mankind, therapy remains a problem especially compared to other common infectious diseases. Treatment for most bacterial infections is based on knowledge of

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local antimicrobial susceptibility complemented by susceptibility testing of the organism infecting that particular patient. In contrast, *H. pylori* therapy is most often chosen empirically without specific knowledge of the local susceptibility patterns or an attempt to culture the organism from the infected patient. Worldwide the success rates of anti-*H. pylori* therapy are often poor and significantly inferior to what would be achieved by patient-specific susceptibility-based therapy [1, 2]. This is difficult to understand in that *H. pylori* can be cultured in vitro and any hospital that has provisions for culture and susceptibility testing should be able to provide this service. Despite the widespread availability of bacterial culture for other common pathogens, it is often impossible for the average or even the exceptional clinician to obtain culture and susceptibility testing for *H. pylori*.

H. pylori cannot be cultured from blood, sputum, or urine. The effort required for obtaining an appropriate specimen for culture is not an insurmountable barrier as cultures can be obtained using a gastric biopsy, fluid from a gastric aspirate, or gastric mucus obtained by tube or brush. Possibly because specimens for culture were not easily available, the outcome of the infection was not immediately demonstrable, and the initial focus was on peptic ulcer disease, the infectious disease community abrogated responsibility for the diagnosis, treatment, and development of treatment regimens of the infection to gastroenterologists. Unfortunately, gastroenterologists had no experience in, or history of, successful development of antimicrobial therapies and have continued to foster a trial-and-error approach. The general lack of susceptibility data to accompany treatment trials resulted in delay in understanding the reason for apparent success or failure [3]. Investigators blamed treatment failures on the presence of the presentation (e.g., non-ulcer dyspepsia vs. peptic ulcer) or the presence of *H. pylori* virulence factors such as CagA or the patient (e.g., smoking). In retrospect, when susceptibility data became available, the vast majority of these alternate hypotheses had to be discarded as most proved to be surrogates for differences in antibiotic use. The problem was worsened in that some gastroenterology journals focused on comparative studies that attempted to demonstrate superiority of one regimen over another. This was done despite knowledge that in the presence of susceptible infections, the regimens being compared were all highly effective and essentially equivalent. Many meta-analyses were done but could provide no additional insights as they generally ignored the fact that any differences detected were the results of differences in resistance patterns and not in the regimens [1]. The results were often presented as differences in relative risks which could report whether the regimes were similar or different but ignored whether the overall results were unacceptably low [1, 4]. This led the authors to conclude that increasing the duration of therapy did not improve the outcome of triple therapy when this question could not be addressed by the studies which had unacceptably low cure rates because of the presence of resistance or that one regimen was superior to another despite both achieving unacceptably low success rates [5]. Only after approximately 30 years of examining different therapies in thousands of patients was sufficient data accumulated regarding the effectiveness of different regimes in relation to the presence of resistance to allow rational susceptibility-based therapeutic decisions to be made and effectiveness to be estimated based on local susceptibility data [1, 3, 6].

40.2 Why Are *H. pylori* Infections So Difficult to Cure?

H. pylori occupy a number of different microenvironments within the stomach that vary from residing within the highly acidic mucus on the gastric surface to being attached to epithelial cells and forming a biofilm to residing within gastric epithelial cells. Each microenvironment offers different challenges for effective antimicrobial therapy. The intragastric milieu also experiences a wide range of changes in pH, volume, and composition

throughout the day and with each meal. On average, most *H. pylori* appear to live in an environment where the average pH is approximately 3.5 [7–9]. At this pH they do not replicate. Replication occurs at about pH 6–7, such that many organisms are in environments where the bacteria are resistant to antimicrobials effective only during cell division [10]. In addition, the very large number of bacteria in the stomach essentially ensuring that small populations of resistant strains will be present for those antibiotics in which the spontaneous rate of mutations in the genes responsible for resistance is relatively high [10–12].

Multidrug therapy is generally required to reach the organism in its different microenvironments and to prevent the development of resistance [11]. Because few antimicrobials are effective at low pH, effective therapy requires that gastric acid secretion be inhibited which also reduces dilution of the orally administered and topically active agents. Increasing the local pH to 6 will also encourage the organisms to divide and become susceptible to killing with agents such as amoxicillin which are effective only for replicating organisms.

While the trial-and-error strategy of antimicrobial therapy has resulted in identification of a number of effective regimens, no single regimen has been proven to be effective in all areas or all patients. The major barrier has been the rapid development of resistance related to the general overuse of antibiotics worldwide. The lack of detailed knowledge of resistance patterns has resulted in regimens continuing to being recommended long after they have become ineffective [13–15].

40.3 Causes of Treatment Failure

The three most common reasons for treatment failure relate to problems with the organism (i.e., antimicrobial resistance), with the clinician (i.e., poor choice of drugs, doses, number of drug administrations, or treatment duration), or with the patient (i.e., failure to complete the regimen as prescribed due to side effects or failure to understand the importance of completing therapy).

40.4 Evidence-Based Therapy Is Susceptibility-Based Therapy

The frequent failure to link susceptibility data with the results of clinical trials resulted in the lack of data needed to understand the strengths and weakness of many treatment regimens [1]. As noted previously, many decades of the trial-and-error approach were needed before sufficient susceptibility data was acquired to allow clinicians to understand why therapies succeeded or failed and prospectively predict which regimen would most likely be effective for an individual patient, region, or society. A number of multidrug regimens are now available that will reliably cure 95 % or more of compliant patients with susceptible infections [1] (Table 40.1).

The most commonly used anti-*H. pylori* antimicrobials worldwide are amoxicillin, clarithromycin, metronidazole (or tinidazole), tetracycline hydrochloride, one of the new generation fluoroquinolones (e.g., levofloxacin), furazolidone, a bismuth salt, and rifabutin. These are generally prescribed with a proton pump inhibitor (PPI). Resistance does not develop to bismuth and rarely develops to amoxicillin, tetracycline, or furazolidone. Rifabutin is rarely used clinically except in the treatment of tuberculosis and thus rates of resistance in the general population are still low. In contrast, resistance to clarithromycin and fluoroquinolones has increased to where in most regions neither should be used as an empiric therapy. Metronidazole resistance is high in most of the world except Japan and is unique in that the presence of resistance as identified by in vitro testing is not an "all or none" phenomenon and can be partially overcome by increasing the dose and duration of therapy [16, 17]. However, cure rates greater than 75 % with resistant infections are rare except when given as part of four-drug bismuth-containing therapies. In this special situation, metronidazole resistance can often be completely overcome (see below) [17].

Failed therapy typically results in development of resistance with clarithromycin, metronidazole, fluoroquinolones, and probably rifabutin. This most likely represents selection of

Table 40.1 Recommended regimens for *Helicobacter pylori* eradication therapy

Treatment	Drugs, dosages, and duration
Empiric therapies	
Concomitant therapy	Amoxicillin (1 g), clarithromycin (500 mg), and tinidazole (500 mg) or metronidazole (500 mg) plus a PPI (40 mg omeprazole equivalent per dose) all given twice daily for 14 days
Sequential therapy (not recommended as concomitant is superior)	Amoxicillin (1 g) plus a PPI twice daily for 7 days, followed by clarithromycin (500 mg) and tinidazole (500 mg) or metronidazole (500 mg) plus a PPI (40 mg omeprazole equivalent per dose) all twice daily for a further 7 days (total 14 days)
Sequential-concomitant hybrid therapy	Amoxicillin (1 g) plus a PPI twice daily (40 mg omeprazole equivalent per dose) for 7 days, followed by amoxicillin (1 g), clarithromycin (500 mg) and tinidazole (500 mg) or metronidazole (500 mg) for a further 7 days (total 14 days)
Bismuth quadruple therapy	Bismuth subsalicylate or bismuth subcitrate 2 tablets and tetracycline hydrochloride (500 mg) both four times daily with meals and at bedtime plus metronidazole/tinidazole (500 mg) three times daily with meals and a PPI twice daily for 14 days
For prepackaged bismuth quadruple therapy	PYLERA® for 14 days, add a PPI b.i.d. (40 mg omeprazole equivalent per dose)
Susceptibility-based therapies	
Triple therapy when *H. pylori* infection is known to be susceptible to clarithromycin	Amoxicillin (1 g) and either clarithromycin (500 mg) or tinidazole (500 mg) or metronidazole (500 mg) plus a PPI all given twice daily for 14 days (40 mg omeprazole equivalent per dose)
Fluoroquinolone therapy when *H. pylori* is known to be susceptible to fluoroquinolones	Fluoroquinolone (e.g., levofloxacin 500 mg once daily), plus a PPI and amoxicillin 1 g twice daily for 14 days (40 mg omeprazole equivalent per dose)
Empiric salvage therapy	
Furazolidone quadruple therapy: with or without tetracycline	1. Bismuth subsalicylate or bismuth subcitrate 2 tablets and tetracycline hydrochloride (500 mg) both four times daily with meals and at bedtime plus furazolidone 100 mg t.i.d., with means and PPI twice daily for 10–14 days
	2. Bismuth subsalicylate or bismuth subcitrate 2 four times daily with meals and at bedtime plus furazolidone 100 mg and amoxicillin 1 g t.i.d., with meals plus a PPI twice daily for 10–14 days
Rifabutin triple therapy	Rifabutin (150 mg daily), amoxicillin (1.5 g q.8.h.), and pantoprazole 80 mg (or an equivalent PPI) q.8.h. for 12–14 days
High-dose PPI-amoxicillin dual therapy	PPI (e.g., rabeprazole 20 mg, esomeprazole 40 mg) plus amoxicillin (500 mg or 750 mg) all four times daily at approximately 6 h intervals for 14 days

a preexisting minor population. However, resistance to amoxicillin, tetracycline, or furazolidone occur rarely following treatment failure, and these drugs can often be used again successfully.

40.5 General "Rules" for Choosing a Regimen

As with other infectious diseases, susceptibility-based therapy yields the best outcomes [1]. With essentially all regimens, the cure rate is best when the drugs are administered for 14 days and the PPI is given as a double dose (e.g., 40 mg of omeprazole or equivalent at least twice a day) [3, 6, 18]. Exceptions are discussed under each regimen. Single-drug and dual-drug therapies, with the exception of PPI plus amoxicillin dual therapy, have proven to provide poor cure rates and are not recommended.

40.6 *H. pylori* Therapies

40.6.1 Triple Therapies

The basic regimen consists of a PPI (e.g., 40 mg omeprazole or equivalent), amoxicillin (1 g), and a third drug (e.g., clarithromycin, metronidazole, or levofloxacin) twice a day for 14 days (Table 40.1). Shorter duration regimens provide lower cure rates with clarithromycin or metronidazole and typically fail to achieve even 90 % cures with fluoroquinolones. For infections with strains susceptible to the antimicrobials chosen, one can anticipate that the regimen will reliably cure >90 %, typically >95 % of infections in patients adherent with the treatment [1]. Resistance to clarithromycin and fluoroquinolones cannot be overcome by increasing the dose or duration of therapy (i.e., it is all or none). Those patients with resistant infections effectively receive only two of the three drugs (e.g., clarithromycin is eliminated) thus receiving only dual therapy with PPI plus amoxicillin. At the doses used in triple therapy, this dual therapy will cure less than 50 % of patients. In western countries, where rapid PPI metabolizers are frequent, cure rates generally fall to less than 20 % (see Sect. 40.6.4). As noted previously, the cure rate with PPI, amoxicillin, and metronidazole triple therapy for 14 days with infections resistant to metronidazole can be as high as 75 %, which is however unacceptable [16]. Triple therapies are now obsolete as empiric therapies. However, triple therapies can still remain excellent choices for use as susceptibility-based therapy.

40.6.2 Four-Drug Therapies

40.6.2.1 Bismuth Quadruple Therapy

The original four-drug therapy was the combination of a PPI, a bismuth, tetracycline HCl, and metronidazole (often called quadruple therapy or bismuth quadruple therapy) [19] (Table 40.1). Cure rates of >90 %, often >95 %, are expected when given for 14 days with good adherence. At least 50 % of patients will experience side effects (e.g., nausea, abdominal pain, etc.), and poor adherence has often been the most important factor limiting its effectiveness [19]. As will be discussed below, the time invested in patient education, regarding side effects to expect and the importance of completing therapy is time well spent. This regimen has proven to be highly effective despite metronidazole resistance, because resistance can often be overcome provided one use full-dose metronidazole (e.g., 1,500 or 1,600 mg) and 14-day therapy [17, 19]. The regimen is complex requiring multiple pills be given three or four times a day. Attempts to simplify this by packaging in blister packs and by combining several drugs into single capsules have been done, but there are as yet no convincing data that this has translated into either improved compliance or outcome.

This regimen is often a first choice in patients unable to take penicillin or in areas where clarithromycin and metronidazole resistance preclude use of empiric triple therapies. If the patient can take penicillin and metronidazole resistance is not present, there is no reason not to use a

simpler and better tolerated triple therapy. Bismuth quadruple therapy is also the first choice for retreatment (e.g., second-line therapy) following treatment failure with a regimen containing different antimicrobials.

Bismuth quadruple therapy is the least well-studied regimen in terms of comparing outcome with susceptibility and with compliance [19]. It can likely be simplified (e.g., twice a day bismuth instead of four times a day). Clearly, a well thought out multi-region development program is needed to best understand and optimally utilize this regimen.

Alternate regimens based on bismuth quadruple therapy include replacing metronidazole with furazolidone (100 mg t.i.d.) or tetracycline with amoxicillin (1 g t.i.d.) [20, 21].

40.6.2.2 Bismuth Furazolidone Quadruple Therapy

Although furazolidone is no longer widely available, it remains available in many regions where multidrug-resistant *H. pylori* are common such as in China and the Middle East. Furazolidone-tetracycline or furazolidone-amoxicillin quadruple therapies have proven to be highly effective despite the presence of *H. pylori* resistant to clarithromycin, metronidazole, and fluoroquinolones [20, 21]. The keys to successful therapy include 14-day therapy and full-dose tetracycline and furazolidone (Table 40.2). Furazolidone is a monoamine oxidase inhibitor (MAO inhibitor) and interacts with many drugs and foods (e.g., soy sauce, aged cheese, etc.) [22]. Side effects are common and patient instructions regarding food avoidances are needed [22]. Nonetheless, when given for 14 days, treatment failures are rare making it the *piece de resistance* for therapy after multiple treatment failures in motivated patients.

Table 40.2 Recommendations

1. Use susceptibility-based therapy whenever possible (based on culture or molecular methods)
2. Avoid clarithromycin, metronidazole, or fluoroquinolone-containing triple therapies unless the infection is proven to be susceptible to the antibiotics given
3. Empiric therapy should generally consist of a four-drug regimen
4. Therapy should be administered for 14 days
5. Patients must be educated about the importance of completing therapy and the nature of expected side effects
6. Confirm of cure should follow treatment, preferably with a locally validated noninvasive test such as a urea breath test (at least 4 weeks after) or stool antigen test (at least 6 weeks after the end of therapy)

40.6.3 Non-bismuth Quadruple Therapies

This group consists of the combination of a PPI, amoxicillin, metronidazole, and clarithromycin given together or sequentially (concomitant therapy, sequential therapy, and hybrid sequential-concomitant therapy) [3, 6, 23, 24] (Table 40.1). Optimal duration is 14 days.

40.6.3.1 Sequential Therapy

Sequential therapy has become an obsolete regimen that was devised to overcome clarithromycin resistance [3, 6]. It consists of dual PPI-amoxicillin therapy followed by PPI-clarithromycin-metronidazole triple therapy. Originally it was devised as a 5 + 5 or 10-day therapy and subsequently optimized as 7 + 7 or 14-day therapy [25]. Its development is the poster child of how the trial-and-error methods failed as an effective means of developing a new therapeutic regimen [25]. Initially, sequential therapy appeared effective and that illusion was maintained by the primary emphasis focusing on showing it to be superior to triple therapy in regions where clarithromycin resistance had been proven to make triple therapy ineffective. Unfortunately, it was not recognized that the low prevalence of metronidazole resistance in the same regions hid the fact that the regimen was undermined by metronidazole resistance. The failure to routinely include susceptibility testing led to repetitive experiments in the same population showing that indeed sequential therapy was superior to triple therapy (which was why it was originally developed). However, when used in Southern Italy or in other regions, it often failed to provide acceptable cure rates [19]. Only then was it discovered that it was markedly less effective in the presence of metro-

nidazole resistance. Sequential therapy is now considered an obsolete regimen and has been replaced by concomitant therapy.

40.6.3.2 Concomitant Therapy

Concomitant therapy is fundamentally identical to giving clarithromycin triple therapy and metronidazole triple therapy simultaneously. It is therefore effective in the presence of either clarithromycin or metronidazole resistance but becomes ineffective in the presence of dual clarithromycin-metronidazole resistance, which in most regions is rare. Treatment success is also duration dependent and is optimal with 14-day therapy [3, 6, 23, 24]. Concomitant therapy is widely used as an empiric therapy; however, if susceptibility data showed the infection was sensitive then a triple therapy would be more appropriate. One can estimate the prevalence of dual clarithromycin-metronidazole resistance by multiplying the known prevalence of each resistance rate. For example, in the Houston population, clarithromycin resistance and metronidazole resistance are approximately 15% and 25%, respectively, which precludes the use of either clarithromycin or metronidazole triple therapies. However, the prevalence of dual resistance is less than 5%, making empiric concomitant therapy an excellent empiric choice for treatment-naïve patients [1, 3, 6].

40.6.3.3 Hybrid and Reverse Hybrid Therapy

Hybrid therapy is a combination of sequential and concomitant therapy in which dual PPI-amoxicillin therapy is followed by all four drugs (PPI-amoxicillin-clarithromycin-metronidazole) as a 7+7 or 14-day therapy [26]. Reverse hybrid therapy reverses the order such that a 7-day concomitant regimen was followed by 7-day dual therapy. Both can be thought of as a 7-day concomitant therapy with either a dual-therapy lead-in or tail. This regimen has not yet been evaluated in areas with high rates of either clarithromycin or metronidazole resistance, but theoretically it is more complex than concomitant therapy, contains the same drugs, and would be used only as an empiric regimen. As such, pending more studies, we believe that concomitant therapy is the currently preferred choice.

40.6.4 PPI-Amoxicillin High-Dose Dual Therapy

As noted above, if the intragastric pH can be maintained reliably at 6 or greater, it is theoretically possible to overcome dormancy, encouraging all *H. pylori* to replicate which makes them highly susceptible to amoxicillin. The pharmacokinetics of amoxicillin suggest that if given every 6 h (a maximum of 8 h is allowed at night), amoxicillin will be present whenever the organism becomes susceptible making it possible to achieve >90 cure rates with 14-day dual therapy [27]. Dual therapy was of great interest in the early to mid-1990s but eventually failed in that reliable cures above 80% could not be achieved. The importance of pH control has become increasingly well documented [27]. The effectiveness of this approach has been repeatedly demonstrated in Asia where a high proportion of the population consists of slow or moderate PPI metabolizers (e.g., related to CYP2C19 polymorphisms), plus a high prevalence of corpus gastritis, both of which promote high pH with PPI therapy [28, 29]. Achieving this high pH consistently has generally required every 6 h PPI and amoxicillin administration and is improved by the use of PPIs that are not highly metabolized by the CYP2C19 pathway (e.g., rabeprazole or esomeprazole) or by a new selective potassium-competitive acid blocker. Higher doses of more rapidly metabolized PPIs are needed than when using slowly metabolized PPIs. In this regimen amoxicillin is given at 500 or 750 mg every 6 h. However, this has not yet proven to be a reliable strategy in the west where rapid and ultrarapid PPI metabolizers are common, but has not been evaluated in most western populations using the every 6 h strategy. The duration of 14 days has proven to be best. It is also recommended that acid food and drinks (e.g., soft drinks) be prohibited during treatment [30]. If reliable pH control can be ensured with the new selective potassium-competitive acid blocker, it may be possible to reduce the frequency of administration of amoxicillin to twice or three times a day.

40.7 Patient Education to Enhance Adherence

Noncompliance with recommended drug therapy has been a recognized issue impeding successful treatment since the time of Hippocrates. Recently, it has been suggested that the term "compliance," which implies obedience, be replaced with "adherence" to reflect the active participation of both the doctor and patient in their commitment to the therapy chosen. Adherence is an especially important problem with long-term therapies for chronic conditions such as treatment of HIV infections or tuberculosis. Most *H. pylori* eradication therapies are short (i.e., 1 or 2 weeks) but are frequently associated with minor but noticeable side effects. Poor adherence is one of the major causes of low eradiation rates and thus an important problem. The considerable literature regarding adherence focuses on features such as the doctor-patient relationship (i.e., trust), the importance of education about the goals and benefits of therapy, and the adverse effects of treatment failure (e.g., risk of gastric cancer, recurrence of peptic ulcer, etc.) [31–34]. Factors that have proven important in predicting reduced adherence include the complexity of the regimen (e.g., the number of medications, dosing intervals, duration of therapy, etc.), the expected side effects (e.g., bad taste in the mouth with clarithromycin), and the importance of tailoring the education about the regimens to the age, sex, ethnicity, educational level, cultural, and socioeconomic background of the individual patient [31–34].

There have been a few *H. pylori*-specific studies including some randomized controlled trial. Based on the successful program used by de Boer when working with bismuth quadruple therapy [35], we recommend establishing a trust-based doctor-patient relationship including:

(a) Taking a detailed medical history and adequate time for follow-up visits
(b) Explaining in simplistic terms the effects of the infection on the stomach, the potential outcomes of the infection, and how cure of the infection results in healing of the damage and prevention of ulcers and ulcer recurrences and reducing or eliminating the risk of gastric cancer
(c) Providing a description of the complexities of the regimen chosen, the necessity for adherence to the treatment schedule, and completing the regimen, including a commitment to try
(d) Providing a clear description of the medications and plan for dosing provided in writing and, if possible, providing appropriate containers (pill boxes or blister packs) arranged according to the dosing plans in relation to meals and bedtime
(e) Providing a description of the adverse effects, such as feeling unwell (nausea, headaches, taste disturbances, loose stools, etc.), which is an expected consequence of treatment

Adherence should be monitored by discussion and by counting the remaining pills, which are asked to be returned by the patient at the end of the treatment.

It has not been clearly defined what rate of treatment completion defines compliance, and it most likely varies according to the type of regimen chosen as well as the individual and/or population rate of antibiotic resistance. In a previous study, once the cutoff for completion of the prescribed BMT (bismuth subsalicylate, metronidazole, and tetracycline) triple therapy was set at 60 %, 96 % of those having taken their medications at above this rate yielded successful *H. pylori* eradication vs. only 69 % of the rest [36]. Lee and colleagues [37] performed a randomized controlled trial using this cutoff to evaluate an enhanced compliance program in which 125 patients received 2 weeks of bismuth metronidazole tetracycline triple therapy. The program included pharmacist counseling and written information on the infection, the effect of eradication, follow-up phone calls, and supplied medication calendars and pillboxes and their instructions of use. Those enrolled in the adherence program had a slight improvement in adherence defined as completing greater than 60 % of their treatment compared to controls (e.g., 95 % vs. 89 %). However, raising the cutoff point to 90 % resulted in a statistically

significant difference in eradication rates (i.e., 89 % vs. 67 %, $p < 0.01$). Importantly, 20 % refused to participate.

Another randomized trial included 76 patients receiving non-bismuth-based (lansoprazole, amoxicillin, and clarithromycin) triple therapies, half of whom received counseling, written educational leaflets, compliance charts, and follow-ups [34, 37, 38]. Adherence to treatment occurred in 92.1 % of those receiving training vs. 23.7 % in controls, when the cutoff point was set at 100 %. *H. pylori* eradication rates were 94.7 % vs. 73.7 %, respectively, favoring training where 100 % of those with successful eradication had taken more than 65 % of their medications. Pharmacoeconomic analysis deemed the intervention cost-effective. Collectively, patient counseling and follow-up represent time well spent in relation to treatment success.

40.8 Recommendations

The most reliable method and highest cure rates are obtained by basing therapy on the results of patient-specific susceptibility testing (Table 40.2). All empiric therapy must be based on knowledge of the local susceptibility patterns tempered by data obtained from the patient concerning their antibiotic exposure. Clinical trials without provisions for susceptibility testing or without stopping rules to limit exposure to poor regimens should not be done. Without susceptibility testing, the trial fails to provide useful information except for the population treated in the trial, and without stopping rules, the exposure to risk would be excessive. If there are no options other than to give empiric therapy, only those regimens proven to be effective locally should be used.

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Triple Therapy

41

Ju Yup Lee

Abstract

Triple therapy has long been used as the first-line therapy for *Helicobacter pylori* (*H. pylori*) infection, but its efficacy has been continuously decreasing over the past 10 years. The main cause of decrease in the *H. pylori* eradication rate of conventional triple therapy is increasing resistance of *H. pylori* to clarithromycin. The *H. pylori*-associated factors affecting the eradication rate of conventional triple therapy are antibiotic resistance, colonization density of *H. pylori* in the gastric mucosal membrane, and virulence factors of *cagA* and *vacA*. Host factors are medication adherence, excess gastric acid secretion, cytochrome P450 gene polymorphism (CYP2C19), treatment duration, drug side effects, underlying gastroduodenal disease, gastritis pattern, smoking, diabetes, obesity, etc. Due to decrease in the *H. pylori* eradication rate of the triple therapy, the current standard first-line treatment for *H. pylori* infection, it has become necessary to develop a new first-line treatment capable of increasing the *H. pylori* eradication rate.

Keywords

Triple therapy • Clarithromycin • Amoxicillin • Resistance

41.1 Introduction

Triple therapy has been considered worldwide the standard first-line treatment for *Helicobacter pylori* (*H. pylori*) infection. After the first presentation of the proton pump inhibitor (PPI)-based triple therapy using the combination of two antibiotics (amoxicillin and clarithromycin) in 1997 by the European Helicobacter Study Group in Maastricht as an *H. pylori* eradication therapy [1], it has been used worldwide, including the

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USA and Europe, as the first-line *H. pylori* eradication therapy. In South Korea as well, triple therapy has been recommended as the first-line treatment for *H. pylori* eradication therapy as the 1998 official recommendation [2, 3]. Also, the latest revised version (2013) of the guidelines specifies that in the current situation in which no therapy better than the conventional triple therapy has yet been developed, the conventional triple therapy is recommended as the standard first-line treatment [4]. However, the recent trend of decrease in the eradication rate with the standard triple therapy due to increasing antibiotic resistance necessitates urgent countermeasures [5]. This work is intended to investigate the eradication rate change patterns of conventional triple therapy, reasons for declining treatment success, and factors influencing the eradication rate of triple therapy.

41.2 Current Status of the Eradication Rates of Standard Triple Therapy

The eradication rates of triple therapy in its earlier years were recognized to be high, ranging between 80 and 90 %. However, with increasing antibiotic resistance, its eradication rate has been continuously decreasing. According to the previous reports, including meta-analyses, only ~18 % exceed the eradication rate of 85 % on an intention-to-treat (ITT) analysis, with ~60 % falling short of 80 % [6]. As such, in Western Europe, standard triple therapy is by now considered "legacy therapy" [6]. In fact, recent European guidelines recommend it as the first-line treatment only when the prevalence of clarithromycin resistance is under 20 % [7].

The situation is not different in South Korea. A study reviewing 19 Korean domestic papers between 2005 and 2013 reported that the eradication rate of standard triple therapy was 90 % or higher in 1997–1998 but kept decreasing afterward, reaching the level of under 80 % in 2009 and 75.8 % in 2012 [8]. In contrast, other studies reported that no great differences were observed in the eradication rate of standard triple therapy for the past 5–11 years [9–11]. Such discrepancies are considered to be attributable to the regional differences in antibiotic resistance or different definition criteria for eradication [9–14]. Taking all these regional differences into consideration, a meta-analysis was performed with a total of 104 studies (38 randomized studies and 66 observational studies) published in South Korea between 1998 and 2013, including abstracts, on standard triple therapy in first-line treatment covering 42,124 patients. The eradication rate on an ITT analysis was 74.6 % (95 % confidence interval [CI], 72.1–77.2 %) and on a per-protocol (PP) analysis 82.0 % (95 % CI, 80.8–83.2 %), with both analyses showing a significantly decreasing trend over 16 years from 1998 to 2013 (Fig. 41.1). Additionally, the eradication rates of 7- and 14-day triple regimens were 81.1 and 85.3 % on a PP analysis [12].

From these results, it may be concluded that despite regional differences, the overall trend clearly demonstrate that the efficacy of standard triple therapy has been decreasing in South Korea, which suggests the necessity for establishing a new efficient standard treatment.

41.3 Factors Influencing the Eradication Rate of Standard Triple Therapy

With the eradication rate of standard triple therapy decreasing, many research efforts have been put to identify the reasons for this phenomenon. Factors that may affect eradication rate can be largely categorized into *H. pylori* factors and host factors. Table 41.1 lists the factors influencing eradication rate identified so far [15]. Of them, the factor reported to be the main cause of eradication failure is antibiotic resistance, clarithromycin resistance in particular [16–20].

41.3.1 *H. pylori* Factors

41.3.1.1 Antibiotic Resistance

For the past two decades, clarithromycin has been used to treat bacterial infections affecting the

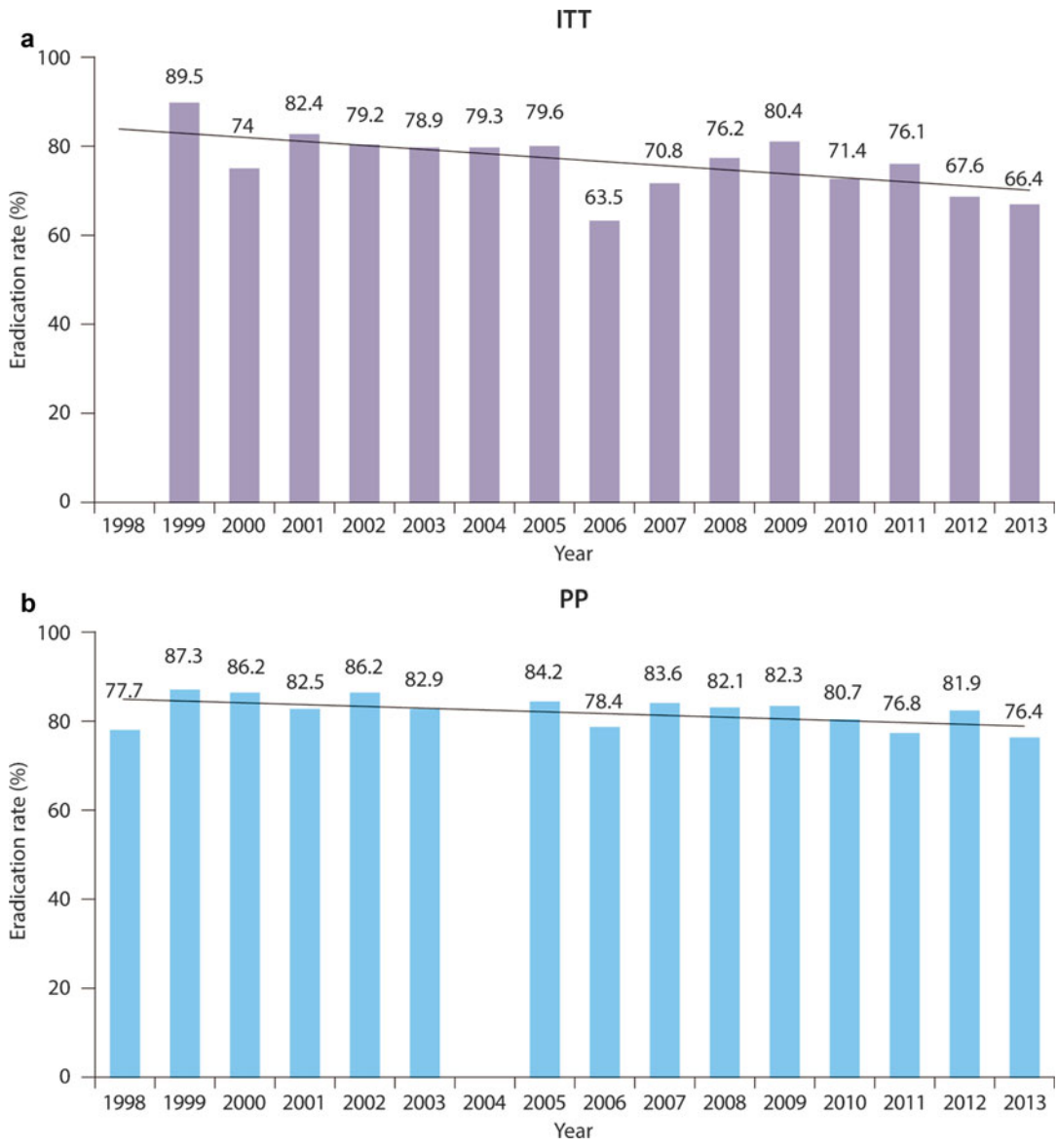

Fig. 41.1 Latest 16-year trends of eradication rate of standard triple therapy as reported in a meta-analysis in South Korea. On both ITT analysis (**a**) and PP analysis (**b**), significant decreases in eradication rate are observed ($p < 0.001$ for ITT, $p = 0.003$ for PP). *ITT* intention to treat, *PP* per protocol (Modified from Gong et al. [12])

respiratory system, and the decrease in eradication rate due to increasing prevalence of clarithromycin resistance of *H. pylori* has been reported globally. As such, clarithromycin resistance is the main cause of eradication failure [16–20].

Despite regional differences, many regions in the USA, Europe, and advanced countries in Asia exhibit high clarithromycin resistance of 20 % or higher. In particular, central, western, and southern European countries exhibit clarithromycin resistance rates exceeding 20 % nationwide [21]. A multicenter study in Japan also reported the clarithromycin resistance rate in 2002 to be 18.9 %, which rapidly increased to 27.2 % in 2006 [22]. The European average amoxicillin resistance rate is reported to be lower than 2 %

Table 41.1 Factors influence the *H. pylori* eradication therapy

Host factors	*H. pylori* factors
Medication adherence	Antibiotic resistance
Excess gastric acid secretion	Density of *H. pylori* in the stomach
Cytochrome P450 gene polymorphism	*cagA* status
Treatment duration	*vacA* alleles status
Drug side effects	*dupA* status
Underlying gastroduodenal disease (PUD vs. NUD)	Bacterial coccoid forms
Gastritis pattern (antral vs. pangastritis)	
Smoking	
Diabetics	
Obesity	

Adapted from Zullo et al. [15]
H. pylori Helicobacter pylori, *PUD* peptic ulcer disease, *NUD* non-ulcer dyspepsia

[23–25], while those in Africa, Asia, and Latin America are reported to be ranging between 6 and 59 % [26, 27]. The *H. pylori* resistance rates toward primary antibiotics since 2000 was over 20 % for clarithromycin, over 40 % for metronidazole, and over 10 % for quinolone in South Korea [28]. Looking more closely at the clarithromycin resistance rates in South Korea, the strains isolated in Hanyang University Hospital and Seoul National University Hospital in 1987 and 1994 were as low as 0 % and 2.8 %, respectively, which rapidly increased to 13.8 % in 2003 [29]. The resistance rates of strains isolated in Seoul National University Bundang Hospital, which was opened in 2003, rapidly increased from 22.9 % (2003–2005) to 25.5 % (2006–2008) and 37.0 % (2009–2012) [30]. As presented above, the antibiotic resistance of *H. pylori* strains, especially clarithromycin-resistant strains are continuously increasing, acting as the major cause of failure of *H. pylori* eradication with standard triple therapy.

41.3.1.2 Other *H. pylori* Factors

High colonization density of *H. pylori* in the gastric mucosal membrane is associated with decrease in eradication rate [31–33], and coccoid form *H. pylori* can also decrease eradication rate due to its low antimicrobial susceptibility [34, 35]. Eradication rate is also affected by the *H. pylori* virulence factor *cagA*. A meta-analysis estimated that the eradication rate in the *cagA*-negative *H. pylori* is lower than that in the *cagA*-positive *H. pylori* by 11 % (95 % CI, 3–19 %, $p = 0.011$) [36]. Moreover, *vacA* s1m1 allele is known to have higher antimicrobial susceptibility than *vacA* s2m2 allele [37]. Shiota et al. [38] reported that another virulence factor called duodenal ulcer promoting gene (*dupA*) is an independent risk factor for eradication failure.

41.3.2 Host Factors

41.3.2.1 Medication Adherence

A low medication adherence of the patient is one of the important factors for eradication failure because the maximum efficacy of antibiotics relies on the administration of adequate dose for an optimal period of time [39]. Since triple therapy involves the concurrent medication of three different drugs, medication adherence rate tends to fall due to more frequent occurrence of side effects. Graham et al. [6] reported that side effects compel about 12 % of patients to stop medication [6]. Therefore, physicians should provide patients with detailed information about side effects that may occur during medication and recommend the continuation of medication in case of mild side effects. Meta-analyses estimated the patient compliance rate of standard triple therapy at 94–96.8 %, which is very high [40, 41]. If patient compliance rate is lower than 80 %, eradication is very likely to fail, and the prevalence of antibiotic resistance is also likely to increase accordingly [42].

41.3.2.2 Excess Gastric Acid Secretion

Gastric acidity regulation is an important factor for *H. pylori* eradication because insufficient inhibitory regulation of gastric acid destabilizes antibiotics such as clarithromycin or amoxicillin, thereby decreasing their eradication rates. An adequate inhibition of gastric acid being an important factor for eradication success, CYP2C19 genotype can be a determinant factor for the success in eradica-

tion in the PPI-based standard triple therapy. PPI is known to be usually metabolized by CYP2C19 via the hepatic cytochrome p450 system [43] (Fig. 41.2). CYP2C19 is classified into extensive metabolizer (EM), intermediate metabolizer (IM), and poor metabolizer (PM) depending on gene polymorphism. PMs are known to have about 20-fold bioavailability compared to EMs owing to extremely slow PPI metabolism [44]. Asians and Caucasians have remarkably different CYP2C19 genotypes, and PM accounts for only 3–4% among Caucasians and African Americans, 14% among Koreans and Chinese, and 22.3% among Japanese [44, 45]. According to a meta-analysis of three previous studies on CYP2C19 genotypes, omeprazole was influenced by CYP2C19 genotypes, but rabeprazole was not [46–48]. In a more recent meta-analysis of randomized clinical studies, PMs showed higher eradication rates than EMs, and while eradication rate was influenced by the CYP2C19 genotype under omeprazole and lansoprazole medication, it was not the case under rabeprazole and esomeprazole medication [49]. While the eradication rate of triple therapy using lansoprazole and rabeprazole was not influenced by CYP2C19 genotype in a South Korean study [50], other studies reported that the eradication rate was significantly higher in PMs compared to EMs in triple therapy including pantoprazole and esomeprazole [14, 51]. However, it is not easy to examine CYP2C19 genotype in clinical settings. Therefore, regulating the type and dose of PPI was presented as a more practical approach in the 2nd Asia-Pacific Consensus Guideline [52].

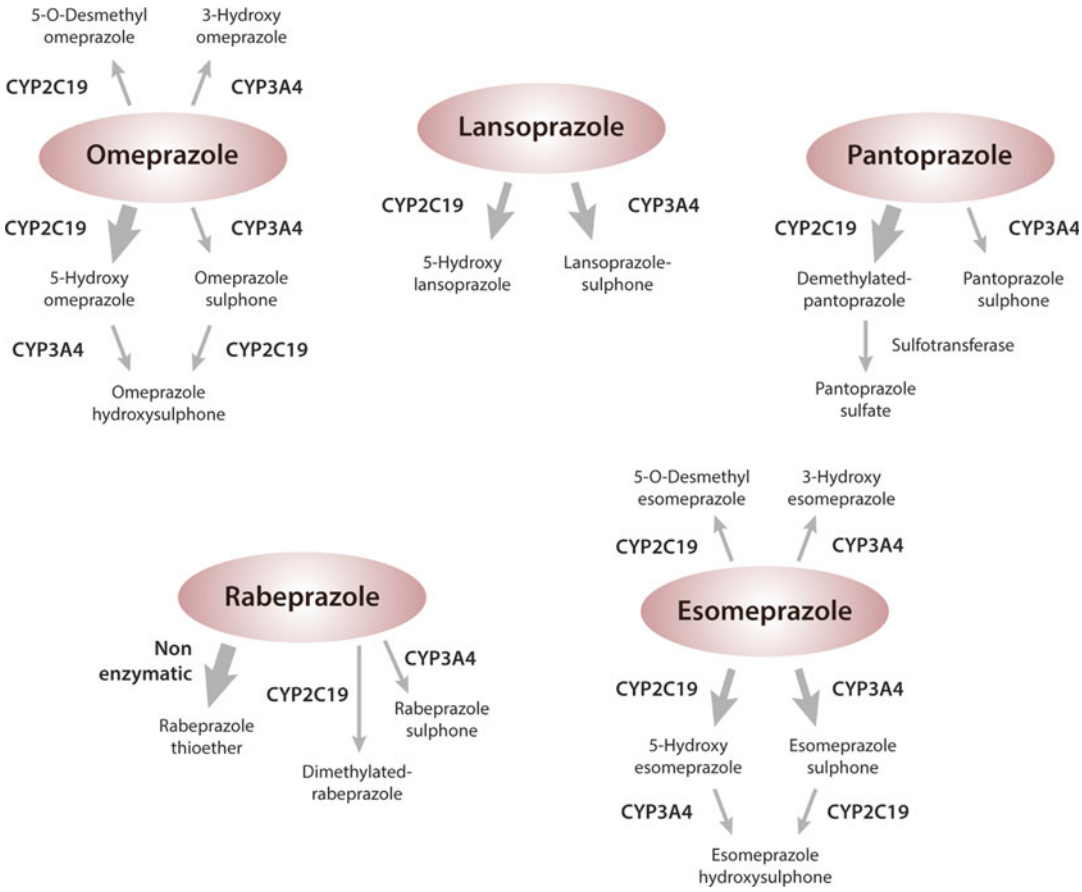

Fig. 41.2 Metabolic pathway related to cytochrome P450 isoenzyme (CYP2C19 and CYP3A4) according to the types of proton pump inhibitor (PPI) (Adapted from Sugimoto and Furuta [43])

41.3.2.3 Treatment Duration

In order to counteract the declining eradication rate, extension of eradication treatment duration to a 14-day regimen was considered; Calvet et al. [53] performed a meta-analysis and reported that duration extension increased the eradication rates. The increase in eradication rate through extended eradication treatment in 10- and 14-day regimens was 4 % and 6 %, respectively, according to report in countries including the USA. According to some other reports, however, extending the duration of eradication treatment yielded unfavorable results in terms of cost-effectiveness, and due to the increase in side effects through the prolonged medication and subsequent decrease in medication adherence, no statistically significant difference in eradication rate was yielded by the extension [54, 55]. Looking at the eradication rates according to the treatment duration in South Korea, a prospective multicenter study conducted in 2007 yielded the finding that the duration extension from 7 to 14 days did not result in significant increase in eradication rate [56], whereas a meta-analysis performed in 2008 estimated the increase in eradication rate at about 10 % as the treatment duration was prolongs from 7 to 14 days [57].

Negative views prevail as to the effect of extended triple therapy in overcoming clarithromycin resistance. Clarithromycin eradicates *H. pylori* by binding to ribosome, and resistance impedes the binding to ribosome. Consequently, the question may be raised whether this deficit can be improved by increasing the administration dose and duration. In other words, it has yet to be clarified through further study whether a simple prolongation of eradication treatment can raise eradication rate [8, 58].

41.3.2.4 Drug Side Effects

Zullo et al. [40] performed a meta-analysis and estimated the incidence of side effects of standard triple therapy at 9.8 % and the rate of discontinuation of medication due to severe side effects at 0.007 %. Tong et al. investigated seven studies in a meta-analysis and reported the incidence of side effects of standard triple therapy to be approximately 8.7 % (6.0–17.1 %) [59]. Side effects of standard triple therapy are bloating, heartburn, loss of appetite, altered taste (bitter taste in the mouth), nausea and vomiting, abdominal pain, headache, indigestion, diarrhea, constipation, itching, etc. The most recent meta-analysis performed in South Korea reviewed 41 studies (24 randomized clinical studies and 17 observational studies) covering 8,018 patients estimated the incidence of drug side effects at 24.0 %, with the most common side effect being altered taste, followed by soft stool or diarrhea, abdominal discomfort, and nausea. The cases of discontinuing eradication treatment due to severe side effects accounted for 1.81 %, demonstrating that most patients endure well side effects [12].

41.3.2.5 Underlying Gastroduodenal Disease

According to some reports, patients with non-ulcer (functional) dyspepsia exhibit lower eradication rates than patients with peptic ulcer disease [60]. This difference is ascribable to different *H. pylori* strains involved. Dyspeptic patients are easily infected by strains with low virulence (*cagA* negative; *vacA* s2 or m2 genotype) and low proliferation rate. Such strains are known to have low antimicrobial susceptibility. Patients with non-ulcer dyspepsia are also reported to exhibit higher clarithromycin resistance rates than patients with peptic ulcer disease, but there is no consensus about this point [21]. There is also a report that patients with non-ulcer dyspepsia exhibit higher incidence of *H. pylori* reinfection compared to peptic ulcer patients, and this has yet to be clarified in further research [61].

41.3.2.6 Gastritis Patterns

Pattern of *H. pylori*-associated gastritis is also one of the factors affecting eradication rate. It was reported that pangastritis was associated with lower eradication rate compared to antral gastritis, which did not change when the standard triple therapy was extended to 14 days [62, 63].

41.3.2.7 Smoking

Many studies pointed out that smoking is associated with *H. pylori* eradication failure. A meta-analysis reviewing 22 clinical studies covering

5,538 patients [64] reported that compared to non-smokers, smokers showed higher potential for treatment failure in 7-day standard triple therapy, estimating the difference at 8.4 % [64]. Smoking reduces blood flow in the stomach and mucus secretion, increases gastric acid secretion, and changes the specific metabolic activity of cytochrome P450 isoenzyme involved in PPI metabolism, and all these physiological changes are considered to contribute to increasing the eradication failure rate [64]. However, smoking-related decrease in eradication rate appeared under clarithromycin-amoxicillin medication, but not under clarithromycin-tinidazole medication [65]. Additionally, no noticeable smoking-related decrease in eradication rate was observed in 14-day triple therapy or sequential therapy [66].

41.3.2.8 Other Factors

Some studies reported that chronic diseases affected treatment outcomes, such as low eradication rate in diabetic patients [67], high clarithromycin resistance in patients with chronic renal diseases [68], and high eradication rate in patients with chronic liver diseases [69], but the effects of such chronic diseases on eradication rate are no consistent in these studies and still lack evidence.

Body mass index (BMI) can also affect eradication rate, and a report on obesity (BMI >25) was associated with decreased eradication rate with 7-day standard triple therapy [70].

Several studies reported that social drinking is associated with eradication success. Drinking alcohol of moderate alcohol content (5–40 %) decreases gastric acid secretion and has antimicrobial effect on *H. pylori*, and strengthens the prostaglandin-mediated mucosal defense, thus contributing to *H. pylori* eradication [71–74]. In most studies, however, alcohol intake did not influence eradication rate [75].

The association of *H. pylori* eradication rate with age is different from study to study. Some studies reported that aged patients showed higher eradication rate, ascribing it to the increase in intestinal metaplasia and gastric mucosal atrophy with increasing age [76–79]. Specifically, intestinal metaplasia creates an adverse environment for *H. pylori*, leading to decreased colonization density, which is more favorable for eradication, and gastric mucosal atrophy decreases gastric acid secretion, thus promoting the antimicrobial activity of the drug that increases in high pH. In contrast, a study on patients with functional dyspepsia or peptic ulcer disease who underwent standard triple therapy reported that the older patent group (≥45 years) showed significantly lower *H. pylori* eradication rate [20]. The mechanism of decreasing eradication rate in elderly patients has not yet been known, but there is a report that eradication rate is lowered in case of many comorbidities [80].

Conclusions

Standard triple therapy has long been the first-line treatment for *H. pylori* infection, but its eradication rate has been continuously decreasing for the past 10 years. The major cause of eradication rate is increase in clarithromycin resistance. There is a need for establishing a new first-line treatment capable of increasing eradication rate.

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Quadruple Therapy

42

Ju Yup Lee

Abstract

Bismuth quadruple therapy (BQT) has been used as the second-line rescue therapy in cases where standard triple therapy fails as the first-line treatment. *Helicobacter pylori* (*H. pylori*) eradication rates under 7-day regimen are in the range of 63–81 %, and no clearly decreasing trend has yet been observed. Studies on the feasibility of extending the treatment duration of BQT to a 14-day regimen have presented different results. The main problem in extending the treatment duration is increase in side effects. The most recent guidelines recommend BQT as first-line treatment in regions with clarithromycin resistance of 20 % or higher. However, its outcomes are not very promising, with eradication rate lower than 80 %. Increase in metronidazole resistance is considered a contributing factor for the potential failure of BQT. The risk for decrease in eradication rate is especially high in regions where metronidazole resistance exceeds 30 %. Due to the unsatisfactory outcomes of BQT as first- or second-line treatment, which is ascribable to increase in antimicrobial resistance, there is an urgent need for developing a new eradication treatment method.

Keywords

Quadruple therapy • Bismuth • Metronidazole • Resistance

42.1 Introduction

Classical quadruple therapy is defined as a 7- or 14-day regimen consisting of PPI standard dose b.i.d (twice a day) + bismuth 120 mg q.i.d. (four times a day) + tetracycline 500 mg q.i.d. + metronidazole 500 mg t.i.d (three times a day). Because it contains bismuth, it is usually called bismuth quadruple therapy (BQT) in differentiation with the concomitant non-bismuth quadruple therapy

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(CNBQT), currently in wide use, in which proton pump inhibitor (PPI), clarithromycin, amoxicillin, and metronidazole are simultaneously used.

Bismuth has long been used for the treatment of gastritis and peptic ulcer for the effects of bismuth in protecting the gastric mucosa and curing related diseases. It is known to increase the antimicrobial therapeutic effects and decreasing antimicrobial resistance to *H. pylori* eradication treatment by reducing the colonization density of its strains because it can directly induce their dissolution [1, 2]. An additional advantage of bismuth is that it does not provoke antimicrobial resistance. The classical bismuth-containing quadruple therapy has usually been used as second-line treatment in case of failure of clarithromycin-containing first-line triple therapy. More recently, its role as the first-line treatment has become important since the Maastricht 2012 Consensus Report [3], and the latest version of Korean guidelines [4] recommended BQT as the first-line empiric treatment in regions with clarithromycin resistance exceeding 20 %.

This work is intended to investigate the treatment achievements of BQT as the first- and second-line treatments for *H. pylori* infection and antimicrobial resistance affecting BQT.

42.2 Efficacy of Bismuth Quadruple Therapy as Second-Line Treatment

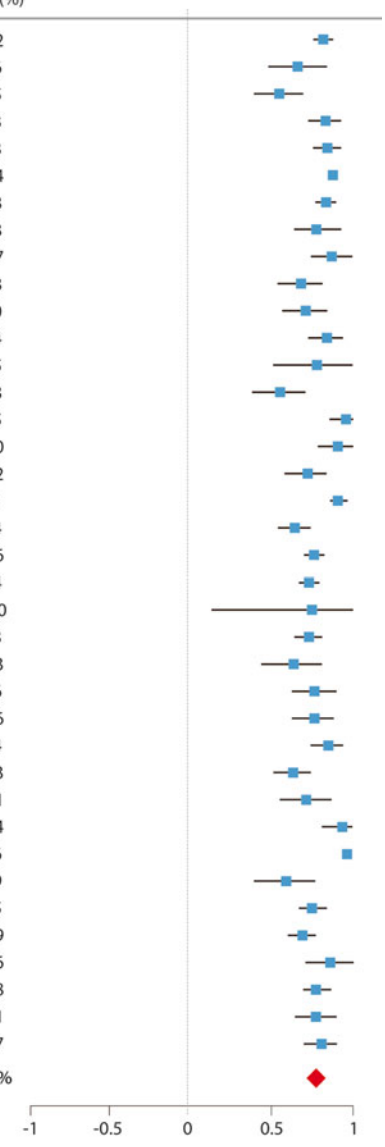
The eradication rate of BQT applied as rescue therapy in case of treatment failure with the first-line standard triple therapy (PPI + amoxicillin + clarithromycin) was estimated at 78 % (95 % confidence interval [CI], 75–81 %; 33 studies covering 3,131 patients) on overall average of the domestically published reports [5–43] (Fig. 42.1). The eradication rates in 7-, 10-, and 14-day regimens were reported to be 76 % (95 % CI, 72–80 %; 29 studies, 2,097 patients), 77 % (95 % CI, 60–93 %; 2 studies, 142 patients), and 82 % (95 % CI, 76–88 %; 12 studies, 831 patients), respectively [5].

Korean reports on the eradication rates of BQT widely vary, ranging from 63 to 81 % in 7-day regimen, and no clearly decreasing trend has yet been observed [44] (Fig. 42.2). Under prolonged regimen (from 7 to 14 days) as well, the eradication rates widely varied, ranging between 69 and 96 %. According to the study conducted in 2010 by Lee et al. [26] with 227 patients who failed with standard triple therapy, the eradication rate was considerably higher under 2-week regimen (Intention to treat [ITT], 82.6 %) compared to 1-week regimen (ITT, 64.3 %). In contrast, according to the results of the randomized controlled trial (RCT) with 169 patients published in 2012 by Yoon et al. [49], no significant difference between 1- and 2-week regimens were observed. As the current clinical practice of extending the treatment duration to 14 days carries the risks of increasing side effects and decreasing medication adherence, the issue of medication duration needs to undergo further consideration. Specifically, given the difficulties with BQT medication involving high-dose levels and including two drugs that have to be taken four times a day, coupled with relatively frequent side effects (36.4 %) [50], there is an urgent need for developing a new rescue therapy with simplified medication and fewer side effects.

42.3 Efficacy of Bismuth Quadruple Therapy as First-Line Treatment

Theoretically, bismuth-containing agents are easily delivered to the gastric mucosa of patients with dyspepsia with relatively mild inflammatory responses, thus rapidly reaching *H. pylori*, and have low antimicrobial resistance rate, and there have hence been arguments that bismuth-based quadruple therapy should replace the conventional PPI-based triple therapy for *H. pylori*-associated gastritis [51]. A recently performed meta-analysis reviewing a total of 12 RCTs in order to compare standard triple therapy and BQT as first-line treatment reported the eradication rate of BQT to be much higher than that of standard triple therapy (77.6 % vs. 68.9 %; relative risk [RR] 1.13; 95 % CI, 1.07–1.18) [52] (Table 42.1 and Fig. 42.3). In this analysis, when

Study	Year	Duration (days)	Patients (n)	Eradicated patients (n)	Eradication rate (%)
Boixeda et al. [6]	2002	7	140	115	82
Cheon et al. [7]	2006	7	29	19	66
Cheon et al. [8]	2006	7	44	24	55
Choung et al. [9]	2004	7 or 14	71	59	83
Chung et al. [10]	2011	7 or 14	199	166	83
Chung et al. [11]	2007	7	87	73	84
Chung et al. [12]	2012	7	37	29	78
Chung et al. [13]	2009	7	507	445	88
Elizalde et al. [14]	1998	7	31	27	87
Finizio et al. [15]	2005	7	50	34	68
Franceschi et al. [16]	2011	7	50	35	70
Georgopoulos et al. [17]	2002	7	49	41	84
Gisbert et al. [18]	2008	7	38	21	55
Gisbert et al. [19]	1999	7	9	7	78
Gomollon et al. [20]	1999	7	21	20	95
Kang et al. [21]	2006	7	30	27	90
Kang et al. [22]	2007	14	53	38	72
Kim et al. [23]	2003	14	94	86	91
Kuo et al. [24]	2009	7	83	53	64
Kuo et al. [25]	2010	7	190	144	76
Lee et al. [26]	2010	7 or 14	227	167	74
Magaret et al. [27]	2001	14	2	2	100
Mantzaris et al. [28]	2005	7 or 14	115	84	73
Marko et al. [29]	2005	7	27	17	63
Michopoulos et al. [30]	2000	14	38	29	76
Michopoulos et al. [31]	2012	14	45	34	76
Moon et al. [32]	2012	7	57	48	84
Nista et al. [33]	2003	7	70	44	63
Nista et al. [34]	2004	7	35	25	71
Ozcay et al. [35]	2004	7	16	15	94
Park et al. [36]	2004	14	107	103	96
Peitz et al. [37]	1998	7	29	17	59
Perri et al. [38]	2003	7 or 14	200	146	75
Rokkas et al. [39]	2009	10	120	83	69
Sicilia et al. [40]	2000	10	22	19	86
Uygun et al. [41]	2008	14	100	78	78
Wu et al. [42]	2011	7	62	50	81
Wu et al. [43]	2006	7	47	36	77
				Total	78%



Heterogeneity: Tau2 = 0.01; Chi2 = 208.49, df = 37 ($p < 0.00001$); I2 = 82%

Test for overall effect: Z = 44.91 ($p < 0.00001$)

Fig. 42.1 Intention-to-treat (ITT) eradication rate of the bismuth quadruple therapy (BQT) applied as a rescue therapy after the failure of standard triple therapy as the first-line treatment. The overall eradication rate of BQT is shown to be 78 % (Modified from Marin et al. [5])

10-day BQT and 7-day standard triple therapy were compared, BQT showed better outcomes, and there were no differences in side effects and patient compliance [52]. However, given the fact that selection and exclusion criteria were different from study to study and individual studies used different PPIs and, above all, the doses of the antibiotics used in BQT slightly varied among the studies, comparing these studies has the disadvantage of great heterogeneity, and particular attention should thus be paid to this issue when interpreting the results. In fact, in the study by Gomollon et al. [53], in which BQT was reported to have eradication rate as low as 68.8 %, 250 mg metronidazole was administered three times a day, and Malfertheiner et al. [64] used three-in-one

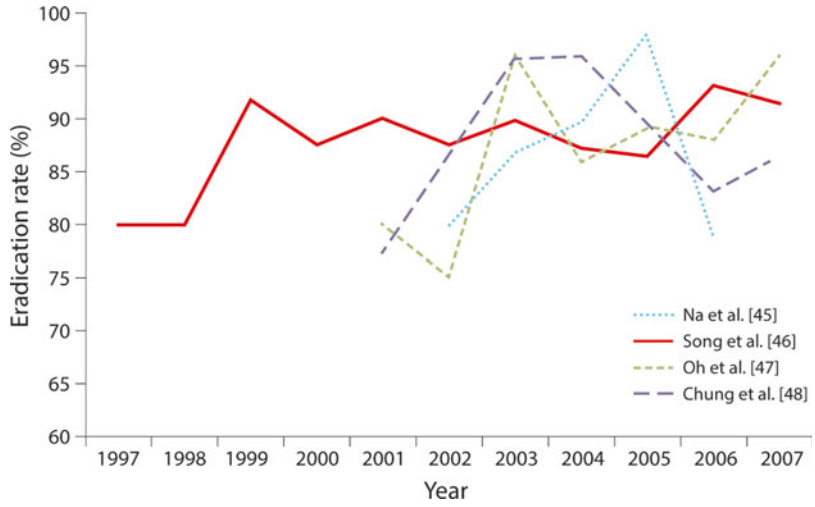


Fig. 42.2 Changes in the per-protocol (PP) eradication rate in South Korea of bismuth quadruple therapy (BQT) used as the second-line rescue therapy. No noticeable decreasing trend in eradication rate is observed (Modified from Kim and Jung [44])

capsule combining bismuth, metronidazole, and tetracycline, which may have contributed to higher medication adherence.

The overall evaluation of BQT eradication rate is lower than 80 %. Especially in South Korea, BQT shows no advantage over triple therapy in efficacy. Therefore, while its use as first-line treatment in regions with high clarithromycin resistance may be considered, no great effects are to be expected.

42.4 Factors Affecting the Bismuth Quadruple Therapy Eradication Rate: Antimicrobial Resistance

Antimicrobial resistance is known to be the main cause of *H. pylori* eradication failure. For standard triple therapy, the key issue is clarithromycin resistance. Antibiotics included in BQT are tetracycline and metronidazole. Because tetracycline resistance is reported to be low globally [65–68], metronidazole resistance may be said to be the main factor affecting BQT's eradiation rate [66]. It is known that metronidazole resistance can be overcome by increasing administration dose or extending medication duration to 10–14 days [69]. For example, a prospective study conducted in China reported that eradication rate over 90 % could be achieved with 14-day maximum-dose BQT regimen as first-line empiric treatment (30 mg lansoprazole b.i.d., 220 mg bismuth b.i.d., 500 mg tetracycline q.i.d., and 400 mg metronidazole q.i.d.) without regard to metronidazole resistance [70]. Gerrits et al. [71] reported that metronidazole resistance may decrease the efficacy of metronidazole-containing eradication treatment, but cannot affect the overall outcomes. In a more recent meta-analysis as well [52], eradication rates analyzed from four studies presenting the results related to antimicrobial susceptibility yielded the findings that standard triple therapy eradication rates were 88 % and 14.3 % in cases with or without clarithromycin resistance, respectively, thus demonstrating the extent to which clarithromycin resistance affect standard triple therapy eradication rate. In contrast, BQT eradication rates with or without metronidazole resistance were not significantly different: 92 % without metronidazole resistance and 84.2 % with metronidazole resistance. Further research is necessary to investigate the discrepancies between such in vitro metronidazole resistance and actual eradication treatment outcomes. One feasible hypothesis is the

Table 42.1 Comparison of standard triple therapy and bismuth quadruple therapy used as the first-line empiric treatment for *H. pylori* infection in terms of treatment success, compliance, and side effects

Author (year, location)	Therapy	Duration (days)	Patients (*n*)	Eradicated patients (*n*)	ITT (%)	Compliance (%)	Side effects (%)
Gomollon et al. [53] (2000, Spain)	Triple	7	49	40	81.9	98	40
	Quadruple	7	48	33	68.8	100	42
Calvet et al. [54] (2002, Spain)	Triple	7	171	132	77.0	94	59
	Quadruple	7	168	139	83.0	91	59
Katelaris et al. [55] (2002, Australia/NZ)	Triple	7	134	104	78.0	97	–
	Quadruple	7	134	110	82.0	94	–
Mantzaris et al. [56] (2002, Greece)	Triple	10	78	61	78.2	96	75
	Quadruple	10	71	46	64.8	93	78
Laine et al. [57] (2003, USA/ Canada)	Triple	10	137	114	83.2	94	59
	Quadruple	10	138	121	87.7	91	59
Pai et al. [58] (2003, India)	Triple	10	35	29	82.9	100	11
	Quadruple	10	33	24	72.7	94	15
Jang et al. [59] (2005, South Korea)	Triple	7	75	59	78.7	–	7
	Quadruple	7	74	53	71.6	–	10
Uygun et al. [60] (2007, Turkey)	Triple	14	120	69	57.5	96	11
	Quadruple	14	120	84	70.0	91	13
Ching et al. [61] (2008, UK)	Triple	7	50	46	92.0	100	90
	Quadruple	7	44	40	91.0	86	95
Songur et al. [62] (2009, Turkey)	Triple	14	113	37	32.7	91	–
	Quadruple	10	119	56	47.1	87	–
Zheng et al. [63] (2010, China)	Triple	7	85	54	63.5	100	60
	Quadruple	10	85	76	89.4	100	42.3
Malfertheiner et al. [64] (2011, Europe)	Triple	7	222	123	55.0	>95	51
	Quadruple	10	218	174	80.0	>95	47
Overall	Total patients (*n*): 2,753			ITT eradication rate of CTT: 68.9 %			$p < 0.00001$
	Total studies (*n*): 12			ITT eradication rate of BQT: 77.6 %			

– Not reported, *ITT* intention to treat, *CTT* clarithromycin triple therapy, *BQT* bismuth quadruple therapy

difference in oxygen partial pressure. Specifically, metronidazole-resistant *H. pylori* strains can be mistaken as metronidazole-sensitive strains in an environment with low in vitro oxygen partial pressure [32]. Irrespective of such discrepancies, BQT eradication rates are known to fall below 90 % in regions with metronidazole resistance rates exceeding 30 %, which implies that BQT cannot be a suitable alternative in regions with high antimicrobial resistance [72].

Looking at South Korean reports, the BQT eradications rates of BMT (bismuth + metronidazole + tetracycline) regimen were 100 % in the absence of metronidazole resistance and 77.8 % when there was metronidazole resistance [73]. The same for BMA (bismuth + metronidazole + amoxicillin) regime were reported to be 91.7 and 70 % [74]. While the metronidazole and tetracycline resistance rates of *H. pylori* strains have recently been increasing in South Korea [68, 75], no great changes have been reported on the eradication rate of second-line standard rescue therapy [44]. However, in consideration of the fact that second-line standard rescue therapy involves a smaller number of patients than first-line standard treatment and in the current lack of standard reference

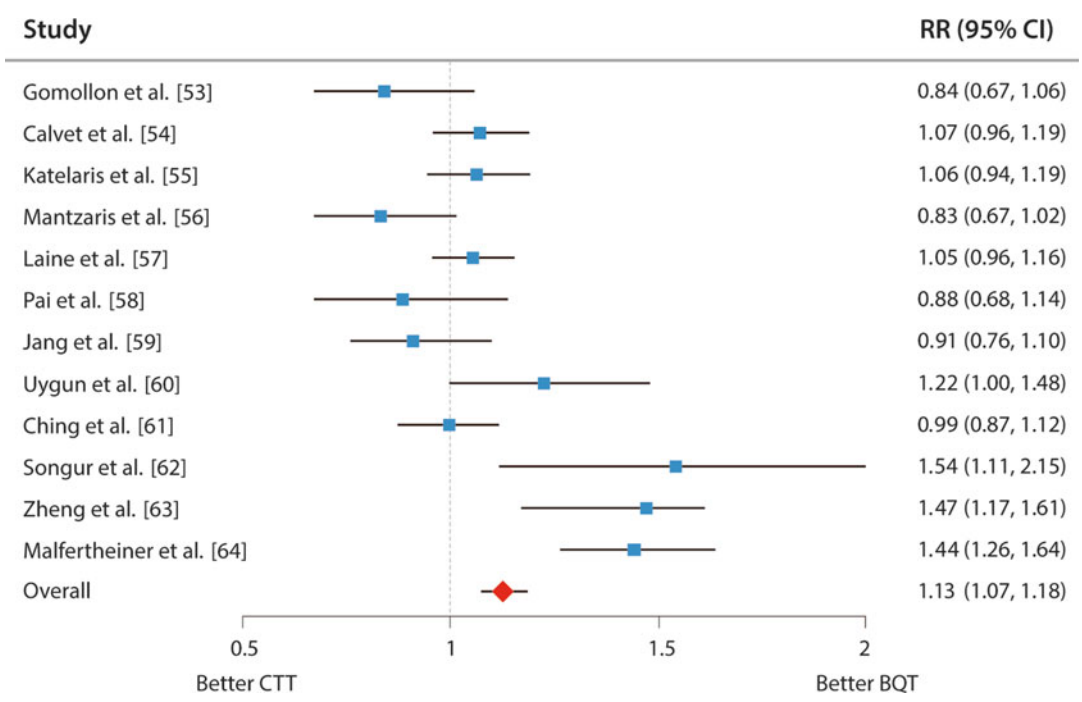


Fig. 42.3 Forest plot representing the analytic comparison of relative risks (RR) associated with the ITT eradication rates of bismuth quadruple therapy (BQT) and standard triple therapy used as the first-line treatment. The RR of BQT is 1.13 (95 % CI, 1.07–1.18). *ITT* intention to treat, *CTT* clarithromycin triple therapy, *BQT* bismuth quadruple therapy (Modified from Venerito et al. [52])

concentrations for antimicrobial sensitivity testing as well as various unclarified influential factors, such as in vivo manifestations of antimicrobial sensitivity results, further research with a larger number of patients is necessary [76].

Conclusions

Although BQT has been applied as second-line rescue therapy and in regions with high clarithromycin resistance as first-line treatment, its treatment performance is not satisfactory, with eradication rate below 80 %. Increasing metronidazole resistance is considered the principal factor for affecting BQT eradication rate, and in South Korea, tetracycline resistance is also relatively high (10–15 %), which may exert much influence on further decrease in BQT eradication rate. Therefore, there is an urgent need for intensifying research for developing new first- and second-line therapies capable of replacing BQT.

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Sequential Therapy

43

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Abstract

Sequential therapy consists of the first half and last half. In the first half, proton pump inhibitor (PPI) and amoxicillin are administrated. Then, PPI, clarithromycin, and antibiotics of nitroimidazole family (metronidazole or tinidazole) are administrated in the last half. This regimen was developed in the theoretical background that amoxicillin in the first half attenuates the cell wall of *Helicobacter pylori*, inhibits the development of efflux channel, and finally it could overcome the clarithromycin resistance. It is though that sequential therapy has higher eradication rate than standard triple therapy and shows similar adverse events rate with standard triple therapy. Therefore, sequential therapy is recently suggested as an alternative regimen for standard triple therapy of which the eradication rate is continuously decreasing all over the world. However, this regimen also has some limitations in that the dosage is complex and it is difficult to find an appropriate second-line treatment when this regimen failed.

Keywords

Helicobacter pylori • Eradication • Sequential therapy

43.1 Introduction

The eradication rate of standard triple therapy for *Helicobacter pylori* (*H. pylori*) infection has been continuously decreasing due to the increase of antibiotic resistance all over the world [1]. One of several regimens which appeared as an alternative is sequential therapy. Sequential therapy consists of the first half and last half. In the first half, proton pump inhibitor (PPI) and amoxicillin are administrated. Then, PPI, clarithromycin, and antibiotics of nitroimidazole family (metronidazole or tinidazole) are administrated in the last half. At the present time, European guideline recommends sequential therapy as an alternative first-line eradication therapy in the areas which show high resistance rate to clarithromycin [2].

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However, in the guidelines of Asia and the USA, this regimen is not adopted for the reason that the supporting data is insufficient in each corresponding area [3, 4].

43.2 Theoretical Background of Sequential Therapy

The following two reasons have been suggested for expectation that the eradication rate of sequential therapy would be superior to that of standard triple therapy. First, the effect of eradication therapy is known to be inversely proportional to the density of *H. pylori* in the stomach [5]. Dual therapy (PPI + amoxicillin) of the first half of sequential therapy decreases the density of *H. pylori* and thus it maximizes the effect of subsequent triple therapy (PPI + clarithromycin + nitroimidazole) [6]. Second, one of the main mechanisms for the development of clarithromycin resistance in *H. pylori* eradication is that *H. pylori* produces efflux channel which pumps clarithromycin out of cell membrane and hampers the binding of clarithromycin to ribosome [7]. Amoxicillin which is administrated in the first half of sequential therapy attenuates the cell wall of *H. pylori* and inhibits the development of efflux channel. Thus, it could overcome the clarithromycin resistance [6]. However, it is not clear whether the reason that sequential therapy is superior to standard triple therapy is indeed due to the aforementioned mechanism or it is simply due to the concomitant administration of main antibiotics which are used for *H. pylori* eradication.

43.3 Types of Sequential Therapy

The dose of PPI, amoxicillin, and clarithromycin in sequential therapy is the same as that of standard triple therapy. However, the types and dosage of antibiotics of nitroimidazole family slightly vary with studies. Early studies which were performed in Europe centering on Italy used tinidazole as antibiotics of nitroimidazole family. However, succeeding studies in Asia used metronidazole more commonly than tinidazole. Metronidazole is almost similar to tinidazole except for the fact that action duration of it is shorter than that of tinidazole. Recent meta-analysis reported that there is no difference in the eradication rate of sequential therapy according to these two antibiotics [8]. The most common dosage for tinidazole and metronidazole is 500 mg b.i.d. (bis in die, twice a day) and 500 mg t.i.d. (ter in die, three times a day), respectively.

The duration of sequential therapy also varies with studies. The most common regimen is 10-day regimen which consists of each 5-day first half and last half. Fourteen-day regimen in which 2 days were prolonged in each half is the second common regimen.

43.4 Outcome of Sequential Therapy

43.4.1 Eradication Rate

A country which reported the outcome of sequential therapy in literature for the first time is Italy. At that time, the authors reported surprising results that eradication rate in intention-to-treat (ITT) analysis was 98 % [9]. Thereafter, open-label study and double-blind randomized clinical trial which compared sequential therapy with 7-day or 10-day standard triple therapy consistently reported the superiority of sequential therapy to standard triple therapy, and thus sequential therapy received attention from the academic community [10, 11]. Thereafter, many studies regarding sequential therapy were performed, and meta-analyses which summarized these studies also reported that eradication rate of sequential therapy was about 91.0–93.5 % in ITT analysis and it was superior to that of standard triple therapy [12–14]. However, these meta-analyses have geographic limitation in that most studies which were included in the analyses were performed in Italy. Therefore, another meta-analysis was recently performed for studies regarding only Asian adults. The authors concluded that sequential therapy is also superior to standard triple therapy in Asian adults. However,

disappointingly, the absolute eradication rate was 81.8 % in ITT analysis, and this was approximately 10 % lower than previous eradication rates of Western studies [8].

43.4.2 Adverse Events

Because sequential therapy consists of more kinds of antibiotics than standard triple therapy, there has been a concern that the rate of adverse events of sequential therapy would be higher than that of standard triple therapy. However, although the rate of adverse events of sequential therapy varied as 8.4–22.6 % according to studies, most meta-analyses concluded that the rate of adverse events of sequential therapy was not significantly higher than that of standard triple therapy [8, 13, 14].

43.5 Limitations of Sequential Therapy

43.5.1 The Complexity of Regimen

There are many kinds of drugs to be administrated in sequential therapy. In addition, the drugs which are administrated in the first half and the last half are different. Therefore, there is a possibility that the compliance of patients could decrease [15]. Considering that compliance is the second leading factor which affects the eradication rate following antibiotic resistance, this is a very important problem. One meta-analysis reported that the compliance of sequential therapy and standard triple therapy is 97.4 % and 96.8 %, respectively [12]. One review article reported that when eight studies in which the data regarding the compliance was available were taken together, the rate of the patients who showed compliance higher than 90 % was not different between sequential therapy and standard triple therapy (92.6 % vs. 94 %) [16]. However, considering that the compliance in the real field is generally lower than that in the clinical studies, the possibility that the compliance of sequential therapy is lower than that of standard triple therapy cannot be excluded.

43.5.2 Measures After Treatment Failure

Because sequential therapy uses clarithromycin and nitroimidazole which are the most important drugs in the *H. pylori* eradication in the same time, great difficulty is anticipated to select the second-line treatment when sequential therapy failed as the first-line treatment. European guideline suggests levofloxacin-based triple therapy as second-line regimen when first-line sequential therapy failed. However, the eradication rate of levofloxacin-based triple therapy as second-line treatment was approximately 77.5 % [17]. Therefore, one literature suggested quadruple therapy including PPI, bismuth, tetracycline, and levofloxacin as an alternative. However, because the number of the subjects was only 24, the evidence level of that study is not high [17].

43.5.3 Antibiotic Resistance

To demonstrate that one of the main benefits of sequential therapy compared with standard triple therapy is to overcome clarithromycin resistance by administration of amoxicillin in the first half, the comparison of eradication rate according to antibiotic resistance of each *H. pylori* strain is required. However, actually there are just a few studies which evaluated antibiotic resistance in sequential therapy. One meta-analysis reported the eradication rate of sequential therapy, and standard triple therapy for clarithromycin-resistant strain of *H. pylori* was 70.7 % and 33.8 %, respectively [14]. Most studies reported that even though the eradication rate of sequential therapy decreases for clarithromycin-resistant strain of *H. pylori*, the absolute eradication rate of sequential therapy is much higher than that of standard triple therapy [10, 11, 18–20]. However, because the total number of clarithromycin-resistant strains is only dozens when we combine all these studies, the results should be interpreted with caution. There is limited number of studies which reported the result of sequential therapy in metronidazole-resistant strain. One meta-analysis reported that the eradication rate of sequential

therapy and standard triple therapy for metronidazole-resistant strains was 96.0 % and 67.68 %, respectively [14].

43.5.4 The Quality of Individual Study

Since 2000s, the number of studies which evaluated the efficacy of sequential therapy in the first-line treatment for *H. pylori* infection amounted to dozens. However, meta-analyses regarding these studies pointed out that studies in which the number of subjects is more than 100 were rare, the heterogeneity among studies is high, and the quality of study design is relatively low [8, 12–14]. Although we have to interpret the results considering that antibiotic resistance varies with area worldwide and the administration duration of standard triple therapy was long, multi-countries study in Latin America in which the number of subjects was largest among studies regarding sequential therapy suggested that 14-day standard triple therapy was even superior to 10-day sequential therapy [21]. Therefore, to conclude that sequential therapy is really superior to standard triple therapy, well-designed randomized controlled trials with larger number of subjects would be required.

Conclusions

Sequential therapy is recently suggested as an alternative regimen for standard triple therapy of which the eradication rate is continuously decreasing all over the world. Taken together many studies that were performed to date, it is though that sequential therapy has higher eradication rate than standard triple therapy and shows similar adverse events rate with standard triple therapy. Although areas except Europe worldwide do not recommend sequential therapy as first-line treatment for *H. pylori* infection, if high-quality studies which evaluate antibiotic resistance in each area were performed, there is a possibility that sequential therapy becomes new standard regimen for *H. pylori* eradication. However, this regimen also has some limitations in that the dosage is complex and it is difficult to find an appropriate second-line treatment when this regimen failed.

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Concomitant Therapy and Hybrid Therapy

44

Hyuk Yoon

Abstract

Concomitant therapy and hybrid therapy were developed to improve the eradication rate of sequential therapy. Concomitant therapy is a regimen in which antibiotics of standard triple therapy and nitroimidazole are administrated at the same time. This therapy has strength in that it is as superior as sequential therapy to standard triple therapy in the eradication rate and the usage is simpler than sequential therapy. Therefore, there is a possibility that this regimen might emerge as an alternative for standard triple therapy in the first-line treatment of *Helicobacter pylori* infection in the future. However, the limitations that the data regarding effect for antibiotic-resistant strains is lacking and there are few drugs to try when this regimen fails as the first-line treatment are the same as sequential therapy. Hybrid therapy is a regimen in which amoxicillin which is administrated in the first half of sequential therapy is continuously administrated during the last half. This therapy is not a regimen which is widely studied. Therefore, this regimen is difficult to be generally used in the daily clinic.

Keywords

Helicobacter pylori • Eradication • Concomitant therapy • Hybrid therapy

44.1 Introduction

Concomitant therapy is a regimen in which antibiotics of standard triple therapy and nitroimidazole are administrated at the same time. Therefore, this regimen is also called as bismuth non-containing quadruple therapy. The first result of concomitant therapy was reported in 1998 in Germany and Japan; the eradication rate in intention-to-treat (ITT) analysis was high as 96% and 93.3%,

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respectively [1, 2]. Thereafter, studies regarding concomitant therapy were performed all over the world as a better alternative than sequential therapy for first-line *Helicobacter pylori* (*H. pylori*) treatment. In addition, there is a regimen called as hybrid therapy which combines sequential therapy and concomitant therapy. Hybrid therapy is a regimen in which amoxicillin which is administrated in the first half of sequential therapy is continuously administrated during the last half. Similar to concomitant therapy, this regimen was also developed to improve the eradication rate of sequential therapy. In this chapter, I will review concomitant therapy mainly and discuss hybrid therapy together.

44.2 Theoretical Background of Concomitant and Hybrid Therapies

There are some critical eyes on the sequential therapy behind the suggestion of concomitant therapy. That is doubt about whether the high eradication rate of sequential therapy is indeed originated from the administration order of antibiotics included in this regimen [3]. If the high eradication rate of sequential therapy is not stemming from the administration sequence of antibiotics and it is rather due to the absolute total number of antibiotics, administration of these four drugs at the same time as carpet bombing would be better than sequential administration in complex, since the former would increase compliance of patient and therefore the eradication rate might be better [4].

The theoretical background for hybrid therapy is that the effect of dual administration of proton pump inhibitor (PPI) and amoxicillin is in proportion to administration duration of these drugs [5]. This hypothesis was tested in Taiwan study in 2011 for the first time [6].

44.3 Outcome of Concomitant and Hybrid Therapies

44.3.1 Eradication Rate

The first meta-analysis for concomitant therapy which was published in 2009 reported the analysis for 771 patients using nine studies; the eradication rate in ITT and in per-protocol (PP) analysis was 89.7 % and 92.9 %, respectively [7]. Five studies included in this analysis were randomized controlled trials comparing concomitant therapy and standard triple therapy. Eradication rate in ITT analysis was 90.8 % in concomitant therapy and 79 % in standard triple therapy. That is, concomitant therapy was superior to standard triple therapy. Meta-analysis regarding 1,723 patients using 15 studies was published in 2011. The mean eradication rate by ITT analysis was 90 % and this was similar to the previous meta-analysis [8]. In succeeding meta-analysis which was published in 2012 by the same group, the eradication rate by ITT analysis was mean 88 % in 2,070 patients using 19 studies [9]. When the studies from Latin America and Turkey in which the eradication rate was 74 % and 75 %, respectively [10, 11], were excluded, the heterogeneity among studies was low and eradication rate by ITT analysis was over 90 %.

There are just a few studies which compared the eradication rate of concomitant therapy and sequential therapy. Studies from Taiwan, China, and Spain which compared 10-day sequential therapy and 10-day concomitant therapy all concluded that there was no significant difference in the eradication rate between the two regimens [12–14]. However, if the purpose of concomitant therapy is to simplify the complexity of sequential therapy, to compare the eradication rate of 10-day sequential therapy and 5-day concomitant therapy would be more reasonable. The above-mentioned multinational study from Latin America compared the eradication rate of 14-day standard triple therapy with 10-day sequential therapy as well as 5-day concomitant therapy; the eradication rate between sequential therapy and concomitant therapy was not different [10]. A Taiwanese study which compared 7-day standard triple therapy, 10-day sequential therapy, and 7-day concomitant therapy also reported that there was no difference in the eradication rate between sequential therapy and concomitant therapy [15].

The duration of concomitant therapy varies from 3 to 10 days. In a meta-analysis, concomitant therapy showed high eradication rate even by short period of administration. However, there was a trend that the eradication rate was higher

by long administration (7–10 days) than by short administration (3–5 days) [8]; the eradication rate was 85 %, 89 %, 93 %, and 92 % by 3-day, 5-day, 7-day, and 10-day administration, respectively.

As opposed to sequential therapy and concomitant therapy which appeared in the literatures already since late 1990s, studies regarding hybrid therapy have been performed since 2010s. The first study which reported the result of hybrid therapy is multicenter study in Taiwan. The authors performed 14-day hybrid therapy in which they administrated PPI and amoxicillin during the first 7 days, and they added clarithromycin and metronidazole to PPI and amoxicillin during the remaining 7 days. The results were surprising; the eradication rate by ITT analysis and PP analysis was 97.4 % and 99.1 %, respectively [6]. These were approximately 5 % improved results compared to 14-day sequential therapy which was performed in the same institutions (eradication by ITT analysis 91.9 %, eradication by PP analysis 93.9 %) [16]. Hybrid therapy also showed significantly higher eradication rate than sequential therapy in an Iranian study which compared 10-day sequential therapy and 14-day hybrid therapy [17]. However, to the contrary, a Korean study reported that there was no difference in the eradication rate between 14-day hybrid therapy and 14-day sequential therapy (eradication by ITT analysis 81.1 % vs. 79.8 %, $p=0.821$; eradication by PP analysis 85.9 % vs. 82 %, $p=0.489$) [18]. In addition, in a study which compared 14-day hybrid therapy and 14-day concomitant therapy in Italy and Spain, there was no difference in the eradication between the two regimens (eradication rate by ITT analysis 90 % vs. 91.7 %, $p=0.35$; eradication by PP analysis 92 % vs. 96.1 %, $p=0.07$) [19]. Other than that, multicenter study in Italy compared 5-day concomitant therapy, 10-day sequential therapy, and 14-day hybrid therapy. Although there was no significant difference in the eradication rate, the absolute eradication rate of hybrid therapy was a bit low (eradication rate of concomitant/sequential/hybrid therapy 85.5/91.1/80 % by ITT analysis, 91.6/92.1/85.7 % by PP analysis) [20]. Another Italian study reported that the eradication rate of 10-day sequential therapy, 14-day concomitant therapy, and 14-day hybrid therapy is similar, and the eradication rate of 5-day concomitant therapy was merely low [21]. The results of the succeeding study in Taiwan which tried shortening of administration duration of 14-day hybrid therapy were recently published. There was no difference in the eradication rate among 10-day, 12-day, and 14-day hybrid therapy (eradication rate by PP analysis 95 %, 95.1 %, 93.4 %, respectively) [22]. In summary, because the number of studies regarding hybrid therapy is yet small and in studies which compared hybrid therapy with sequential therapy and concomitant therapy the administration duration of each regimen was various, it is hard to draw general conclusions. However, it is though that the eradication rate of hybrid therapy is generally excellent compared to standard triple therapy.

44.3.2 Adverse Events

According to the first meta-analysis in 2009, the adverse events of concomitant therapy was not different with that of standard triple therapy (27–51 % vs. 21–48 %); serious adverse events were not reported in any studies [7]. Therefore, although three kinds of antibiotics are administrated in the same time, we could say that concomitant therapy is not different with standard triple therapy in the aspect of adverse events. It is known that the adverse events of hybrid therapy are also not different with those of sequential therapy and concomitant therapy which are the main comparators of hybrid therapy [17–19].

44.4 Limitations of Concomitant Therapy

44.4.1 Measures After Eradication Failure

Because the kinds of antibiotics used in concomitant therapy and hybrid therapy are the same with those of sequential therapy, the limitation that great difficulty is anticipated to select the second-line treatment when these regimens failed as the first-line treatment is the same with sequential therapy.

44.4.2 Antibiotic Resistance

Studies regarding whether concomitant therapy is effective in *H. pylori* strains show clarithromycin resistance or not are scarce. A few studies reported that clarithromycin resistance did not affect the eradication rate of concomitant therapy [12, 23, 24]. However, because the number of clarithromycin-resistant strains which were included in these studies was very small, the results should be interpreted with caution. The data regarding whether concomitant therapy could overcome metronidazole resistance is also very lacking. Although a few studies reported that the eradication rate of concomitant therapy is irrespective of metronidazole resistance [23, 24], there is also a study against this suggestion [1].

There has been much little information in the literature regarding the effect of hybrid therapy for antibiotic-resistant *H. pylori* strains than concomitant therapy. Some studies reported that eradication rate of hybrid therapy for clarithromycin and metronidazole resistant strains was 75–100 % and 72.7–100 %, respectively [6, 19]. However, because the number of resistant strains included in these studies was very small, it is hard to put a meaning.

In summary, studies regarding whether concomitant therapy and hybrid therapy are effective for antibiotic-resistant *H. pylori* strains or not are very limited. Therefore, for the present, we can just infer it from the results of studies regarding sequential therapy which uses the same kinds of antibiotics with concomitant therapy and hybrid therapy.

Conclusions

Concomitant therapy has strength in that it is as superior as sequential therapy to standard triple therapy in the eradication rate, and the usage is simpler than sequential therapy. Therefore, there is a possibility that this regimen might emerge as an alternative for standard triple therapy in the first-line treatment of *H. pylori* infection in the future. However, the limitations that the data regarding effect for antibiotic-resistant strains is lacking and there are few drugs to try when this regimen fails as the first-line treatment are the same as sequential therapy. Hybrid therapy which combines concomitant therapy and sequential therapy is not a regimen which is widely studied. Therefore, this regimen is difficult to be generally used in the daily clinic.

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Tailored Therapy Based on Antibiotic Resistance

45

Yong Hwan Kwon

Abstract

The cure rates of *Helicobacter pylori* (*H. pylori*) eradication therapy using a proton pump inhibitor (PPI) and antimicrobial agents such as amoxicillin (AMX), clarithromycin (CAM), and metronidazole (MNZ) are mainly influenced by bacterial susceptibility to antimicrobial agents, and the eradication rates have gradually decreased because of the increased prevalence of *H. pylori* strains resistant to antimicrobial agents. The prevalence of antibiotic (e.g., CAM, MNZ, tetracycline, AMX, and furazolidone) resistance varies among different countries; it appears to be partly determined by geographical factors. Since the worldwide increase in the rate of antibiotic resistance represents a problem of relevance, local surveillance of antibiotic resistance assessed by *H. pylori* culture and antimicrobial susceptibility testing is warranted to guide clinicians in their choice of therapy. Recently developed molecular tests offer an attractive alternative to culture and allow for the rapid molecular genetic identification of *H. pylori* and resistance-associated mutations directly from biopsy samples or bacterial culture material. In the future, tailored therapy based on use of these techniques may allow for successful therapy for *H. pylori* infection.

Keywords

Helicobacter pylori • Antibiotic resistance • Eradication • Amoxicillin • Clarithromycin • Metronidazole

45.1 Introduction

Helicobacter pylori (*H. pylori*) is considered to be the main pathogen that causes benign peptic ulcer and functional dyspepsia related to *H. pylori*-associated inflammation [1–3]. The current International Agency for Research on Cancer (IRAC) of World Health Organization (WHO)

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classifies *H. pylori* as class a carcinogen, and the eradication of *H. pylori* infection is necessary for the treatment and prevention of *H. pylori*-induced disease [4]. Since Marshall and Warren revealed the existence of *H. pylori* in the stomach, numerous clinical trials of eradication of *H. pylori* infection were performed, but the results have remained unsatisfactory for a decade [5, 6]. The effectiveness of the most commonly recommended regimens has declined, and, especially, the effectiveness of the triple regimen containing clarithromycin (CAM) or metronidazole (MNZ), proton pump inhibitor (PPI), and amoxicillin (AMX) has decreased with a corresponding increase in CAM resistance or MNZ [7–16]. Recently, bismuth-containing quadruple regimen and triple therapy based on fluoroquinolone have been adopted as the most frequent rescue regimen after failure of first eradication of *H. pylori*, but the eradication rate of these regimens reported variously and reached up to 60–95 % [17–23]. From now on, to manage with the increase of antibiotic resistance in *H. pylori*, we discussed about the mechanism of antibiotic resistance in *H. pylori* and the efficacy of the tailored therapy for its eradication based on antibiotic susceptibility.

45.2 The Recent Guideline of *H. pylori* Eradication

For several years, meta-analyses and systemic reviews have reported that the effectiveness of the triple regimens containing CAM has decreased over time, corresponding with the increase in CAM resistance [15]. This literature regarding *H. pylori* eradication treatments, the recommended "legacy triple therapy" consisting of a PPI, CAM, and AMX, used as first-line therapy for eradication of *H. pylori* and showed unacceptable eradication rates in the world, recently [5, 24–30].

According to Maastricht guideline reported from European *Helicobacter* Study Group, it is not advisable to use standard triple therapy in areas with CAM resistance of over 15–20 % [31]. It recommends first-line eradication treatment using a CAM- or MNZ-based regimen and an alternative eradication using a bismuth-containing quadruple treatment in areas where prevalence of CAM- or MNZ-resistant strains is low and a bismuth-containing quadruple treatment in areas of high resistance [31]. Recent reported studies showed the new four-drug CAM and MNZ-containing regimens (e.g., sequential, concomitant, or hybrid regimens and 14-day CAM triple therapy in the absence of CAM resistance,) all have similar high levels of successful eradication rate [24, 28, 32–35]. After failure of standard triple therapy, bismuth quadruple regimen and triple levofloxacin protocol are recommended. However, the Maastricht IV Consensus Report recommends that, after a second failure, if culture and susceptibility testing is not possible, molecular genetic tests should be conducted to detect *H. pylori* [31].

45.3 The Patient-Specific Therapy: Why Tailored Therapy Is Needed

Recently, the eradication rates of *H. pylori* infection have decreased resulting in 30–40 % [5, 29, 36–39]. The cure rates of *H. pylori* infection are influenced by several factors such as antibiotic susceptibility, insufficient inhibition of acid secretion (e.g., cytochrome P4502C19 (CYP2C19) genotype, PPI dose, and PPI treatment schedule), bacterial genotypes that reduce virulence (e.g., *cagA*-negative strains and the *vacA* s2 genotype), the environment (e.g., smoking), and compliance [7, 40–42]. In all of these factors, the eradication failure was influenced mainly due to an increase in antimicrobial resistance, especially to CAM [5, 14, 15, 36, 37, 39, 42–45]. Numerous studies have shown that antibiotic resistance substantially impairs the efficacy of anti-*H. pylori* therapy [36, 46]. In the *H. pylori* infection associated with geographic areas, the prevalence of resistance rates appears to be partly determined by geographical factors; the prevalence of CAM resistance and MNZ resistance reported from 11.1 % to 92.4 % and 17.0 % to 70.0 %, respectively, while AMX resistance reported 1.0–2.0 % in worldwide [6, 7, 9, 12, 39, 47–50].

However, the clinical relevance of antibiotic resistance in *H. pylori*-associated diseases is still challenged. Most of all, the critical issue is concerned to resistance of CAM in *H. pylori*. Resistance to CAM is known to be a major cause of failure to eradicate *H. pylori* infection, i.e., point mutations in the 23S rRNA gene at position 2142 or 2143 (A2143G, A2142G, A2142C of domain V) [14, 27, 45, 51, 52]. These resistances seem to substantially reduce the efficacy of all macrolide-containing regimens. The success rates of macrolide-containing dual therapies (with a PPI or bismuth compound) decreased from 68 % (61–75 %) for macrolide-susceptible strains to 33 % (22–45 %) for macrolide-resistant strains [36, 37, 53, 54]. The success rates for macrolide-containing triple therapies (with a PPI and AMX, MNZ, or tinidazole) are high in patients with macrolide-susceptible strains (86 %, 80–92 %), but remain low (25 %, 12–38 %) for macrolide-resistant strains [36, 37, 39, 55]. In the previous reported studies concerned about the resistance to MNZ, the eradication rate of triple therapy was not affected by the MNZ resistance in *H. pylori* [36, 37, 39, 54, 55]. But, there were contradictive reports that the resistance to MNZ in *H. pylori* does have substantial effect on eradication rate. Resistances to AMX, tetracycline, furazolidone, rifabutin, ciprofloxacin, or other related fluoroquinolones have also been held responsible for therapy failure with these drugs [39, 53, 56–59]; however, there are not enough data available yet to make an accurate estimate of the effect of these resistances on treatment success (Table 45.1).

45.4 Detection of Antibiotic Resistance in *H. pylori*

For the purpose of identifying the resistance of *H. pylori*, numerous techniques have been developed to detect antibiotic resistance in *H. pylori*. These methods can be divided in culture and nucleic acid-based assays (Fig. 45.1). Antibiotic susceptibility in *H. pylori* is usually assessed by culture-based methods (e.g., agar dilution, disc diffusion, E-test, breakpoint susceptibility testing, broth micro-dilution method) [39, 60–62], but since knowledge of antibiotic resistance mechanisms in *H. pylori* is growing, several (non-)invasive nucleic acid-based tests have been developed, e.g., restriction fragment length polymorphism (RFLP), mismatch polymerase chain reaction (PCR), immunoassays, real-time PCR, and fluorescent in situ hybridization (FISH) [51, 63–66].

45.4.1 Culture-Guided Method for Antibiotic Susceptibility Test in *H. pylori*

Agar dilution is a reliable method to assess antibiotic susceptibility in *H. pylori*. The US National Committee for Clinical Laboratory Standards (NCCLS) has approved this technique as the method of choice to detect resistance to all commonly used antibiotics for anti-*H. pylori* therapy [67] (Table 45.2). In the agar dilution assay, antibiotic susceptibility is assessed by growing *H. pylori* on agar plates containing two-fold serial dilutions of the antibiotic [39] (Fig. 45.2). This method can also be done in broth, by the so-called broth micro-dilution method [68]. Since both methods are time consuming and not used on a daily basis, the protocols have been simplified; bacteria are grown either in agar or broth containing a critical concentration of the antibiotic necessary to define antibiotic resistance (breakpoint susceptibility testing) [39, 68]. Another cheap and simple method to assess antibiotic susceptibility is the disc diffusion method [39]. In this assay, antibiotic discs are placed on an agar plate with bacteria, and, after incubation, antibiotic susceptibility is determined by measuring the inhibition zone [39]. E-test is a commercially available quantitative variant of the disc diffusion method [60, 69]. However, the results of antibiotic resistance identified by agar dilution, disc diffusion, and E-test were not always consistent [39, 60, 68, 69]. These tests have the methodological difficulty and cost associated with culture and MIC

Table 45.1 Mode of action, resistance mechanisms, and prevalence of resistance among antimicrobials used for treatment of *H. pylori* infection

Antimicrobial	Commonly used compound	Resistance rates	Mode of action	Mechanism of resistance
Nitroimidazoles	MNZ, tinidazole	20–95 %	Reduction of prodrug by nitroreductases leads to formation of nitro anion radicals and imidazole intermediates and subsequent DNA damage	Absence of imidazole reduction caused by reduced or abolished activity of electron transport proteins (e.g., RdxA, FrxA, FdxB)
Macrolides	CAM, erythromycin	0–50 %	Binds 23S rRNA ribosomal subunit, resulting in inhibition of protein synthesis	Point mutations in 23S rRNA genes
Penicillins	AMX	0–30 %	Binding of beta-lactam antibiotic to penicillin-binding proteins (PBP) inhibits cell division	Decreased binding of AMX to PBP D (tolerance) or PBP1A (resistance caused by point mutation in the *PBP1A* gene) and reduced membrane permeability (resistance)
Tetracyclines	Tetracycline	0–10 %	Binding to ribosome prevents association with aminoacyl-tRNA and subsequent protein synthesis	Point mutations in 16S rRNA genes and reduced membrane permeability
Fluoroquinolones	Ciprofloxacin, moxifloxacin, levofloxacin	0–20 %	Inhibition of DNA gyrase and topoisomerases, interfering with DNA replication	Point mutations in the DNA gyrase gene, *gyrA*
Rifamycins	Rifabutin	0–2 %	Binding to RNA polymerase, resulting in transcription inhibition	Point mutations in the RNA polymerase gene, *rpoB*
Nitrofurans	Furazolidone	0–5 %	Reduction of prodrug by nitroreductases, leads to formation of nitro anion radicals and subsequent DNA damage	Unknown
Proton pump inhibitor	Omeprazole, lansoprazole, pantoprazole	Not reported	Inhibits the proton motive force of the bacterium and destabilizes its site of colonization in the stomach	Unknown
Bismuth	Bismuth subcitrate, bismuth subsalicylate, ranitidine bismuth citrate	Not reported	Inhibits protein, ATP, and cell membrane synthesis	Unknown

Adapted from Gerrits et al. [39]
AMX amoxicillin, *CAM* clarithromycin, *MNZ* metronidazole, *RdxA* oxygen-insensitive NAD(P)H nitroreductase, *FrxA* NAD(P)H flavin nitroreductase, *FdxB* ferredoxin-like protein, *PBP* penicillin-binding proteins

tests [39]. However, there are many limitations in using bacterial culture and susceptibility testing to identify resistance in a clinical practice before initiation of actual eradication therapy because it is difficult to culture *H. pylori* and because it takes approximately 10 days to isolate and culture *H. pylori* and measure the minimal inhibitory concentration.

45.4.2 Nucleic Acid-Based Methods for Antibiotic Susceptibility Test in *H. pylori*

The use of PCR, a fast and inexpensive, culture-free method, for susceptibility test was proven to improve the eradication rate when used to create

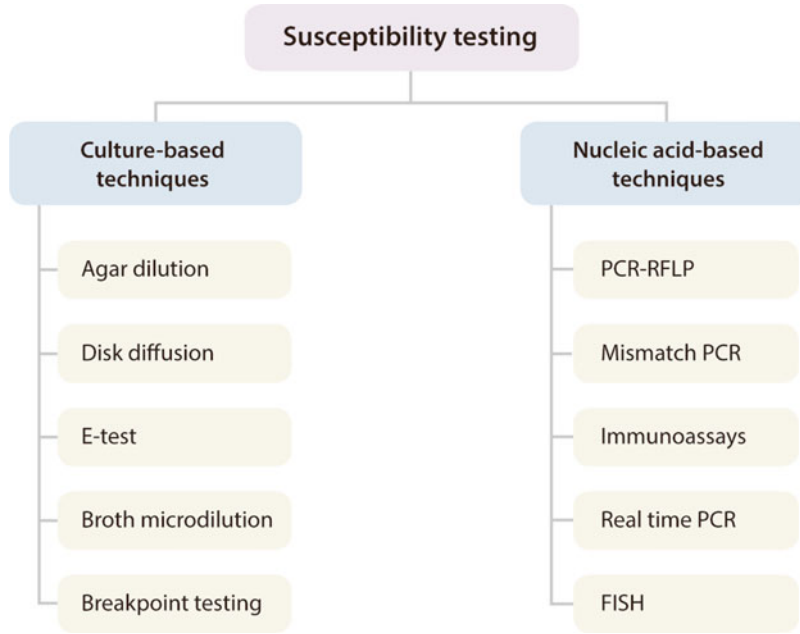

Fig. 45.1 Detection of antibiotic resistance in *H. pylori*. Antibiotic resistance in *H. pylori* can be assessed by culture and nucleic acid-based techniques. *PCR-RFLP* PCR-restriction fragment length polymorphism, *FISH* fluorescent in situ hybridization

tailored therapy. They can now be replaced by rapid molecular methods aiming to the genotypic detection of *H. pylori* and its susceptibility to macrolides. They include a standard PCR and other PCR-based methods including PCR-RFLP, PCR-DNA enzyme immunoassay (DEIA), PCR oligonucleotide ligation assay (OLA), and PCR-line probe assay (LipA) [70, 71]. Real-time PCR assays, representing a powerful advancement of the basic PCR method, have been also developed and are commercially available [71, 72]. These PCR-based culture-free methods are accurate in detecting genotypic CAM resistance (sensitivity and specificity reportedly exceeding 80–90 %) [73] and offer rapidly results as they can be applied directly to biopsy specimens. PCR-RFLP is a PCR-based assay that is commonly used to detect mutational changes in *H. pylori* obtained from biopsy samples or feces [74]. The method is relatively simple and is based on the presence or absence of a restriction site within the amplified DNA fragment. If there is no suitable restriction enzyme available, mutations can be detected using mismatch PCR. Other methods—e.g., DEIA, OLA, preferential homoduplex formation, and LipA—include an additional hybridization step with labeled oligonucleotide probes, specific antibodies, or streptavidin-alkaline phosphatase after the PCR step. It is important that no additional gastric mucosal biopsies are required for PCR testing, as samples originally taken for rapid urease testing have also been utilized [75]. Moreover, freezing specimens for off-site testing may be not necessary, as samples simply stored in a rapid urease test at room temperature for up to 30 days have been successfully used for PCR testing [76]. An alternate approach to DNA amplification and sequencing for the molecular detection of mutations is to use FISH [39]. In this method, fluorescent probes specific to mutations associated with resistance are used for the rapid detection of mutations in paraffin-embedded gastric biopsies. FISH is reported to correlate well with results of culture-based susceptibility testing and has the advantage that it may be offered by pathology services using tissue samples sent for histological analysis [77, 78]. Critically, both PCR-based

Table 45.2 Proposed clinical antimicrobial breakpoints for *H. pylori* Black square represents of the cutoff point of antibiotic-resistant minimal inhibitory concentration (MIC) values

Antibiotics	Resistant (μg/ml)				
A moxicillin	2	1	0.5	0.25	0.125
Azithromycin	4	2	1	0.5	0.25
Ciprofloxacin	4	2	1	0.5	0.25
Clarithromycin	4	2	1	0.5	0.25
M etronidazole	32	16	8	4	2
Tetracycline	16	8	4	2	1
Levofloxacin	2	1	0.5	0.25	0.125
Moxifloxacin	2	1	0.5	0.25	0.125

and FISH methods are highly accurate in detecting genotypic resistance including the heteroresistant status, defined as the coexistence of strains susceptible and resistant to the same antibiotic in the same patient [72, 78, 79].

45.4.3 Which Method Would Be Appropriate for Tailored Therapy in *H. pylori*?

Resistance to CAM is known to be a major cause of failure to eradicate *H. pylori* infection, i.e., point mutations in the 23S rRNA gene at position 2142 or 2143 (A2143G, A2142G, A2142C of domain V) [45, 52, 64]. Therefore, the susceptibility of *H. pylori* strain to CAM can be measured by genetic testing [63, 66, 70, 80, 81]. Like CAM resistance, the quinolone resistance in *H. pylori* is known to be dependent on mutations in the quinolone resistance-determining region (QRDR) of *gyrA* in DNA gyrase or *parC* in topoisomerase IV [16, 39, 82]. With this background, it is believed that a rapid and convenient method for detection of pretreatment *H. pylori* antimicrobial resistance could consist of analyzing the mutation based on nucleic acid techniques [39]. Rapid and culture-free susceptibility tests using PCR or genotypic resistance testing primarily for CAM and levofloxacin are being developed, and it would be easier to perform tailored therapy even in treatment-naïve patients, achieving an eradication rate of >95 % [72]. However, in the case of other antibiotics used for *H. pylori* eradication such as AMX, MNZ, or tetracycline, the loci of mutations causing antimicrobial resistance are known to be alterations in penicillin-binding proteins (PBPs) in AMX resistance, mutational inactivation of the oxygen-insensitive NAD(P)H nitroreductase (RdxA), NAD(P)H flavin nitroreductase (FrxA), ferredoxin-like protein (FdxB) in MNZ resistance, and single-, double-, and triple-base-pair substitutions in the 16S rRNA in tetracycline resistance [36, 59, 83–86]. These point mutations related to resistance to these antibiotics vary, and there is not enough data available to accurately estimate the effect of resistance to these antibiotics. Therefore, culture-guided susceptibility testing (the agar dilution method or E-test) would be a feasible method for selecting the appropriate antibiotic regimens to detect multi-antimicrobial resistance or failure of eradication therapy for *H. pylori* infection under the situation of increased resistance to quinolone and MNZ.

45.5 Efficacy of Tailored *H. pylori* Eradication Therapy Based on Antibiotic Susceptibility

The Maastricht IV Consensus Report recommends that, after a second failure, if culture and susceptibility testing is not possible, molecular genetic tests should be conducted to detect *H. pylori* [31]. But, the clinical evidences of tailored therapy were reduced in first-line eradication (most of all studies, the authors concentrated on the CAM resistance), and there is an increased demand to clarify the efficacy of tailored therapy based on antibiotic susceptibility test. Despite the availability of various methods of susceptibility

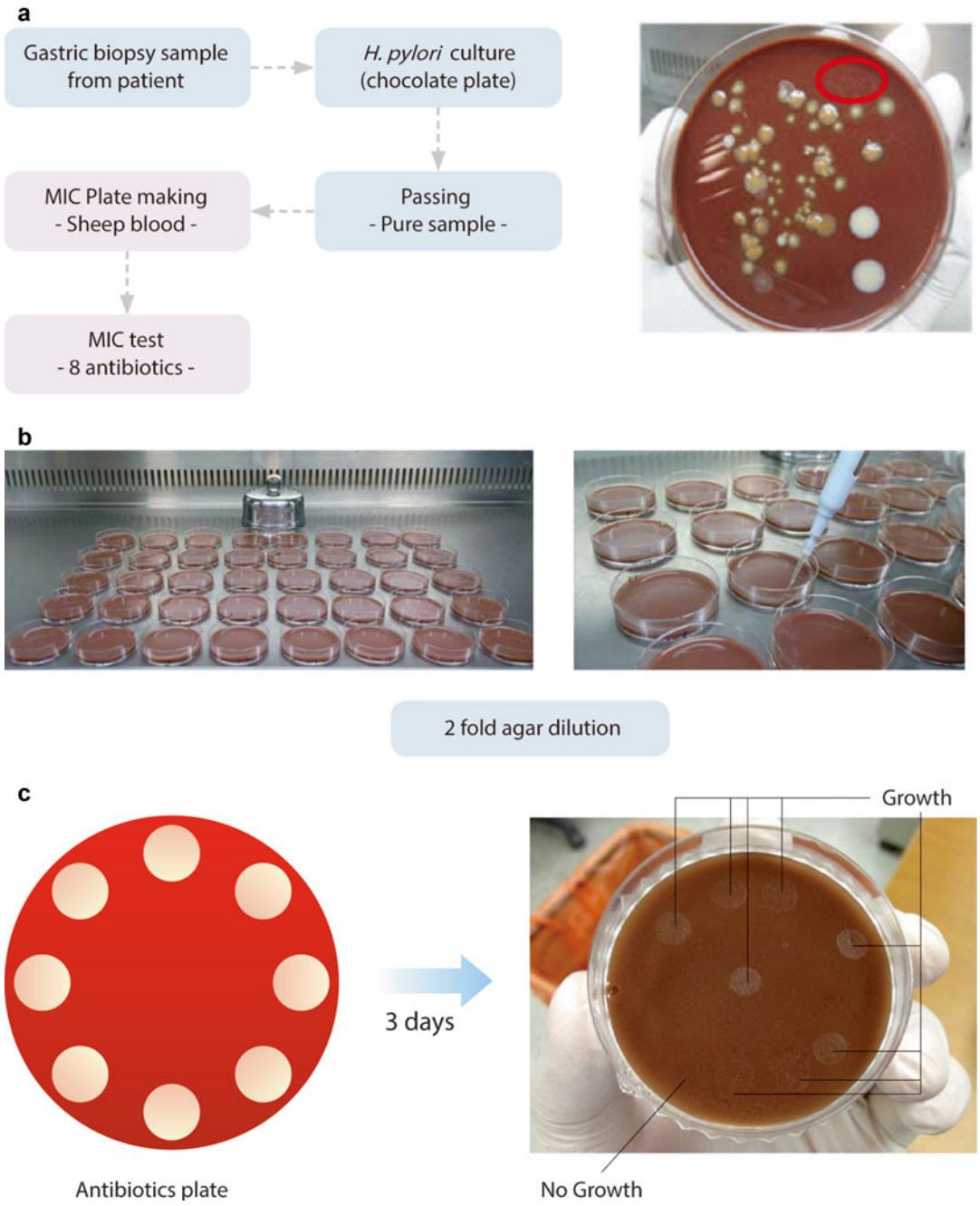

Fig. 45.2 *H. pylori* culture and minimal inhibitory concentration (*MIC*) test. (**a**) For *H. pylori* isolation, gastric biopsy specimens were obtained from the stomach body and antrum during endoscopic examinations. The tissue specimens were cultured on brain-heart infusion agar plates (Difco Laboratories, Detroit, MI., USA) containing 7% horse blood for 3–5 days. Incubation time was extended to 6 or 7 days if the colonies (*red oval*) were too tiny to distinguish. Every *H. pylori* culture was conducted under microaerobic conditions (5% O_2, 10% CO_2, 85% N_2). Cultured bacteria were identified as *H. pylori* by colony morphology, gram staining, and reactions to several enzymatic tests (oxidase, catalase, and urease tests). (**b**) The MIC values of the *H. pylori* isolates to antibiotics were examined using a serial twofold agar dilution method. The bacteria were subcultured on Mueller-Hinton agar supplemented with 5% defibrinated sheep blood for 48 h. The bacterial suspension, adjusted to 1×10^7 CFU, was inoculated directly onto each antibiotic-containing agar dilution plate. (**c**) After 72 h of incubation, the MIC of was determined by proposed clinical antimicrobial breakpoints of *H. pylori*

testing, susceptibility testing is rarely performed, and most patients with *H. pylori* infection receive an empirically chosen treatment. Table 45.3 shows the previous results of culture-guided tailored therapy for eradication of *H. pylori*.

Early evidence on the benefits of molecular-based methods as a genuine basis for tailoring treatment of *H. pylori* infection has been provided by a few recent studies. Two Asian studies have provided data on the potential utility of a tailored therapeutic approach based on the molecular detection of *H. pylori* resistance to CAM. In a landmark study, 300 *H. pylori*-positive patients were randomized to either a 1-week standard regimen or to personalized therapy based on both CYP2C19 and CAM susceptibility status assessed by genetic testing [87]. The intention-to-treat (ITT) eradication rates were significantly higher in the tailored group (96 % vs. 70 %) without an increase of the final per-patient cost for successful eradication. Tailored treatment using a simple PPI/MNZ regimen successfully eradicated the pathogen in 94.3 % vs. 71.4 % using empirical standard treatment [27]. In a larger randomized study (1,232 participants), CAM was replaced by MNZ when genetic resistance to the former was detected by PCR. This tailored approach was significantly more effective as compared to both CAM- and MNZ-based triple therapies given empirically (eradication rates 91.2 %, 75.9 %, and 79.1 %, respectively; $p < 0.001$ for both comparisons) [45]. In a Korean study (114 patients), the ITT eradication rates were 94.7 % (95 % confidence interval [CI], 88.8–100 %) in the culture-guided antimicrobial susceptibility-guided group and 71.9 % (95 % CI, 60.2–83.5 %) in the CAM-based triple therapy group after the initial treatment ($p = 0.002$), whereas the per-protocol (PP) eradication rates were 96.4 % (95 % CI, 91.5–100 %) in the antimicrobial susceptibility-guided group and 73.2 % (95 % CI, 61.5–84.8 %) in the CAM-based triple therapy group ($p = 0.001$) [88].

Many trials are also attempted in *H. pylori* infection by antimicrobial susceptibility in the West. However, many studies reported mixed results regarding the efficacy of tailored therapy based on antimicrobial susceptibility testing after *H. pylori* eradication failure. Toracchio et al. [29] reported that eradication rates in the standard group were, respectively, 81 % and 75 % in 56 patients by PP and ITT analysis, while in the second group they were 98 % and 91 % in 53 patients ($p < 0.05$) in an open label randomized controlled trial. Cosme et al. [89] showed that, in a population with high CAM resistance, a first-line triple

Table 45.3 Summary of the culture-guided tailored therapy data

Line of tailored therapy	Author	Type of therapy	Tailored therapy eradication rates per protocol/intention-to-treat analysis
First	Toracchio et al. [29]	Triple	98 %/91 %
	Molina-Infante et al. [32]	Quadruple	92 %/91 %
	Cosme et al. [89]	Triple	88 %/NR
	Furuta et al. [87]	Triple	97 %/96 %
	Kawai et al. [27]	Triple	94 %/94 %
Second	Yahav et al. [94]	Triple	86 %/63 %
Third	Gasbarrini et al. [91]	Quadruple	77 %/52 %
	Cammarota et al. [90]	Quadruple	92 %/91 %
	Cammarota et al. [90]	Triple	80 %/80 %
	Gomollón et al. [92]	Quadruple	36–52 %/NR
	Vicente et al. [93]	Quadruple	47–74 %/NR
Fourth	Fiorini et al. [68]	Triple	89–90 %/NR

Modified from Cammarota et al. [62]
NR not reported

therapy (including omeprazole, CAM, and AMX) achieves better eradication rates if patients are diagnosed by antimicrobial susceptibility testing (88 % vs. 49 % eradication rate). Molina-Infante et al. [32] compared tailored non-bismuth quadruple (concomitant) therapy to either standard triple therapy (in CAM-susceptible *H. pylori* group) or sequential therapy (in CAM-resistant *H. pylori* group). Tailored concomitant therapy was proven to be better than triple therapy and at least as effective as sequential strategy. Cammarota et al. [90] reported excellent results (90 % success rate) in 94 patients after treatment with a culture-guided, third-line regimen. In contrast, Gasbarrini et al. [91] showed an eradication rate of 77 % or 52 % by the PP or ITT analysis by choosing tailored quadruple therapy as a third-line therapy, based on the in vitro sensitivity to the tested antibiotics. Furthermore, according to two trials conducted in Spain [92, 93], the 14-day quadruple therapy administered as a third-line eradication treatment showed eradication rates ranging from 36 % to 73.6 % depending on the antibiotic regimen used. Taken together, antibiotic resistance rates are rising worldwide and have a significant negative impact on treatment results [12, 16, 34, 48], suggesting that the eradication rate for a third-line therapy could be disappointing even it is administered based on the in vitro sensitivity to the tested antibiotics.

Conclusions

For more than a decade, triple regimens have been the standard of care therapies for *H. pylori* infection. However, in more recent years, worldwide increase in prevalence of macrolide resistance has accounted for the failure of standard therapies for the treatment of *H. pylori* infection. In order to maintain high therapeutic efficacy, regimens with an improved performance against antibiotic-resistant *H. pylori* strains are now recommended as preferred treatment regimens or the fields of genotypic detection of *H. pylori* antimicrobial susceptibility and pharmacogenomics could offer a fascinating new perspective. Accumulating evidence indicates that surveillance of antimicrobial resistance by susceptibility testing is feasible and necessary to inform clinicians in their choice of therapy for management of *H. pylori* infection.

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Levofloxacin or Rifabutin-Based Triple Therapy

46

Hyuk Yoon

Abstract

Levofloxacin-based triple therapy and rifabutin-based triple therapy are regimens which use proton pump inhibitor and amoxicillin as it is in standard triple therapy and replace clarithromycin to levofloxacin and rifabutin, respectively. These two regimens are main ones which we can consider as salvage therapy when first- and second-line eradication therapy fails in *Helicobacter pylori* treatment. Levofloxacin-based triple therapy is known to show higher eradication rate and lower adverse events rate than bismuth quadruple therapy. However, levofloxacin-based triple therapy is challenged by steep increase in resistance and subsequent decrease in eradication rate. With regard to rifabutin-based triple therapy, although resistance rate is very low at the present, whether eradication rate is superior to other regimens is unclear and there is a possibility of fatal adverse events such as bone marrow suppression.

Keywords

Helicobacter pylori • Eradication • Levofloxacin • Moxifloxacin • Rifabutin

46.1 Introduction

When first- and second-line eradication therapy fails in *Helicobacter pylori* (*H. pylori*) treatment, the most frequently mentioned regimens as salvage therapy are levofloxacin-based triple therapy and rifabutin-based triple therapy. These therapies are regimens which use proton pump inhibitor (PPI) and amoxicillin as it is in standard triple therapy and replace clarithromycin to levofloxacin and rifabutin, respectively. Recent European guideline recommends levofloxacin-based triple therapy as second-line therapy for *H. pylori* infection together with quadruple therapy containing bismuth and recommends rifabutin-based triple therapy as one of third-line eradication therapies [1]. Asian guideline also similarly

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comments levofloxacin-based triple therapy and rifabutin-based triple therapy as salvage therapy [2]. The position of the USA is somewhat different with that of Eurasia; the guideline of the USA comments levofloxacin-based triple therapy and rifabutin-based triple therapy as salvage therapy, but it does not recommend them positively [3]. The reason might be due to the limitation that there are scarce studies regarding these regimens in the USA. Like this, although there are some differences, main guidelines worldwide treat levofloxacin- or rifabutin-based triple therapy as one of main salvage therapies. In this chapter, we will review various aspects of levofloxacin-based triple therapy and rifabutin-based triple therapy.

46.2 Levofloxacin Triple Therapy

46.2.1 Theoretical Background

Levofloxacin is antibiotics of fluoroquinolone family and it exerts antibacterial effect by inhibiting DNA gyrase and topoisomerase IV of bacteria [4]. Levofloxacin shows wide antibacterial effects on gram-positive bacteria, gram-negative bacteria, and atypical respiratory pathogen. This antibiotic has received attention since the effect on *H. pylori* infection was demonstrated in the laboratory [5]. Moreover, after levofloxacin was known to be mostly effective for *H. pylori* strains which have resistance to clarithromycin and metronidazole through laboratory and clinical studies, many studies were performed to explore the potency of levofloxacin-based triple therapy for salvage therapy for *H. pylori* infection [6, 7].

46.2.2 Eradication Rate

Levofloxacin-based triple therapy is usually composed of PPI and amoxicillin and levofloxacin 250–500 mg/day instead of clarithromycin in standard triple therapy. The duration of this regimen is 7–14 days. Two meta-analyses regarding levofloxacin-based triple therapy as salvage therapy for *H. pylori* infection were published in 2006. In a meta-analysis which included four randomized controlled trials for 391 subjects, 10-day levofloxacin-based triple therapy was superior to 7-day bismuth quadruple therapy (mean eradication rate 87 % [95 % CI, 82–92 %] vs. 68 % [95 % CI, 62–74 %]; relative risks [RR] for successful eradication 1.41; 95 % CI, 1.25–1.59) [8]. On the other hand, adverse events (RR 0.51; 95 % CI, 0.34–0.75) and drug discontinuation rate due to adverse events (RR 0.30; 95 % CI, 0.10–0.89) of levofloxacin-based triple therapy were lower than those of bismuth quadruple therapy. In the subgroup analysis, the eradication rate of levofloxacin-based triple therapy was higher in 10-day administration than in 7-day administration (mean eradication rate 87 % [95 % CI, 82–92 %] vs. 68 % [95 % CI, 62–74 %]). In the aspect of dosage, there was no significant difference in the eradication rate between 500 mg q.d. (quaque die, every day) and 250 mg b.i.d. (bis in die, twice a day) of levofloxacin. The authors concluded that 10-day triple therapy containing levofloxacin 500 mg q.d. was superior to 7-day bismuth quadruple therapy in the aspect of eradication rate and compliance. Another meta-analysis which included 977 subjects and 14 studies also reported that levofloxacin-based triple therapy was superior to bismuth quadruple therapy in the eradication rate (81 % vs. 70 %; odds ratio [OR], 1.80; 95 % CI, 0.94–3.46), and levofloxacin-based triple therapy showed less adverse events (19 % vs. 44 %; OR, 0.27; 95 % CI, 0.16–0.46) [9]. In this meta-analysis, the eradication rate of 10-day levofloxacin-based triple therapy was higher than that of 7-day levofloxacin-based triple therapy (81 % vs. 73 %, $p < 0.01$).

There are several studies regarding triple therapy using moxifloxacin which is antibiotics of fluoroquinolone family and was developed later than levofloxacin. In a meta-analysis which included four randomized controlled trials regarding 772 subjects, the eradication rate of moxifloxacin-based triple therapy was significantly higher than that of standard triple therapy (mean eradication rate, 84.1 % [318/378] vs. 73.6 % [290/394]; RR, 1.13; 95 % CI, 1.01–1.27; $p = 0.04$) [10]. There was no significant difference in the adverse events between the two regimens (RR, 0.61; 95 %

CI, 0.25–1.48; $p<0.28$). A meta-analysis which compared moxifloxacin-based triple therapy and bismuth quadruple therapy for second-line eradication therapy for *H. pylori* infection in 787 subjects and seven randomized controlled trials also reported that moxifloxacin-based triple therapy is superior to bismuth quadruple therapy in the eradication rate (74.9 % vs. 61.4 %; OR 1.89; 95 % CI, 1.38–2.58; $p<0.001$) [11]. The rate of adverse events and drug discontinuation rate due to adverse events were also lower in moxifloxacin-based triple therapy than in bismuth quadruple therapy (adverse events rate, 10.1 % vs. 27.8 %; drug discontinuation rate, 1.4 % vs. 8.2 %).

46.2.3 Adverse Events

The most common adverse event of levofloxacin is gastrointestinal symptom such as nausea, diarrhea, and constipation. The second common adverse event is one related to central never system such as headache and insomnia. However, most adverse events are mild. Adverse events of moxifloxacin are similar to levofloxacin, and the incidence of adverse events is reported as less than 5 % [12]. The reason that the rate of adverse events of triple therapy containing these fluoroquinolones is higher than that of individual fluoroquinolone is that PPI and amoxicillin which are administrated together also cause various adverse events. Therefore, like the aforementioned meta-analyses, it is not arguable that fluoroquinolone-based triple therapy is superior or at least not inferior to standard triple therapy or bismuth quadruple therapy in the aspect of adverse events and compliance.

46.2.4 Antibiotic Resistance and Limitations

Although European guideline recommends levofloxacin-based triple therapy as one of second-line treatments for *H. pylori* infection, it warned of steeply increasing resistance rate [1]. Actually, resistance to levofloxacin is already a big problem in some countries. For example, the eradication rate of first-line and second-line levofloxacin-based triple therapy in Korea was 69.8 % [13] and 51.6–65.5 % [13, 14], respectively; these are much lower than those from earlier meta-analyses which evaluated the results from mostly European studies. These disappointing eradication rates of levofloxacin-based triple therapy in Korea are related with increase in fluoroquinolone resistance during the same period. The first and second resistance rate to levofloxacin and moxifloxacin was 4.7 % and 16.7 %, respectively, during 2003 and 2005; they sharply increased to 28.1 % and 50.0 % during 2009 and 2012 [15]. Fluoroquinolone resistance in *H. pylori* strain is usually acquired by mutation of *gyrA* gene [16]. The steep increase of antibiotic resistance and subsequent decrease of eradication rate in Korea are presumed to be related with frequent administration of these antibiotics for respiratory and otolaryngologic diseases. Therefore, we should be cautious to use fluoroquinolone-based triple therapy in patients who have history of these diseases, and there are some limitations to use fluoroquinolone-based triple therapy as second-line treatment for *H. pylori* infection in countries where the resistance rate of fluoroquinolone is over 30 %.

46.3 Rifabutin Triple Therapy

46.3.1 Theoretical Background

Rifabutin is one of rifamycin derivatives. This antibiotics is structurally similar to rifampin (rifampicin) which is an antituberculous drug and shares many characteristics with rifampin [17]. Rifabutin exerts bactericidal effect by inhibiting beta-subunit of DNA-dependent RNA polymerase which is encoded by *rpoB* gene in *H. pylori* strain [18]. The reason that rifabutin receives attention in *H. pylori* treatment is that these antibiotics show no cross-resistance with clarithromycin, and because it is not a commonly used antibiotic, the resistance rate is very low in *H. pylori* infection [19]. In addition, it is known that rifabutin shows stable antibiotic activity in low pH in the stomach [20, 21].

46.3.2 Eradication Rate

Rifabutin was usually tried as triple therapy combined with PPI and amoxicillin in *H. pylori* infection. Related studies were mostly performed after 2000 and meta-analysis was recently published [17]. Many studies were performed in European countries, especially in Germany, Italy, and Spain. The dosage is usually 300 mg/day (150 mg b.i.d. or 300 mg q.d.) and treatment duration was mostly 7 or 10 days. The number of subjects in most studies was less than 100, and all studies were regarding over second-line and third-line treatment. When the data regarding 21 studies and 1,008 subjects was analyzed, eradication rate by intention-to-treat (ITT) analysis was mean 73 % (95 % CI, 67–79 %). In the subgroup analysis for 223 patients who underwent rifabutin-based triple therapy as second-line treatment for *H. pylori* infection, eradication rate was mean 79 % (95 % CI, 67–92 %); the eradication rate was higher in studies with long administration (10–12 days) than in the studies with short administration (7 days) (92 % vs. 69 %). In the subgroup analysis for 342 patients who underwent rifabutin-based triple therapy as third-line treatment, eradication rate by ITT analysis was mean 66 % (95 % CI, 55–77 %). There are few studies which directly compared rifabutin-based triple therapy with other regimens. For the present, three studies which compared rifabutin-based triple therapy with bismuth quadruple therapy as second-line treatment for *H. pylori* infection were published. However, the results were contrary to each other [22–24]. There are just two studies which compared rifabutin-based triple therapy with levofloxacin-based triple therapy as third-line treatment. First, the Spanish study compared the effect of 10-day rifabutin-based triple therapy and levofloxacin-based triple therapy in 40 patients who failed second-line treatment of *H. pylori* infection; the eradication rate by ITT analysis was 45 % and 85 %, respectively [25]. The authors reported that levofloxacin-based triple therapy is superior to rifabutin-based triple therapy. However, this study was not randomized controlled study but open-labeled study. In addition, the number of subjects was too small and the eradication rate of rifabutin-based triple therapy group was unexpectedly low. Another study was performed in Korea [26]. The eradication rate of rifabutin-based triple therapy and levofloxacin-based triple therapy was 71.4 % and 57.1 %, respectively; there was no significant difference in the eradication rate between two regimens ($p = 0.656$). However, the total sample size of this study was just over 20 and the study design was retrospective. In addition, although the treatment duration of levofloxacin-based triple therapy was constant as 10 days, treatment duration of rifabutin-based triple therapy varied as 7–14 days. Therefore, it is very difficult to make final conclusion regarding the efficacy of rifabutin-based triple therapy.

46.3.3 Adverse Events

The most common adverse events of rifabutin are rash and gastrointestinal symptoms such as nausea, vomiting, and dyspepsia. Besides, it can cause neutropenia or anemia and the color of urine can be changed as red [27]. These adverse events are known to be dose dependent [28]. According to the meta-analysis, the rate of adverse events in *H. pylori* eradication therapy was mean 22 % (95% CI, 19–25%) [17]. Bone marrow suppression is clinically important adverse event. However, it is known that this adverse event is not common, and it usually occurs when high dose above 600 mg/day is administrated and the treatment duration is long. For *H. pylori* eradication therapy, the rate of bone marrow suppression was reported as 1.5–3.0 % in some studies. However, leukopenia was usually recovered spontaneously in a few days, and there was no case in which it causes serious problem. Infection or adverse events related with leukopenia during the use of rifabutin in *H. pylori* eradication therapy have not been reported to date. However, because the total number of tested subjects is still just around one thousand, there is a possibility that these adverse events occur in the future. Therefore, physician should make sufficient explanation for adverse events to the patient before trying rifabutin-based triple therapy. In addition, they should check complete blood count

when symptoms like fever occur and bone marrow suppression is suspected during treatment. Besides, uveitis was reported when rifabutin was administrated together with clarithromycin, ethambutol, or fluconazole [29]. However, this adverse event is common in immunocompromised patients or in patients who received antituberculous treatment for a long period. There has been no case report regarding *H. pylori* eradication treatment.

46.3.4 Antibiotic Resistance and Limitations

According to the review articles, primary resistance of *H. pylori* for rifabutin was very low as 0.6–1.4% [17, 30]. Considering that *H. pylori* can acquire resistance to rifabutin by point mutation of *rpoB* gene [31], the reason that resistance rate of *H. pylori* to rifabutin is very low is that these antibiotics have been used clinically for the very limited indications. Therefore, if rifabutin were used widely for *H. pylori* treatment, similar to other antibiotics, there is a possibility that resistance rate increases in the future.

In addition, because rifabutin and rifampicin inhibit *H. pylori* by the same mechanism and they show cross-resistance, if a patient has a history of previous exposure to rifampicin due to the reason such as antituberculous treatment, the possibility that the effect for eradication decreases is high [32]. This acts as a very big limitation in the countries which show high incidence of tuberculosis. In addition, the price of this drug is expensive, and we should be cautious about the fact that sometimes serious leukopenia or thrombocytopenia was reported. Even though there has been no report regarding resistance induction after short administration such as in *H. pylori* treatment, if rifabutin were generally used for *H. pylori* infection, there is a possibility that resistance of *Mycobacterium tuberculosis* for rifabutin increases.

Conclusions

Levofloxacin-based triple therapy and rifabutin-based triple therapy are main regimens which we can consider as salvage therapy when first- and second-line eradication therapy fail in *H. pylori* treatment. However, levofloxacin-based triple therapy is challenged by steep increase in resistance and subsequent decrease in eradication rate. With regard to rifabutin-based triple therapy, although resistance rate is very low at the present, whether eradication rate is superior to other regimens is unclear and there is a possibility of fatal adverse events such as bone marrow suppression.

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Probiotics

47

Sung Wook Hwang

Abstract

Various microorganisms are used as probiotics of which lactic acid-producing bacteria such as *Lactobacillus* spp., *Bifidobacterium* spp., etc. are most commonly used. The effects of probiotics are carried out through immunological or nonimmunological mechanisms. Production of antibiotic substances, competition with *Helicobacter pylori* (*H. pylori*) for adhesion receptors, stimulation of mucin production, stabilization of the gut mucosal barrier, etc. are being suggested as nonimmunological mechanisms. Although improvement in *H. pylori* eradication rate, decrease in antibiotic-associated side effects, reduced *H. pylori* colonization, and decreased *Helicobacter*-induced gastritis are being reported in human studies with probiotics, further investigation is needed as results are shown to differ among studies.

Keywords

Helicobacter pylori • Probiotics • *Lactobacillus* • *Bifidobacterium*

47.1 Introduction

Probiotics are "live, nonpathogenic microbial feeds or food supplements that exert a positive influence on their host by altering his microbial balance" [1]. Known as the "father of lactic acid bacteria," Nobel Prize-winning Metchnikoff hypothesized in *The Prolongation of Life* in 1907 that indigested food and feces in the intestine produce toxin and shorten one's life and mentioned that lactic acid fermented milk diminishes toxin and supports prolongation of human life based on the fact that there are many people who live long in Bulgaria and the Caucasus where people regularly consume lactic acid fermented milk. Currently, various microorganisms are used as probiotics, and the most commonly used probiotic is lactic acid-producing *Lactobacillus* spp. Others include *Bifidobacterium* spp., *Bacillus* spp., *Escherichia coli*, *Saccharomyces boulardii*, *Streptococcus thermophilus*, etc. [2].

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The biological mechanisms of action of probiotics are known to include synthesis of antimicrobial substances, competitive inhibition of pathogenic bacteria, modification of toxins, immune regulation, etc. [1, 3].

Probiotics have been continuously studied for its treatment for and preventive effects on inflammatory diseases, such as atopy, acute infectious diarrhea, and inflammatory bowel disease. Probiotics are also being reported to be helpful for the treatment against *Helicobacter pylori* (*H. pylori*) infection [1, 4]. Although there are various antibiotics used as *H. pylori* eradication treatment, their results are insufficient. In addition, as the issue of antibiotic resistance is being raised, the expectation on probiotic supplementation has been increased. In this chapter, we have aimed to explore the effect of probiotics against *H. pylori* infection.

47.2 Mechanism of Action of Probiotics on *H. pylori* Infection

Studies on the mechanism of action of probiotics are being conducted through various in vitro studies. Largely, they are classified as immunological mechanism and nonimmunological mechanism. Different probiotics show various immune responses according to the host's immune status [5].

47.2.1 Immunological Mechanisms

Probiotic immunomodulation is achieved through a balance of pro- and anti-inflammatory cytokines, and animal studies have shown that probiotic reduces gastric inflammation [1, 4]. Bacteria binds to receptors on the surface of epithelial cells, such as Toll-like receptors, initiating various immunological mechanisms [6]. Initially, the secretion of pro-inflammatory cytokines, such as interleukin (IL)-8, causes migration of neutrophils and monocytes to the gastric mucosa. Activated monocytes and dendritic cells then secrete a variety of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and IL-6 [7]. Meanwhile, probiotics eventually regulate the immune response and reduce gastric inflammation by interacting with epithelial cells producing anti-inflammatory cytokines [8]. In 1997, Kabir et al. have reported that *Lactobacillus salivarius* inhibits IL-8 secretion stimulated by *H. pylori* [9]. Several animal studies have shown that probiotics decreased specific IgG antibodies to *H. pylori* [10, 11] and increased IgA production in the intestinal epithelial cells [7, 12]. In addition, it has been reported that large amounts of *Lactobacillus acidophilus* inactivate Jak1/Stat1 pathway reducing *H. pylori*-induced Smad7 transcription and inhibit NF-κB production leading to a reduction of gastric inflammation [13]. However, it is difficult to generalize these effects on immune responses as probiotics show various responses depending on the host's immune status.

47.2.2 Nonimmunological Mechanisms

Upon entrance of bacteria into the stomach, nonimmunological barrier such as gastric acid and gastric mucosal barrier acts as a first line of defense [14]. Studies have reported probiotics effect of strengthening these nonimmunological barriers and suggested the production of antimicrobial substances, coaggregation with the bacteria, competing with *H. pylori* for adhesion receptors, stimulating mucin production and strengthening the gastric mucosal barrier through this mechanism [4].

Several *Lactobacilli* synthesize bacteriocin-related antimicrobial substances, and other substances secreted include those which inhibit bacteria such as organic acids, hydrogen peroxide, and carbon dioxide [15–17]. It has also been reported that large amounts of lactate, which is produced by probiotics, inhibit *H. pylori*. Produced lactate not only shows antimicrobial effects by lowering the pH but also inhibits urease of *H. pylori* [10]. However, these inhibitory effects differ depending on the strain.

H. pylori adhering to the epithelial cells is the most important phase of developing *H. pylori*-associated disease. In a cell line study, it has been reported that *Lactobacillus johnsonii* La1, *L. salivarius*, *L. acidophilus*, etc. inhibit *H. pylori* adhesion to the epithelial cell lines [9, 18]. This inhibitory effect is exerted through nonspecific blockage of receptor sites or specific binding to carbohydrate receptors by probiotics [4] or showing antiadhesive activity through antimicrobial substances produced [14].

Mucous produced by epithelial cells combines with bacteria and interferes with the adhesion of bacteria on the epithelial cells. *H. pylori* gastritis often shows reduced mucous secretion from damaged epithelium [19]. It has been reported that *Lactobacillus plantarum* and *Lactobacillus rhamnosus* increase the expression of MUC2 and MUC3 and mucous secretion from colon epithelial cells [20, 21].

47.3 Probiotic Treatment of *H. pylori* Infection

L. johnsonii La1 and *L. rhamnosus* GG are most commonly used in probiotic studies in animals and human in addition to *Lactobacillus casei*, *L. acidophilus*, *L. brevis*, and *Bifidobacterium* [4]. The effects of probiotics on *H. pylori* infection have been assessed mainly through rapid urease test, urea breath test, serology test, stool antigen test, and biopsy, etc.

47.3.1 Studies Using Animals and Cell Line

Effects of various probiotics on *H. pylori* infection in animal studies have been reported [9, 11, 22–26]. Oral administration of *L. salivarius* by *H. pylori*-infected BALB/c mice has shown preventive and treatment effects [9, 11]. Also, Johnson-Henry et al. showed reduction in *H. pylori* colonization and *H. pylori*-induced gastritis in a C57BL/6 murine model administered with *L. acidophilus* R0052 and *L. rhamnosus* R0011 [22]. A Mongolian gerbil model also showed the effects of probiotics on *H. pylori* infection through probiotics-induced reduction of *H. pylori* colonization, reduction of mucin infection, and change in blood flow and gastrin-somatostatin [23]. Sgouras et al. reported that administration of *L. casei* strain Shirota and *L. johnsonii* La1 in a C57BL/6 model reduced *H. pylori* gastritis. *L. casei* strain Shirota also reduced *H. pylori* colonization [24]. It has also been shown that in clarithromycin-resistant *H. pylori* infection, *L. gasseri* reduced *H. pylori* colonization [25].

Recently, it has been reported that coculture with *H. pylori* and *Lactobacilli* inhibits *H. pylori* growth and that supernatant lacking cells and living *Lactobacilli* both have anti-*H. pylori* activity [26]. Hwang et al. reported that supernatant produced by the cultivation of *L. acidophilus* and *L. plantarum* reduces *H. pylori* infection in the gastric epithelial cell line [27]. The precise mechanisms of the probiotic bacteria-producing molecules effective for the treatment of *H. pylori* infection are needed to be investigated in the further study.

47.3.2 Human Studies

Results of numerous clinical studies have been presented on the role of probiotics regarding treatment of *H. pylori* infection, and well-systematized studies on relatively large number of adult patients have been summarized [28–41] (Tables 47.1 and 47.2). Probiotics have been studied as supplements to *H. pylori* antibiotic treatment or as substitution for antibiotics, and studies have been mainly focused on its ability to improve the eradication rate. Another matter of interest of studies has been how well probiotics could reduce side effects of antibiotics, such as gastrointestinal disorders induced by the change in intestinal bacterial flora.

In 2000, Canducci et al. performed the first human study showing improvement of *H. pylori* eradication rate in the patient group treated with *L. acidophilus* LB [34] (Table 47.1). However, it did not alleviate side effects of antibiotics. Afterward, Armuzzi et al. showed reduction of

Table 47.1 Summary of study results on probiotics-supplemented *H. pylori* infection treatment

Probiotics	*H. pylori* eradication treatment	Number of patients	Study design	Eradication rates	Side effects	References
Lactobacillus acidophilus LB	Clarithromycin + amoxicillin + PPI	120	O, R	↑	↔	Canducci et al. [34]
Lactobacillus GG	Clarithromycin + tinidazole + PPI	120	DB, P, R	↔	↓	Armuzzi et al. [33]
Lactobacillus- and *Bifidobacterium*-containing yogurt (AB-yogurt)	Clarithromycin + amoxicillin + PPI	160	O, R	↑	↓	Sheu et al. [40]
Lactobacillus GG *Saccharomyces boulardii Lactobacillus* spp., *Bifidobacteria*	Clarithromycin + tinidazole + PPI	85	DB, P, R	↔	↓	Cremonini et al. [32]
Lactobacillus casei subsp. *casei* DG	Bismuth + amoxicillin + tinidazole + PPI (second-line quadruple)	70	R	↔	↓	Tursi et al. [31]
Lactobacillus acidophilus HY2177, *Lactobacillus casei* HY2743, *Bifidobacterium longum* HY8001, *Streptococcus thermophilus* B-1 (Will yogurt)	Clarithromycin + amoxicillin + PPI	347	O, R	↑	↔	Kim et al. [30]
Lactobacillus acidophilus HY2177, *Lactobacillus casei* HY2743, *Bifidobacterium longum* HY8001, *Streptococcus thermophilus* B-1 (Will yogurt)	Moxifloxacin + amoxicillin + PPI (second-line quadruple)	337	O, R	↔	↔	Yoon et al. [29]
Lactobacillus reuteri	Levofloxacin + amoxicillin + PPI (second-line quadruple)	90	R	↑	↓	Ojetti et al. [28]

Modified from Patel et al. [4]
PPI proton pump inhibitor, *O* open label, *R* randomized, *DB* double blind, *P* placebo controlled, ↑ increase, ↓ decrease, ↔ no effect

side effects, such as diarrhea and nausea, through *L. rhamnosus* GG administration [33, 42]. Sheu et al. reported that administration of *L. acidophilus* La5 and *B. lactis* Bb12 improves the eradication rate and reduces side effects of antibiotic treatment [40]. Also in Korea, Kim et al. showed improvement in eradication rate with yogurt including *L. acidophilus*, *L. casei*, *Bifidobacterium longum* and *Streptococcus thermophilus* in addition to the standard triple therapy [30]. Effects of probiotics have also been reported in patients with secondary treatment due to *H. pylori* antibiotics tolerance [28, 29], and Ojetti et al. reported that *Lactobacillus reuteri* in addition to levofloxacin, amoxicillin, and esomeprazole secondary triple therapy improved the eradication rate and reduced side effects [28].

A few studies have shown the effects of probiotics on *H. pylori* infection in pediatrics [43, 44]. In 2005, Sykora et al. performed a double-blinded randomized clinical study on 86 *H. pylori*-positive pediatrics and showed improve-

Table 47.2 Summary of study results using probiotics as *H. pylori* infection treatment)

Probiotics	Number of patients	Study design	Results	References
Lactobacillus acidophilus	15	O	Eradication rate ↑, *H. pylori* colonization ↓	Mrda et al. [41]
Lactobacillus johnsonii La1	53	DB, P, R	Eradication rate ↔, urease activity ↓, *H. pylori* colonization ↓	Felley et al. [39]
Lactobacillus johnsonii Lj1	50	DB, P, R	Eradication rate ↔, *H. pylori* colonization ↓, gastritis ↓	Pantoflickova et al. [38]
Lactobacillus- and *Bifidobacterium*-containing yogurt (AB-yogurt)	70	O	Eradication rate ↔, urease activity ↓, *H. pylori* colonization ↓, gastritis ↓	Wang et al. [37]
Bifidobacterium bifidum	79	DB, P, R	Eradication rate ↑, ΔUBT ↓, PG I ↓	Miki et al. [36]
Lactobacillus reuteri ATCC 55730	40	DB, P, R	Eradication rate ↔, ΔUBT ↓	Francavilla et al. [35]

Modified from Patel et al. [4]
O open label, *R* randomized, *DB* double blind, *P* placebo controlled, *UBT* urea breath test, *PG* pepsinogen, ↑ increase, ↓ decrease, ↔ no effect

ment in eradication rate when given milk with *L. casei* DN-114 001 (OAC-LC) in addition to the existing standard triple therapy (clarithromycin, amoxicillin, omeprazole) [44]. In another double-blinded randomized study on 40 pediatric patients, addition of *L. reuteri* ATCC 55730 to clarithromycin, tinidazole, and omeprazole treatment showed reduced antibiotic-related side effects [43].

Various studies have been conducted on whether probiotics can substitute antibiotic treatment (Table 47.2). In 1998, Mrda et al. performed a study on 15 *H. pylori*-positive patients who were given *L. acidophilus* fermented milk. Among 14 patients who completed the study, 6 patients showed *H. pylori* eradication [41]. Afterward, Michetti et al. reported reduced activity through administration of *L. acidophilus* (*johnsonii*) La1 culture supernatant [45], and two other studies on *L. acidophilus* (*johnsonii*) also showed similar results [38, 39]. However, these studies did not show *H. pylori* eradication effects of probiotics. In a double-blinded randomized study performed by Imase et al., inhibition of *H. pylori* activity and density were shown through urea breath test following administration of *L. reuteri* [46], and Francavilla et al. also reported similar study results on *L. reuteri* [35].

47.4 Safety

Probiotics are known to be safe and regarded as GRAS (generally regarded as safe) by the WHO [4]. In fact, *Lactobacilli* and *Bifidobacteria* are part of normal bacterial flora, and it has been shown that *Bifidobacteria* do not have particular side effects when taken by healthy people and do not have any effects on the immune status [47]. However, in 2008, Besselink et al. reported that probiotics intake by acute pancreatitis patients does not reduce infection complications and rather increase the mortality rate [48] and several *Lactobacilli* bacteremia have also been reported [49]. For this reason, caution is needed when patients take probiotics.

Conclusions

Studies are conducted on the role of probiotics in the treatment for *H. pylori* infection. As probiotics are produced as medicine and dairy products, they are safe and easy to take and

show various effects in human studies. However, the effects of probiotics are still controversial as the results differ from study to study. Further studies are needed to investigate probiotics effects on eradication of *H. pylori*.

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Treatment Guidelines

48

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Abstract

Peptic ulcer disease and gastric mucosa-associated lymphoid tissue (MALT) lymphoma are classical indications for *Helicobacter pylori* (*H. pylori*) eradication treatment, which is recommended after early gastric cancer resection to prevent its recurrence. There is as yet no consensus about the prophylactic eradication treatment in case of chronic atrophic gastritis, functional dyspepsia, and family history of gastric cancer, and guidelines of different countries represent different views. The scope of indications for eradication treatment has been expanded. In Japan, for example, *H. pylori*-related gastritis has been added as indication for eradication treatment in an attempt to reduce the risks for *H. pylori*-related gastric cancer to the maximum possible extent. In the treatment of *H. pylori* infection, no firm countermeasures against decreasing eradication rate of standard triple therapy have yet been presented. Continuous research is thus necessary to develop improved alternative therapies and reestablishing corresponding therapy guidelines.

Keywords

Helicobacter pylori • Guideline • Peptic ulcer disease • MALT lymphoma • Early gastric cancer

48.1 Introduction

Helicobacter pylori (*H. pylori*) infection has little tendency to spontaneous cure, and its eradication is only possible through adequate treatment including antibiotics. However, since the majority of individuals infected with *H. pylori* do not show clear clinical symptoms or develop serious diseases such as peptic ulcer disease, gastric cancer, and gastric mucosa-associated lymphoid

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tissue (MALT) lymphoma, it is important to set clear criteria for selecting the treatment target population among those infected. However, researchers hold many different views about the *H. pylori* eradication treatment target group, and each country has its own diagnosis and treatment guidelines to provide adequate treatment for *H. pylori* infection, taking into account national and regional features in establishing standard therapy [1–4]. The purpose of this work is to investigate the eradication treatment target population and *H. pylori* treatment strategies, thereby focusing on the recently revised guidelines.

48.2 South Korean Guidelines (2013)

In South Korea, after the first publication [5] of consensus about the indications for the treatment of *H. pylori* infection in 1998 by the Korean Society of Helicobacter and Upper Gastrointestinal Research (now Korean College of Helicobacter and Upper Gastrointestinal Research), new treatment guidelines were released in 2009 based on research and literature [6], and its new version was released in 2013, revised on the basis of a systematic literature review [1].

The 2013 guidelines strongly recommend eradication treatment for the following target groups: *H. pylori*-infected patients with peptic ulcer disease and gastric MALT lymphoma, patients with endoscopic resection of early gastric cancer (EGC), and patients with chronic idiopathic thrombocytopenic purpura. Eradication treatment is additionally recommended for patients with functional dyspepsia, family history of gastric cancer, patients with atrophic gastritis and intestinal metaplasia, and patients with medical history of peptic ulcer disease under long-term medication of aspirin to prevent recurrence [1] (Table 48.1). In particular, recommended for eradication treatment are also patients with functional dyspepsia (Level of evidence: A, Grade of recommendation: 2), atrophic gastritis and intestinal metaplasia (Level of evidence: C, Grade of recommendation: 2), medical history of peptic ulcer disease under long-term use of low-dose aspirin (Level of evidence: C, Grade of recommendation: 1), thus expanding the indications for eradication treatment in comparison with 2009 guidelines [1].

As first-line treatment, standard triple therapy is recommended in general, and bismuth quadruple therapy is recommended in regions suspected of clarithromycin resistance. In cases where triple therapy fails, bismuth quadruple therapy is recommended as rescue therapy, and if bismuth quadruple therapy fails as first-line treatment, medication of two or more additional antibiotics is recommended [1] (Table 48.2). At the present time, with no therapy better than conventional triple therapy available yet, existing standard triple therapy is recommended as first-line treatment subject to future development of alternative therapy capable of complementing its drawbacks [1]. However, treatment failure rate is increasing due to increasing resistance [7, 8], and a recent meta-analysis [9] highlights the urgency of developing a new therapy, with the eradication rate of triple therapy significantly reduced over the past 10 years.

48.3 Japanese Guidelines (2013)

The Japanese *H. pylori* treatment guidelines revised in 2013 added a broad-spectrum condition termed "*H. pylori*-related gastritis" to the list of indications for eradication treatment with firm intent to provide the infected individuals with early eradication of *H. pylori* at a reversible stage [2] (Table 48.1). Through this expansion of the scope of indications for the treatment of *H. pylori* infection, the entire population infected with *H. pylori* have been included in the eradication treatment target population since February 2013 in Japan with firm intent to reduce the prevalence of gastric cancer and *H. pylori* infection rate to the levels of Western advanced countries in 20–30 years by providing the infected patients with eradication treatment and those at risk of infection with opportunities to prevent it [10]. A systematic literature review in Japan reported that *H. pylori* eradication treatment can reduce

Table 48.1 Indications for *H. pylori* eradication treatment

Country (year)	Indication for *H. pylori* eradication	Country (year)	Indication for *H. pylori* eradication
Korea (2013) [1]	Strongly recommended Peptic ulcer disease Low-grade gastric MALT lymphoma After resection of EGC Idiopathic thrombocytopenic purpura	Japan (2013) [2]	Japanese national health insurance system Peptic ulcer disease After resection of EGC Gastric MALT lymphoma Idiopathic thrombocytopenic purpura *H. pylori*-related gastritis
	Recommended Functional dyspepsia Family history of gastric cancer Atrophic gastritis/intestinal metaplasia Long-term aspirin/NSAIDs	Europe (2012) [4]	Gastroduodenal diseases Peptic ulcer disease MALT lymphoma After resection of EGC Gastritis with preneoplastic conditions Functional dyspepsia Prior to NSAIDs therapy/ additional to PPI therapy In "aspirin" users with history of peptic ulcer In patients on long-term PPI
China (2013) [3]	Strongly recommended Peptic ulcer disease (regardless of activeness or complications) Gastric MALT lymphoma		Extragastric diseases Idiopathic thrombocytopenic purpura Iron deficiency anemia Vitamin B12 deficiency
	Recommended Chronic gastritis with dyspepsia Chronic gastritis with mucosal atrophy/erosion EGC resected endoscopically or by subtotal gastrectomy Long-term use of PPI Family history of gastric cancer Planning to take long-term NSAIDs (including low-dose aspirin) Iron deficiency anemia of unknown causes Idiopathic thrombocytopenic purpura Other *H. pylori*-related disease (lymphocytic, gastritis, Menetrier disease, gastric hyperplastic polyps) Requested by individual patient		

H. pylori Helicobacter pylori, *MALT* mucosa-associated lymphoid tissue, *EGC* early gastric cancer, *NSAIDs* nonsteroidal anti-inflammatory drug, *PPI* proton pump inhibitor

the incidence of gastric cancer by about one-third [11], and prospective studies with 10-year post-treatment follow-ups reported that successful eradication treatment significantly improved histological inflammation or atrophy [12, 13]. The timing for eradication plays an important role in disease prevention. For example, Asaka et al. [14] reported that *H. pylori* eradication treatment at the age of 40 years or earlier can achieve gastric cancer prevention rate close to 100 % because of low prevalence of atrophic gastritis till the age of 40, whereas older individuals (≥50 years) have higher incidence of atrophic gastritis and thus are at higher risks of developing gastric cancers even

Table 48.2 *H. pylori* eradication treatment regimen

		Korea (2013) [1]	Japan (2013) [2]	China (4th, 2013) [3]	Europe (Maastricht IV, 2012) [4]
First-line treatment	Low Clari-R	PPI (standard dose b.i.d.) Clarithromycin (500 mg b.i.d.) Amoxicillin (1,000 mg b.i.d.) For 7–14 days	PPI (standard dose b.i.d.) Clarithromycin (200–400 mg b.i.d.) Amoxicillin (750 mg b.i.d.) For 7 days	PPI (standard dose b.i.d.) Clarithromycin (500 mg b.i.d.) Amoxicillin (1,000 mg b.i.d.)/ metronidazole (400 mg b.i.d.) For 7–14 days	PPI-clarithromycin-amoxicillin/ metronidazole or bismuth quadruple
	High Clari-R	PPI (standard dose b.i.d.) Bismuth (120 mg q.i.d.) Tetracycline (500 mg q.i.d.) Metronidazole (500 mg t.i.d.) For 7–14 days		PPI (standard dose b.i.d.) Bismuth (120 mg q.i.d.) Tetracycline (500 mg q.i.d.) Metronidazole (500 mg t.i.d.) For 7–14 days	Bismuth quadruple if no available: non-bismuth quadruple (either sequential or concomitant)
Second-line treatment	Low Clari-R	PPI (standard dose b.i.d.) Bismuth (120 mg q.i.d.) Tetracycline (500 mg q.i.d.) Metronidazole (500 mg t.i.d.) For 7–14 days	PPI (standard dose b.i.d.) Metronidazole (250 mg b.i.d.) Amoxicillin (750 mg b.i.d.) For 7 days	PPI (standard dose b.i.d.) Bismuth (220 mg b.i.d.) Tetracycline (750 mg b.i.d.) Metronidazole (400 mg b.i.d.) For 10–14 days	Bismuth quadruple or PPI-levofloxacin-amoxicillin
	High Clari-R	Combination of two/more antibiotics without prior exposures experiences		Antibiotics without using first-line treatment	PPI-levofloxacin-amoxicillin

Clari-R clarithromycin resistance, *PPI* proton pump inhibitor, *b.i.d.* bis in die (twice a day), *t.i.d.* ter in die (three times a day)

after eradication treatment, which necessitates posttreatment follow-up with regular endoscopy depending on the evidence and extent of atrophic gastritis [2, 14].

The Kyoto Global Consensus Meeting held on 31 January to 1 February of 2014 produced proceedings indicative of many radical changes regarding *H. pylori*-induced gastritis [15]. The following summarizes the contents of these changes. First, *H. pylori*-induced gastritis was classified as "infectious disease." Even in the absence of symptoms perceived by the patient or disorders related to peptic ulcer disease or gastric cancer, *H. pylori*-induced gastritis itself should be regarded as an infectious disease, and in disease classification, too, *H. pylori* should be classified as a causative factor. Second, *H. pylori*-induced gastritis is defined as a causative factor for *H. pylori*-related dyspepsia. In extension, eradication treatment is recommended as first-line treatment for *H. pylori*-positive dyspepsia because eradication treatment is more efficient than placebo or other treatments in mitigating symptoms of dyspepsia. Third, all *H. pylori*-positive patients are urged to undergo eradication treatment. If a physician verifies *H. pylori* infection in a patient, the physician has to inform the patient of that fact and the necessity for eradication treatment. Kyoto Global Consensus Meeting presents radical changes of imposing nature in diagnosis and treatment of *H. pylori* infection, such as recommending patients with dyspepsia to undergo eradication treatment or including all *H. pylori*-positive patients into eradication treatment target group for the purpose of preventing gastric cancer [15].

The changes made in the Japanese *H. pylori* guidelines and national health insurance coverage reflect Japan's strong will to inherit the nation free of gastric cancer to future generations, not the legacy of 50,000 gastric cancer deaths a year, by providing early rescue for the stomachs colonized by *H. pylori* at a reversible stage, thereby even confronting problems posed by prophylactic eradication treatment [2, 16].

Japan uses lower-dose antibiotics for eradication treatment compared to South Korea or Europe. Triple therapy in 7-day b.i.d. (bis in die, twice a day) regimen is recommended as first-line treatment, consisting of standard-dose proton pump inhibitor (PPI), 750 mg amoxicillin, and 200 mg (or 400 mg) clarithromycin. As second-line treatment, PPI + 750 mg amoxicillin + 250 mg metronidazole in 7-day b.i.d is recommended [2] (Table 48.2). No recommendations are made for 14-day regimen of bismuth-containing quadruple therapy as first- or second-line treatment [17]. The eradication rate in Japan is 70 % in first-line and 90 % in second-line treatments, with overall eradication rate maintained at 95 % or higher via these two therapies [18, 19].

48.4 Chinese Guidelines (2013)

The 4th Chinese National Consensus Report revised in 2013 strongly recommends eradication treatment for peptic ulcer disease and gastric MALT lymphoma [3] (Table 48.1). It is noteworthy that the disease group additionally recommended for eradication treatment includes *H. pylori*-related diseases such as lymphocytic gastritis, gastric hyperplastic polyps [20], and Menetrier disease. In contrast, intestinal metaplasia was not included on the basis of the results of a meta-analysis [21] that *H. pylori* eradication treatment improved the corpus atrophic gastritis, but not intestinal metaplasia [3]. Like South Korea, China recommends 7–14-day regimen of standard triple therapy including clarithromycin and amoxicillin as first-line treatment [3] (Table 48.2). With eradication rate falling short of 80 % [17], however, bismuth quadruple therapy is recommended as first-line therapy in regions with high clarithromycin resistance (≥15–20 %) [3]. China has very high levels of metronidazole and clarithromycin resistance, albeit with regional differences. For example, while metronidazole resistance in Shanghai region is as high as 60–70 %, clarithromycin resistance is much lower (20–38 %) [22]. Due to regional differences in *H. pylori* antimicrobial resistance, many studies have noted that such regional features should be reflected in treatment [23, 24].

48.5 European Guidelines (The Maastricht IV/Florence Consensus Report, 2012)

The Maastricht III Consensus Report included debatable cases, such as patients with atrophic gastritis, family history of gastric cancer patients, and patient's wish, in the target population for strong recommendation of eradication treatment, thus prompting criticism for having excessively expanded the scope of treatment target group [25]. The 2012 revised version (Maastricht IV) reflects the results of continued research activities by including functional dyspepsia, NSAID, aspirin users, gastroesophageal reflux disease, and emphasizing the inclusion of extragastric diseases as indications for *H. pylori* eradication treatment [4] (Table 48.1).

2012 revised version is characterized by the inclusion of clarithromycin resistance in setting up treatment strategies [4] (Table 48.2). Regional division is made between low-resistance and high-resistance regions, using clarithromycin resistance rate of 20% as mark. For low-resistance regions, clarithromycin-containing triple therapy is recommended as first-line empiric treatment and bismuth quadruple therapy as alternative treatment. In case of first-line treatment failure, bismuth quadruple therapy or levofloxacin-containing triple therapy is recommended as second-line treatment. On the other hand, bismuth quadruple therapy is recommended as first-line empiric treatment in high-resistance regions, if not available, non-bismuth quadruple therapy (either sequential or concomitant regimen) is recommended as first-line treatment. Although both sequential and concomitant therapies contain clarithromycin, clarithromycin resistance is assessed to be maintainable at an acceptable level, with the treatment success rates ranging between 75 and 95% as per previous reports. If first-order treatment in sequential regimen fails in regions with high clarithromycin resistance, levofloxacin-containing triple therapy is recommended as second-line treatment. If second-line treatment fails, third-line treatment is recommended without regard to clarithromycin resistance, preceded by culture or antimicrobial susceptibility testing. This is indicative of flexible treatment strategies depending on antimicrobial resistance rate.

Conclusions

From the various guidelines presented above, conclusion may be derived that while gastric ulcer, duodenal ulcer, and gastric MALT lymphoma are classical indications for eradication treatment, post-endoscopic resection of EGC has been added as a new indication for eradication treatment to prevent EGC recurrence. No consensus has yet been reached as to eradication treatment in cases of chronic atrophic gastritis, functional dyspepsia, family history of gastric cancer, and the like, with individual countries holding different views. From the therapeutic perspective, no clearly defined countermeasures against decreasing eradication rate of standard triple therapy have yet been presented. Continuous research is thus necessary to develop new therapies capable of overcoming antimicrobial resistance issue and to reestablish corresponding treatment guidelines.

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Gastric Cancer Screening

49

Su Youn Nam

Abstract

Gastric cancer is one of the most common cancers worldwide, and East Asian countries including Korea, Japan, and China have a higher incidence of gastric cancer. Although there was no randomized controlled study for effectiveness of gastric cancer screening, Korea and Japan have provided government-supported national screening programs for gastric cancer. Several case-control studies and recent cohort studies have reported the effectiveness of radiographic screening for gastric cancer in Japan. Japanese guidelines for gastric cancer screening recommend that radiographic screening was the only effective method because of a lack of studies using endoscopic screening. From analysis of Korean national cancer screening data, endoscopy was more effective than radiography for gastric cancer screening because the accuracy of endoscopy is much higher than that of radiography. The Korean National Cancer Screening Program has recommended either endoscopy or radiography screening for gastric cancer, but the 2015 updated Korean guideline recommends only endoscopy for national cancer screening program. In this chapter, the accuracy and effectiveness of gastric cancer screening by screening modalities and gastric cancer screening guidelines of Korea and Japan will be discussed.

Keywords

Gastric cancer • Screening • Mortality • Case-control study • Cohort study • Stage shift • Early gastric cancer

49.1 Introduction

Gastric cancer is one of the most common cancers and ranks as the third leading cause of cancer-related deaths worldwide [1]. East Asian countries including Korea, Japan, and China have a higher incidence of gastric cancer

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than other countries. Korea and Japan have provided government-supported national screening programs for gastric cancer. Since 1965, the Japanese government has provided annual radiographic screening. The South Korean government has provided biennial gastric cancer screening using endoscopy or radiography to people over 40 years, since 1999 [2]. There was no randomized controlled study for effectiveness of gastric cancer screening. However, several case-control studies and recent cohort studies have reported the effectiveness of radiographic screening for gastric cancer in Japan [2, 3]. In 2008, the Japanese guidelines for gastric cancer screening through evidence-based methods reported that radiographic screening was the only effective method because of a lack of studies using endoscopic screening [3]. From analysis of Korean national cancer screening data, endoscopy was reported to be more effective than radiography for gastric cancer screening because the accuracy of endoscopy was much higher than that of radiography [4]. Before the 2015 Korean National Cancer Screening Program (NCSP) has recommended either endoscopy or radiography screening for gastric cancer but the updated Korean gastric cancer screening guideline in 2015 recommends only EGD for national cancer screening program based on a meta-analysis for the effect of endoscopic screening on gastric cancer mortality [5].

The main modalities for gastric cancer screening are esophagogastroduodenoscopy (EGD) and contrast imaging study. EGD allows direct visualization of the entire gastric mucosa and biopsies to confirm the histologic type. Although EGD is more invasive and usually has a higher cost, it is more sensitive for diagnosing gastric lesions. Double-contrast barium radiographs with photofluorography or direct radiography can also identify gastric lesions. However, the rate of false-negative study was reported up to 50% of cases [6]. In early gastric cancer, the sensitivity of radiographic study may be as low as 14% [7]. In this chapter, the accuracy and effect of gastric cancer screening by screening modalities and gastric cancer screening guidelines of Korea and Japan will be discussed.

49.2 Accuracy of Gastric Cancer Screening Methods

49.2.1 Endoscopic Screening

Sensitivity and specificity of endoscopic screening was constantly high in both Korean and Japanese studies [4, 8]. In a large Korean cohort study using 2,250,392 gastric cancer screenees, sensitivity and specificity for 924,822 cases of endoscopic screening were 0.69 (95% confidence interval [CI], 0.67–0.71) and 0.96 (95% CI, 0.96–0.96) [4]. In a Japanese study using 56,676 screenees, sensitivity for 7,388 cases of endoscopic screening was 0.89 (95% CI, 0.70–0.98) in the prevalence method and 0.95 (95% CI, 0.84–0.99) in the incidence method. And specificity was 0.85 (95% CI, 0.84–0.86) in the prevalence method and 0.89 (95% CI, 0.88–0.89) in the incidence method [8].

49.2.2 Radiographic Screening

Sensitivity of radiographic screening for gastric cancer was far different between the Korean and the Japanese study (Fig. 49.1). A large Korean study showed very low sensitivity (0.37) and high specificity (0.96) [4]. Sensitivity from a Costa Rican study by Rosero-Bixby (58%) was better 58% than that of the Korean study [9]. A Japanese study showed higher sensitivity ranging from 0.69 to 0.93 and lower specificity ranging from 0.88 to 0.91 than that of the Korean study [8, 10–13]. Pooled sensitivity of radiographic screening except that of the Korean study was 0.84 [5].

Possible explanations for the different sensitivities are the screening intervals (2 years in Korea vs. 1 year in Japan), the definition of positive findings (only gastric cancer-suspected lesions were regarded as positive findings in the Korean study but all abnormal findings were considered as positive findings in Japanese studies),

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Choi (2012)	1,196	68,853	2,067	1,693,793	0.37 [0.35, 0.38]	0.96 [0.96, 0.96]
Rosero-Rixby (2007)	38	163	27	6,600	0.58 [0.46, 0.71]	0.98 [0.97, 0.98]
Fukao (1992)	356	17,375	158	138,049	0.69 [0.65, 0.73]	0.89 [0.89, 0.89]
Abe (2000)	315	15,684	77	152,417	0.80 [0.76, 0.84]	0.91 [0.91, 0.91]
Ishida (1994)	53	6,703	10	29,055	0.84 [0.73, 0.92]	0.81 [0.81, 0.82]
Hamashima (2013)	48	2,001	6	14,772	0.89 [0.77, 0.96]	0.88 [0.88, 0.89]
Yamamoto (2010)	227	11,114	19	112,137	0.92 [0.88, 0.95]	0.91 [0.91, 0.91]
Higashiyama (2010)	782	39,953	57	391,107	0.93 [0.91, 0.95]	0.91 [0.91, 0.91]

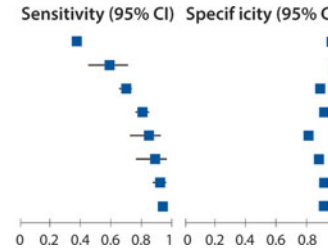

Fig. 49.1 Meta-analysis on the accuracy of radiographic screening. *TP* true-positive, *FP* false-positive, *FN* false-negative, *TN* true-negative, *CI* confidence interval (Adapted from Park et al. [5])

or the film-reading system (one reader in Korea vs. two well-trained readers in Japan).

49.2.3 Comparison of Sensitivity Between Endoscopic and Radiographic Screening

One Korean and one Japanese study showed the sensitivity of endoscopic and radiographic screening at the same time [4, 8]. A huge Korean cohort study showed higher sensitivity for endoscopic screening compared to radiographic screening (69.0 % vs. 36.7 %) and similar specificity (96.0 % vs. 96.1 %) [4]. A Japanese case-control study also showed similar pattern [8].

49.3 Effect of Gastric Cancer Screening on Gastric Cancer Mortality

49.3.1 Endoscopic Screening

Two case-control and two cohort studies evaluated the effect of endoscopic screening on gastric cancer mortality in the general population [14–17]. In a case-control study by Hamashima et al., odds ratio (OR) for endoscopic screening within 36 months of the date of diagnosis was 0.70 [14]. The Korean NCSP report by Cho enrolled a baseline cohort between 2002 and 2003 and investigated gastric cancer development up to 2008 and gastric cancer death up to 2011 [15]. Endoscopic screening decreased gastric cancer mortality (adjusted OR, 0.43). A Japanese cohort study by Matsumoto showed that the relative risk (RR) of gastric cancer mortality was 0.35 [16]. A Japanese cohort study by Hosokawa demonstrated that the RR for gastric cancer death in the screened group was 0.35 [17]. Three Japanese studies and a large Korean study constantly showed that endoscopic screening reduced gastric cancer mortality.

49.3.2 Radiographic Screening

The effect of radiographic screening on gastric cancer mortality was far different among nations. A case-control study by Oshima et al. reported that the OR of gastric cancer mortality in the radiographic-screened group was estimated at 0.52 (90 % CI, 0.30–0.91) in men and 0.49 (90 % CI, 0.24–0.99) in women [18]. A case-control study in Venezuela demonstrated that radiographic screening reduced gastric cancer mortality [19]. In a case-control study from Miyagi prefecture in Japan, radiographic screening reduced gastric cancer mortality in men but not in women [20]. Another case-control study by Tsubono et al. reported a reduction in mortality of 79 % in the radiographic-screened group [21]. However, radiographic screening did not reduce gastric cancer mortality in a study by Hamashima et al. [14]. Cho et al. conducted a large-scale case-control study from the Korean NCSP. Radiographic screening showed only a 7 % reduction in gastric cancer mortality [15].

A population-based cohort study by Inaba et al. reported that radiographic screening did not reduce gastric cancer mortality signifi-

cantly in both men and women [22]. In a prospective cohort study by Mizoue et al., radiographic screening reduced gastric cancer mortality in men but not in women [23]. In a population-based prospective cohort study by Lee et al., 40 % reduction in gastric cancer death was noted in the screened group [24]. Another Japanese cohort study reported that radiographic screening reduced gastric cancer mortality [25]. A cohort study from Costa Rica suggested that gastric cancer mortality was about twofold higher in unscreened groups than screened groups [9]. A cohort study by Matusmoto demonstrated that gastric cancer mortality was 0.13 % in the unscreened group and 0.07 % in the radiographic-screened group [16].

Cohort studies from Japan and Costa Rica showed constant reduction of gastric cancer mortality in the radiographic-screened group. However, the effect from case-control studies has discrepancy by nations. Japan and Venezuela studies suggested significant reduction of gastric cancer mortality, but a huge Korean case-control study showed just 7 % reduction in radiographic-screened group. The main cause of this discrepancy seems to originate from the sensitivity difference of radiographic screening. The sensitivity was 69–83 % in the Japanese studies but 37 % in the Korean study.

49.4 Guidelines

49.4.1 Updated Korean Guideline 2015

The Korean government has provided gastric cancer screening program for the general population since 1999 even if the effectiveness or harms of gastric cancer screening using EGD and radiographic screening had not been fully evaluated. Recently the Korean multidisciplinary expert committee for developing a gastric cancer screening guideline systematically reviewed the evidence regarding the benefits and harms of gastric cancer screening and developed an evidence-based clinical guideline [5] (Table 49.1). They found a "low"-level evidence that gastric cancer screening using EGD or upper gastrointestinal (UGI) series could reduce gastric cancer mortality in the general population between 40 and 74 years. The benefits of gastric cancer screening using EGD were substantially higher than its harms, while the benefits of radiographic screening were moderately higher. Therefore, this guideline recommended biannual EGD for asymptomatic adults from 40 to 75 years (recommendation B). Radiographic screening in asymptomatic adults between 40 and 74 years could be recommended based on clinicians' judgment regarding the patient's risk and the patient's preference (recommendation

Table 49.1 Updated Korean gastric cancer screening guideline

		Substantial	Benefit size		
			Moderate	Small	Zero/negative
Evidence level	High	A	B	C	D
	Moderate	A	B	C	D
	Low	B (EGD from 40 to 74 years)	C (radiographic screening from 40 to 74 years)	C	D (asymptomatic adults older than 85 years)
	Very low	I	I	I (adults aged between 75 and 84 years)	I

Recommendation grade A means strong recommendations for screening; B means moderate recommendations for screening; C means selective recommendation considering clinical decision and screenee's favorites; D means no recommendation for screening; I means insufficient evidence to estimate the size of benefit and harm
EGD esophagogastroduodenoscopy

C). Gastric cancer screening had insufficient evidence (recommendation I) for adults aged between 75 and 84 years and was not recommended for asymptomatic adults older than 85 years (recommendation D).

49.4.2 Japanese Guideline

The Japanese committee evaluated four methods for gastric cancer screening (photofluorography, endoscopy, serum pepsinogen testing, and *H. pylori* antibody testing) and reported the screening guideline 2008. The studies that evaluated mortality reduction from gastric cancer included five case-control and two cohort studies for radiographic screening. Gastric cancer screening using photofluorography was recommended. The other methods were not recommended for population-based screening due to insufficient evidence. However, the recent Japanese committee suggested the effectiveness of endoscopic screening, and some provinces have used both endoscopic and radiographic study for gastric cancer screening.

Conclusion

Korea and Japan, high-prevalence areas of gastric cancer, have provided national screening programs for gastric cancer. Whereas the Japanese government has provided annual radiographic screening, the South Korean government has provided biennial gastric cancer screening using endoscopy or radiography to people over 40 years. Even if there was no randomized controlled study for the effectiveness of gastric cancer screening, several case-control studies and recent cohort studies have reported the effectiveness of radiographic screening for gastric cancer in Japan. However, recent population-based screening studies demonstrated reduced gastric cancer death in the endoscopic screening group. Furthermore, endoscopic screening was more sensitive than radiographic screening and constantly reduced gastric cancer mortality, whereas the sensitivity of radiographic screening was variable and mortality reduction was also variable by study area. Therefore, the 2015 updated Korean guideline recommends only EGD for gastric cancer screening because of the low sensitivity and minimal reduction of gastric cancer death of radiographic screening in Korea.

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Recrudescence and Reinfection After *H. pylori* Eradication Treatment

50

Nayoung Kim

Abstract

Helicobacter pylori (*H. pylori*) recurrence can be classified into two classes: recrudescence and reinfection. Recrudescence is a recurrence of the same infection after antimicrobial treatment, which occurs as first-year relapses. On the other hand, reinfection is defined as a state wherein a patient becomes infected with a new *H. pylori* strain after the patient went through successful eradication therapy. DNA fingerprinting technique is utilized to distinguish between recrudescence and reinfection. However, this technique cannot be done for every single analysis, so numerous researches define reinfection as a *H. pylori*-positive state after 12 months of *H. pylori*-negative condition during a monitoring period once an eradication treatment is over. In terms of *H. pylori* reinfection, worldwide annual reinfection rate is reported to be 0–23.4 % on adults and 1.9–9.6 % on pediatrics, but the rates are less than 1 % in the developed countries, while they are high among developing nations. The risk factors of this recurrence were noncompliance to the initial therapy, presence of children in the household, male, and low income. *H. pylori* reinfection rate will further decrease, if socioeconomic condition and public hygiene continue to ameliorate.

Keywords

Recrudescence • Reinfection • *Helicobacter pylori*

50.1 Introduction

Helicobacter pylori (*H. pylori*) recurrence is an important factor to determine the efficacy of antimicrobial treatment, because if there is a high conversion ratio of patients who were previously determined to be *H. pylori*-negative into *H. pylori*-positive, then applied antimicro-

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bial treatment would be useless. *H. pylori* recurrence can be classified into two classes: recrudescence and reinfection [1]. Recrudescence is a recurrence of the same infection after antimicrobial treatment, which usually occurs as first-year relapses. On the other hand, reinfection is defined as a state wherein a patient becomes infected with a new *H. pylori* strain after the patient went through successful eradication therapy [2]. For the recurrence-subtype classification, DNA fingerprinting technique is utilized to distinguish between recrudescence and reinfection. However, this technique cannot be done for every single analysis, so numerous researches define reinfection as an *H. pylori*-positive state after a patient has maintained 12 months of *H. pylori*-negative condition during the monitoring period, since the end of successful eradication treatment [2–4]. According to the previous research, reinfection rates of adults and pediatrics before 2000 were 0–100 % and 2.0–13.5 %, respectively [2]. However, the reinfection rates of adults and pediatrics were both below 2 % in the developed nations, while they were both higher than 10 % in the developing countries [2]. Thus, the research shows that the results are different based on nations and time period [2]. This chapter is going to discuss on the mechanism of *H. pylori* reinfection and how *H. pylori* recurrence rates of adults and pediatrics are different among nations.

50.2 Recrudescence After *H. pylori* Eradication Therapy

Recrudescence is a recurrence of the same infection after antimicrobial treatment, which usually occurs as first-year relapses [5]. This arbitrary definition was confirmed by Take et al.'s report which has been performed among 1,609 patients with a mean follow-up of 4.7 years [6]. They compared the strains by random amplification of polymorphic DNA fingerprinting and showed that during the first year after the successful treatment, six of ten strain couples were genetically identical, while none of the four strain couples tested at a later follow-up were identical [6]. This study confirmed that first-year relapses are more likely to be a recurrence of the same infection. Nevertheless, such a recurrence may occur at various rates. In Morocco, the relapse rate was 1 of 239 at 6 months [6], while in Latin America, 1-year relapse rate was 125 of 1,091 (11.5 %) patients [7].

The reasons of recrudescence are noncompliance to the initial therapy, presence of children in the household [7], the low efficacy of used antibiotics, and the inaccuracy of *H. pylori* diagnosis 4 weeks after eradication therapy [8]. In other words, recrudescence is highly possible when *H. pylori* eradication was not successful due to the low efficacy of antibiotics, and proper diagnostic method after *H. pylori* eradication is important [8]. For example, there is a high possibility of a false-negative result if rapid urease test is conducted on patients with either atrophic gastritis or intestinal metaplasia, so conducting modified Giemsa stain histology from the corpus of the stomach gives more accurate result [9–11]. Like this, false-negative rate of diagnoses after *H. pylori* eradication increases mainly because the sensitivity of *H. pylori* infection diagnoses is not highly enough due to *H. pylori* reduction after the eradication therapy. Especially, *H. pylori* converts itself from spiral bacilli into cocci to survive when it is exposed to antibiotics. *H. pylori* in coccus form will not activate its metabolism until its surrounding environment gets better to return itself to bacillus form, and *H. pylori* will not be detected once it is in the coccus form. Thus, administering antibiotics with high efficacy for enough periods and conducting precise *H. pylori* diagnostic methods are necessary to reduce recrudescence. The *H. pylori* recrudescence rates in Korea after administering triple therapy (standard dose of proton pump inhibitor, one gram of amoxicillin and 500 mg of clarithromycin) as twice a day for 7 days were low such as 3.49 % (3/86) in 2008 [12] and 4.9 % (17/348) in 2013 [3], based on the results of *Campylobacter*-like organism (CLO) test, Giemsa histology, and *H. pylori* culture tests from the antrum and corpus.

Table 50.1 Reinfection rates of various countries after successful *H. pylori* eradication

Author	Year [reference]	Population	Country	Annual reinfection rate (%)
Rollan et al.	2000 [15]	Adults	Netherlands	1.49
Hildebrand et al.	2001 [16]	Adults	Bangladesh	23.4
Adachi et al.	2002 [17]	Adults	Japan	1.23
Seo et al.	2002 [18]	Adults	Japan	0.00
Gisbert et al.	2006 [19]	Adults	Spain	1.04
Lee et al.	2008 [12]	Adults	Korea	2.94
Kim et al.	2013 [3]	Adults	Korea	3.51
Kim et al.	2014 [1]	Adults	Korea	Within 2 years: 9.3
				After 2 years: 2.0
Bruce et al.	2015 [20]	Adults	Alaska, USA	Cumulative rate within 2 years: 16.1
Kato et al.	1998 [21]	Children	Japan	5.36
Leal-Herrera et al.	2003 [22]	Children	Mexico	9.55
Farrell et al.	2004 [23]	Children	UK	1.91

50.3 Reinfection After *H. pylori* Eradication Therapy

H. pylori infection rate tends to be low in the developed countries and high in the developing countries, and the rate is different based on age, region, and ethnicity. Developed countries, such as the USA or Europe, show *H. pylori*-positive rate as 10–15 % at the age of 3–5, and the rate increases as the age increases. In comparison, *H. pylori*-positive rate in the developing countries, like Saudi Arabia, was already 40–60 % before the age of 10 [13]. Also, the rate among South American nations is currently reported to be 75–83 % [14]. *H. pylori* reinfection rates are different among countries by up to 23 % [1, 3, 12, 15–23] (Table 50.1), and these rates show similarity with *H. pylori* infection rate, which is low among the developed countries and high among the developing countries [2] (Fig. 50.1). In other words, the reinfection rate on adults and pediatrics before 5 years old was 0.5–2 % in the developed nations [24–29], while the rate under the same conditions was 23.4 % in the developing countries [21]. The similarity of low infection and reinfection rates is attributed to high socioeconomic condition and good public hygiene [16]. This situation can be referred to the researches on Mexico and Peru, where *H. pylori*-positive rates were high previously. According to the researches about *H. pylori* reinfection rate of Mexico and Peru in 2003, their rates were 3.2 % and 7.6 %, respectively [22, 30]. These values indicate that the socioeconomic and public hygiene conditions of those two nations have improved. Similarly, *H. pylori* reinfection rate reduction can be seen in Korea, too. According to Kim et al., *H. pylori* reinfection rate in South Korea was 13 % in 1998 [31], but the rate was reduced up to 3.51 %, after an average of 37.1 months (18–95 months) of follow-up on 331 patients who underwent standard triple therapy [3].

50.4 Risk Factors of Reinfection After *H. pylori* Eradication Therapy

Recently, Bruce et al. performed an interesting study to determine rates of reinfection in three groups followed for 2 years after successful treatment in 229 persons: American Indian/Alaska Native (AI/AN) persons living in urban (group 1) and rural (group 2) communities and urban Alaska non-Native persons (group 3) [20]. *H. pylori* reinfection occurred in 36 persons; cumulative reinfection rates were 14.5 %, 22.1 %, and 12.0 % for groups 1, 2, and 3, respectively [20].

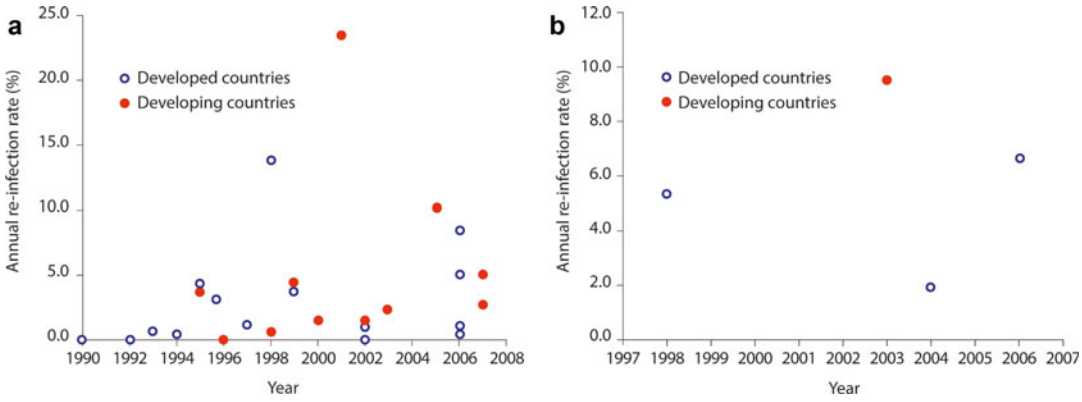

Fig. 50.1 Reinfection distribution of adults (**a**) and pediatrics (**b**) in the developed countries and in the developing countries (Adapted from Zhang et al. [2])

Study participants who became reinfected were more likely to have peptic ulcer disease ($p=0.02$), low education level ($p=0.04$), or a higher proportion of household members infected with *H. pylori* compared to participants who did not become reinfected ($p=0.03$) [20]. In addition, in South Korea the odds ratio (OR) of reinfection rate in men was 2.28 (95 % confidence interval [CI], 1.05–5.00; $p=0.037$), which is higher than that in women, and the OR of low income (≤$5,000) was 3.54 (95 % CI, 1.05–5.00; $p=0.038$), which was also higher than high income (>$5,000) [3], based on a multivariate analysis on factors that affect *H. pylori* reinfection in South Korea, so the risk factors of reinfection are the same as the risk factors for *H. pylori* infection [32–35]. These data imply that the reduced annual *H. pylori* reinfection rate (3.51 %) in South Korea is a true reinfection, instead of recrudescence.

Conclusions

H. pylori recurrence can be classified into two classes: recrudescence and reinfection. Recrudescence is a recurrence of the same infection after antimicrobial treatment, which usually occurs as first-year relapses. On the other hand, reinfection is defined as a patient being infected with a new *H. pylori* strain after the patient went through successful eradication therapy. There is a similarity between infection and reinfection rates of *H. pylori*, which is attributed to socioeconomic condition and public hygiene. Annual *H. pylori* reinfection rates based on worldwide reports are 0–23.4 % for adults and 1.9–9.6 % on pediatrics and less than 1 % in the developed countries and high in the developing countries. This suggests that there is no need to worry about *H. pylori* reinfection in the developed countries, while proper hygiene management after eradication therapy is necessary among the developing nations.

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Part VIII

Consequences of *H. pylori* Eradication

Peptic Ulcer Disease

51

Jinjoo Kim

Abstract

Peptic ulcer disease (PUD) used to recur frequently, cause various complications, and show high mortality rate. However, after the discovery of *Helicobacter pylori* (*H. pylori*), *H. pylori* eradication has dramatically reduced the recurrence rate of PUD. *H. pylori* eradication combined with gastric acid inhibition promotes ulcer healing more rapidly than gastric acid inhibition only. *H. pylori* eradication also prevents various PUD complications, such as bleeding, perforation, and obstruction, to occur. Therefore, PUD patients with *H. pylori* infection should get *H. pylori* eradication for better ulcer healing and prevention of its recurrence and complications.

Keywords

Helicobacter pylori • Peptic ulcer disease • Gastric acid

51.1 Introduction

Gastric mucosal damage with excessive gastric acid and pepsin secretion leads to the development of peptic ulcer disease (PUD). Previously, gastric acid inhibition was the sole mainstay therapy and the recurrence and complication rate of PUD has been quite high. However, after the discovery of *Helicobacter pylori* (*H. pylori*), it has helped to understand the pathophysiology of PUD and it has been a big turnover. In this chapter, the relationship between *H. pylori* eradication and PUD healing/recurrence/complication would be discussed.

51.2 How to Influence on the Peptic Ulcer Healing

According to the cohort study by Gisbert et al. [1] with 212 duodenal ulcer patients, 22 patients (10.4%) have recurred after 1 month following *H. pylori* eradication and only three patients have recurred after 2 months, reporting 98.1% cure rate. According to several studies similar to this study, duodenal ulcer has been healed faster with *H. pylori*

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eradication than with acid inhibition therapy alone [2–5]. Also, not only simple duodenal ulcer but also refractory duodenal ulcer to gastric acid inhibition therapy has been healed successfully with *H. pylori* eradication even after 4 years later [6–17].

There are much fewer studies on the relationship between gastric ulcer and *H. pylori* eradication. According to the study by Labentz and Börsch [18], 84.9 % of *H. pylori*-eradicated gastric ulcer patients have been cured but only 60 % of non-*H. pylori*-eradicated gastric ulcer patients have been cured. Several studies also showed that *H. pylori* eradication helps to heal gastric ulcer successfully, even without persistent gastric acid inhibition therapy [19–30]. Several studies showed that persistent *H. pylori* infection was significantly related to the unsuccessful gastric ulcer healing, and this tendency was more accentuated with nonsteroidal anti-inflammatory drug (NSAID) usage [31–33].

51.3 How to Influence on the Peptic Ulcer Recurrence

PUD has been notorious for its high recurrence rate before the discovery of *H. pylori* [34–38]. The significant relationship between successful *H. pylori* eradication and lower PUD recurrence rate had been reported in several studies [39, 40].

In terms of duodenal ulcer, one meta-analysis by Hopkins et al. [41] showed that the recurrence rate was 67 % without *H. pylori* eradication and it dropped to 6 % with successful *H. pylori* eradication. Similarly, in terms of gastric ulcer, Hopkins showed that the recurrence rate was 59 % without *H. pylori* eradication and it dropped to 6 % with successful eradication [41, 42]. Therefore, *H. pylori* eradication is definitely helpful to reduce the recurrence rate in both duodenal and gastric ulcer.

51.4 How to Influence on the Complications of Peptic Ulcer Disease

PUD has been known to cause several significant complications, such as bleeding in 15–20 %, perforation in 5 %, and obstruction in 2 % [43].

51.4.1 Bleeding

Murray et al. [44] reported high rebleeding rate in patients with persistent ranitidine 150 mg prescription, and Jensen et al. [45] also showed 10 % rebleeding rate in patients with ranitidine. According to one study comparing patients with gastric acid inhibition and *H. pylori* eradication, 12 % of patients with gastric acid inhibition showed rebleeding and only 2.3 % of those with successful *H. pylori* eradication did. Therefore, based on these previous studies, *H. pylori* eradication prevents not only the recurrence of PUD but also the rebleeding rate of PUD [46–49].

51.4.2 Perforation

There are only a few studies on the relationship between PUD perforation and *H. pylori* eradication. According to the study of patients with simple repair of PUD perforation, those with cimetidine treatment showed significantly better prognosis, but this study has not considered *H. pylori* infection as a factor [50]. NSAID has been well known to increase the risk of PUD perforation more than *H. pylori* infection [51–53]. According to several studies with PUD perforation patients, *H. pylori* infection rate ranged from 50 to 80 % [54–58]. According to one randomized study with PUD perforation patients in Hong Kong, 38 % of patients with gastric acid inhibition only recurred and only 5 % of patients with successful *H. pylori* eradication recurred [59].

51.4.3 Obstruction

Obstruction due to PUD occurs not that often, which is less than 5 %, and studies on the relationship between PUD obstruction and *H. pylori* infection are quite limited so far [60–63]. PUD patients with obstruction showed from 45 to 90 % *H. pylori* infection rate, and several studies showed that obstruction has been successfully relieved after *H. pylori* eradication or

after both *H. pylori* eradication and balloon dilatation [63–65].

Conclusions

The relationship between *H. pylori* eradication and PUD has been discussed so far. *H. pylori* eradication has been shown to reduce PUD recurrence and complications. Therefore, *H. pylori* eradication is the standard therapy for PUD patients with *H. pylori* infection to heal PUD more effectively and to reduce recurrence and complications.

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Atrophic Gastritis and Intestinal Metaplasia

52

Hyuk Yoon

Abstract

Currently, many international guidelines recommend *Helicobacter pylori* (*H. pylori*) eradication in patients with precancerous lesions such as atrophic gastritis (AG) and intestinal metaplasia (IM). However, there are still many controversies in whether *H. pylori* eradication improves AG and IM. Taking the results of related studies together, it seems likely that *H. pylori* eradication improves AG to some extent. However, a dominant opinion is that IM which progressed to a certain level is already an irreversible status (point of no return). Therefore, in order to obtain an optimal effect for gastric cancer prevention, eradication of *H. pylori* in the earlier and reversible stage of AG and IM is recommended.

Keywords

Atrophic gastritis • Eradication • *Helicobacter pylori* • Intestinal metaplasia

52.1 Introduction

Helicobacter pylori (*H. pylori*) is well known as a major cause of gastric cancer. Therefore, whether eradication of *H. pylori* could reverse atrophic gastritis (AG) and intestinal metaplasia (IM) which are precancerous lesions and could inhibit the progression of these lesions are clinically important topics. In this chapter, we will evaluate the effect of *H. pylori* eradication on the precancerous lesions. First, we will introduce key individual studies. Second, we will summarize related meta-analyses. Third, we will review major guidelines all over the world. Finally, after mentioning the limitations of related studies, we will conclude.

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52.2 Key Individual Studies

H. pylori eradication is the basis of treatment of AG and IM. However, it is still a controversial topic with a question of whether *H. pylori*

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eradication can improve AG and IM [1–16] (Table 52.1). Several clinical studies reported that eradication of *H. pylori* can improve AG and IM which are the main precancerous lesions. The cohort study by Correa et al. is the first well-designed study in this field. The authors treated *H. pylori* infection in 795 adults who lived in an area with high risk for gastric cancer in Colombia and had precancerous lesions and followed them up for 12 years. The rate of regression or delayed progression of AG and IM was significantly higher in patients who showed successful eradication of *H. pylori* than in patients who failed to eradicate *H. pylori* [17, 18]. A randomized clinical study for 3,365 people in China also suggested that *H. pylori* eradication significantly reduced the prevalence of AG and IM after an 8-year follow-up [19]. Another Chinese study reported that *H. pylori* eradication is effective in delaying progression of IM after a 5-year follow-up [20]. However, a recent Taiwanese study suggested that *H. pylori* eradication in mass population did not decrease IM after a 4-year follow-up even though it significantly reduced AG [21]. In addition, a famous randomized clinical study by Wong et al. showed that *H. pylori* eradication decreased the

Table 52.1 Histologic parameters of AG and IM after *Helicobacter pylori* eradication

	Study arms (*n*)		Follow-up (months)	Histologic parameters			
				AG		IM	
Author, year (country)	Eradicated	Not eradicated		Antrum	Corpus	Antrum	Corpus
Sung et al. 2000 (China) [1]	226	245	12	Yes	Yes	Yes	Yes
Kim et al. 2000 (Korea) [2]	41	16	24	No	No	Yes	Yes
Annibale et al. 2000 (Italy) [3]	25	7	6	Yes	Yes	Yes	Yes
Ohkusa et al. 2001 (Japan) [4]	115	48	12–15	Yes	Yes	Yes	Yes
Ruiz et al. 2001 (Colombia) [5]	29	21	72	Yes	No	No	No
Ito et al. 2002 (Japan) [6]	22	22	60	Yes	Yes	Yes	Yes
Annibale et al. 2002 (Italy) [7]	40	0	6–12	Yes	Yes	Yes	Yes
Yamada et al. 2003 (Japan) [8]	87	29	10–50	Yes	Yes	Yes	Yes
Iacopini et al. 2003 (Italy) [9]	10	0	12	Yes	No	Yes	No
Kamada et al. 2003 (Japan) [10]	37	8	36	Yes	No	No	No
Wambura et al. 2004 (Japan) [11]	107	118	12	Yes	Yes	Yes	Yes
Lahneret al. 2005 (Italy) [12]	38	36	48–137	Yes	Yes	Yes	Yes
Lu et al. 2005 (China) [13]	92	87	36	Yes	Yes	Yes	Yes
Kamada et al. 2005 (Japan) [14]	1,787	233	12	Yes	Yes	Yes	Yes
Toyokawa et al. 2009 (Japan) [15]	241	19	60	Yes	Yes	Yes	Yes

Adapted from Park and Kim [16]
AG atrophic gastritis, *IM* intestinal metaplasia

incidence of gastric cancer only in subjects without initial precancerous lesions such as AG and IM [22]. These findings imply that *H. pylori* eradication may not be effective for prevention of gastric cancer in a high-risk group with precancerous lesions. Therefore, patients with AG should keep receiving surveillance endoscopy even after *H. pylori* eradication [23]. In addition, a test-to-treat strategy to perform *H. pylori* eradication in the young population before development of AG and IM might be more effective for prevention of gastric cancer [24].

Because a considerable fraction of gastric cancer patients already has advanced stage of AG and IM, we can infer the effect of *H. pylori* eradication on AG and IM by examining the incidence of metachronous gastric cancer in gastric cancer patients who received *H. pylori* eradication. One retrospective Japanese study reported that *H. pylori* eradication in gastric cancer patients who underwent endoscopic resection of early gastric cancer (EGC) decreased the incidence of metachronous gastric cancer [25]. Thereafter, Fukase et al. published the article suggesting that compared with the control group, the odds ratio (OR) for the incidence of metachronous gastric cancer in the patients who received *H. pylori* eradication after endoscopic submucosal dissection of EGC is 0.35 (95% confidence interval [CI], 0.16–0.78, $p=0.009$) [26]. This article has been evidence for recommending *H. pylori* eradication in EGC patients in Japan. However, another retrospective Japanese study concluded that when they followed up the patients who underwent endoscopic submucosal dissection of EGC for a long time, *H. pylori* eradication did not reduce the incidence of metachronous gastric cancer [27]. The results of randomized controlled study which evaluated the effect of *H. pylori* eradication on the incidence of metachronous gastric cancer in 901 patients who underwent endoscopic resection of EGC was recently published in Korea [28]. During the median 3 years of follow-up period, there was no significant difference in the incidence of metachronous gastric cancer between the *H. pylori* eradication group and the control group.

52.3 Meta-analyses

Until now, three meta-analyses evaluated the effect of *H. pylori* eradication on AG and IM. The first meta-analysis by Rokkas et al. in 2007 included 1 randomized controlled study and 7 observational studies of a total of 1,154 subjects [29]. All included studies evaluated the degree of AG and IM using the updated Sidney system. The improvement of AG in the *H. pylori* eradication group was significant compared to the control group; the OR of AG in the *H. pylori* eradication group was 0.554 (95% CI, 0.372–0.825, $p=0.004$) in the antrum and 0.209 (95% CI, 0.081–0.538, $p<0.001$) in the body. However, the OR for IM was not significantly different between the *H. pylori* eradication group and the control group; the OR of IM in the *H. pylori* eradication group was 0.795 (95% CI, 0.587–1.078, $p=0.14$) in the antrum and 0.891 (95% CI, 0.663–1.253, $p=0.506$) in the body. Therefore, the authors concluded that *H. pylori* eradication can improve AG but it cannot reverse IM. A second meta-analysis by Wang et al. in 2011 evaluated 3 randomized controlled studies and 9 observational studies of a total of 2,658 subjects [30]. When the histological changes of gastric mucosa were compared before and after *H. pylori* eradication, the pooled weighted mean difference of AG was 0.12 (95% CI, 0.00–0.23, $p=0.06$) in the antrum and 0.32 (95% CI, 0.09–0.54, $p=0.006$) in the body, respectively. The pooled weighted mean difference of IM was 0.02 (95% CI, −0.12–0.16, $p=0.76$) in the antrum and −0.02 (95% CI, −0.05–0.02, $p=0.42$) in the body, respectively. That is, *H. pylori* eradication could improve only AG in the body among precancerous lesions. Considering that AG generally starts in the antrum and progresses to the body, these results are not readily understandable. However, the authors did not specifically discuss this issue. In contrast to these two meta-analyses which suggested IM as a "point of no return," a more recent meta-analysis by Kong et al. suggested that there were improvements in IM after *H. pylori* eradication [31]. In this meta-analysis, a total of 4,294 subjects and 16 studies were included. The pooled weighted mean difference of AG in the

H. pylori eradication group was 0.25 (95% CI, 0.15–0.35, $p<0.00001$) in the antrum and 0.14 (95% CI, 0.04–0.24, $p=0.008$) in the body; the improvement of AG was significant compared with the control group. IM in the antrum was also improved (the pooled weighted mean difference 0.23 (95% CI, 0.18–0.29, $p<0.00001$)). However, IM in the body was not different (the pooled weighted mean difference −0.01 (95% CI, −0.04–0.02, $p=0.51$)). This meta-analysis has strong points in that it included more subjects than previous meta-analyses and it applied a more strict histologic method in evaluating AG and IM; it included studies which collected more than three pieces of gastric mucosal samples in each area.

Taking these three meta-analyses together, it seems likely that *H. pylori* eradication can improve AG to some extent, but it cannot improve IM, especially advanced IM in the body. However, there are many limitations in these meta-analyses. First, the individual studies included were mostly from Japan and Italy and performed in a single institution. Second, although histological evaluation of antral AG was performed in all studies, many studies did not evaluate body AG and IM. Third, the meta-analysis by Kong et al. included studies showed heterogeneity for AG [30]. Fourth, although gastric carcinogenesis occurs over a long period of time, the follow-up period in most studies was very short, approximately just 1 year. Finally, most studies were observational ones and randomized controlled trials were rare. Therefore, to make a definite conclusion is very difficult in the present situation.

52.4 Guidelines

The guideline which was presented in 2012 by ESGE (European Society of Gastrointestinal Endoscopy), EHSG (European Helicobacter Study Group), and ESP (European Society of Pathology) suggested that *H. pylori* eradication can partially regress AG but it cannot reverse IM [32]. However, at the same time, this guideline recommended *H. pylori* eradication in patients with IM based on the reason that *H. pylori* eradication might delay progression to neoplasm in these patients. For the management of *Helicobacter pylori* infection, the Maastricht IV/Florence Consensus Report which was published in a similar time concluded that there are some controversies in whether *H. pylori* eradication could reverse AG and there was no evidence supporting *H. pylori* eradication could reverse IM [33]. Nevertheless, this report also included AG and IM in the indication of *H. pylori* eradication. With regard to Asian view, guidelines of China, Japan, and Korea in 2013 all recommended *H. pylori* eradication in patients with AG and IM [24, 34, 35].

52.5 Limitations

We can think of several reasons regarding why the effect of *H. pylori* eradication on AG and IM varies with studies. First, interobserver variations in the endoscopic diagnosis of AG and IM are very high. In addition, in many cases these precancerous lesions show different distributions according to each area of the stomach [36, 37]. Therefore, to evaluate the change of these precancerous lesions precisely before and after *H. pylori* eradication is very difficult. Second, it is known that the possibility to show improvement in AG and IM is higher when *H. pylori* eradication was performed in the early stage than in the advanced stage [38]. As an example supporting this notion, in a Japanese study which performed *H. pylori* eradication in 1,674 patients with peptic ulcer and followed them up for a mean 5.6 years, the risk ratio of gastric cancer increased in proportion to the degree of atrophy of baseline gastric mucosa [39]. Third, among the three types of IM, type III (incomplete colonic type) is known to have a high risk for progression to gastric cancer. A study which analyzed the results according to the types of IM showed that the complete type of IM is more reversible after *H. pylori* eradication than the incomplete type of IM [40]. However, most studies which evaluated the effect of *H. pylori* eradication on the precancerous lesions did not classify subtypes of AG and IM. Nevertheless,

some authors suggest that *H. pylori* eradication is meaningful in the precancerous lesions since *H. pylori* eradication makes the gastric mucosa more clear by reversing some portions of the precancerous lesions and therefore relatively small cancer could be more easily detected [41].

Conclusions

Currently, many international guidelines recommend *H. pylori* eradication in AG and IM. However, there are still many controversies in whether *H. pylori* eradication improves AG and IM. Taking the results of related studies together, it seems likely that *H. pylori* eradication improves AG to some extent. However, a dominant opinion is that IM which progressed to a certain level is already an irreversible status (point of no return). Anyway, it is clear that the possibility of improvement of AG and IM is higher when *H. pylori* infection was treated in the early stage of these precancerous lesions than the advanced stage. Therefore, in-depth studies regarding when is the optimal timing to treat *H. pylori* infection in patients with AG and IM are required.

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Gastric Cancer

53

Hyuk Yoon

Abstract

Because gastric cancer develops only in a very small portion of *Helicobacter pylori* (*H. pylori*)-infected population and it takes a very long time from *H. pylori* infection to gastric carcinogenesis, it is very difficult to prospectively demonstrate the effect of *H. pylori* eradication on the incidence of gastric cancer. Nevertheless, the individual studies and meta-analyses to date suggest that *H. pylori* eradication can reduce the relative risk for gastric cancer to approximately two-thirds. Guidelines all over the world also consistently recommend *H. pylori* eradication as an effective strategy for prevention of gastric cancer. However, there are still controversies regarding when the optimal timing for *H. pylori* eradication is and whether we have to treat *H. pylori* infection in all asymptomatic healthy population to prevent gastric cancer. In addition, it is clear that *H. pylori* eradication cannot prevent all gastric cancers. Therefore, after *H. pylori* eradication, we have to consider additional surveillance in patients who have family history of gastric cancer or who have precancerous lesions such as atrophic gastritis or intestinal metaplasia.

Keywords

Eradication • Gastric cancer • *Helicobacter pylori*

53.1 Introduction

Considering that *Helicobacter pylori* (*H. pylori*) is the main cause of gastric cancer, there are no controversies in that the ultimate goal of *H. pylori* eradication is prevention of gastric cancer. However, gastric cancer develops only in a very small portion of *H. pylori*-infected population, and it takes a very long time from *H. pylori* infection to development of gastric cancer. Therefore,

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it is very difficult to prospectively demonstrate the effect of *H. pylori* eradication on the incidence of gastric cancer [1]. In this chapter, to examine the effect of *H. pylori* eradication on gastric cancer prevention, first we will examine the results of the key individual clinical studies divided into the primary prevention of gastric cancer and secondary prevention (prevention of metachronous gastric cancer after endoscopic resection or surgery of gastric cancer). Then, we will discuss the related meta-analyses. Finally, we will review major guidelines all over the world.

53.2 Key Individual Studies

As opposed to the fact that there are some controversies in that whether *H. pylori* eradication can improve atrophic gastritis (AG) and intestinal metaplasia (IM), there are more evidences in that *H. pylori* eradication can prevent gastric cancer [2]. A series of patient-control studies which were published in 1990s reported that the risk of gastric cancer is three- to sixfolds higher in the *H. pylori*-infected subjects compared with normal control and suggested that there is a definite correlation between *H. pylori* infection and incidence of gastric cancer [3–5]. However, to make a definite conclusion regarding whether there is causality between *H. pylori* infection and development of gastric cancer and furthermore whether *H. pylori* eradication could decrease incidence of gastric cancer, instead of cohort studies, large randomized controlled trials are required. However, to perform these studies is very difficult in reality. Nevertheless, there are many studies suggesting that *H. pylori* eradication can decrease incidence of gastric cancer. Looking at the key individual studies, first, Uemura et al. checked the *H. pylori* infection status in Japanese people and followed them up for mean 8 years; they found that gastric cancer developed only in *H. pylori*-infected subjects [6]. Thereafter, the famous study of Wong et al. randomized 1,630 *H. pylori*-infected healthy adults in an area of high risk of gastric cancer in China into placebo-administrated group and *H. pylori*-eradicated group and followed them up for mean 7.5 years. In the subgroup which has no precancerous lesions such as AG and IM, the indigence of gastric cancer significantly decreased in the *H. pylori*-eradicated group than in the placebo group [7]. Takenaka et al. performed *H. pylori* eradication in 1,807 Japanese people and followed them up. The authors reported the incidence of gastric cancer was significantly lower in *H. pylori*-eradicated group than in *H. pylori* non-eradication group. This protective effect of *H. pylori* eradication was especially remarkable for gastric cancer of intestinal type [8]. Another Japanese group made *H. pylori*-infected subjects decide to receive eradication therapy or not by themselves and followed them up for mean 3 years. The authors reported that the cumulative incidence of gastric cancer was lower in the group who underwent *H. pylori* eradication than in the group who did not receive *H. pylori* eradication (relative risk [RR] 0.335) [9]. This preventive effect of *H. pylori* eradication was significant only for differentiated gastric cancer. Recently, the results of observational study in Matsu Island of Taiwan were reported. The authors treated *H. pylori*-infected subjects among the general population in 2004 and followed them up for 4 years. The incidence of gastric cancer in the mainland of Taiwan did not significantly change. The incidence of 5-year mean incidence of gastric cancer in Matsu Island decreased to approximately 25 % (from 40.3/1,000 person-year to 30.4/1,000 person-year) as along with the decrease of *H. pylori* prevalence [10].

In the next step, looking at the studies which evaluated the effect of *H. pylori* eradication after endoscopic resection of early gastric cancer (EGC), first, a retrospective Japanese study reported that *H. pylori* eradication in the patients who underwent endoscopic resection of EGC reduced the incidence of metachronous gastric cancer [11]. Thereafter, Fukase et al. reported that among subjects who underwent endoscopic submucosal dissection for EGC, the RR for metachronous gastric cancer in *H. pylori* eradication group was 0.35 (95 % confidence interval [CI], 0.16–0.78, $p = 0.009$) compared with the control

group [12]. This article had become the key evidence for recommendation of *H. pylori* eradication in EGC patients in Japan. However, subsequent retrospective study reported conflicting results that *H. pylori* eradication could not reduce the incidence of metachronous gastric cancer when they followed up the patients who underwent endoscopic submucosal dissection for EGC for a long period [13]. Recently, the results of randomized controlled trial which analyzed the effect of *H. pylori* eradication for the incidence of metachronous gastric cancer in the patients who underwent endoscopic resection of EGC were reported in Korea [14]. The authors followed up 901 patients for median 3 years. The incidence of metachronous gastric cancer was not significantly different between *H. pylori* eradication group and control group. Recently another Korean study reported that *H. pylori* eradication reduces the incidence of metachronous gastric cancer in the patients who underwent endoscopic resection of EGC [15]. Taken together, the results for whether *H. pylori* eradication could prevent metachronous gastric cancer after endoscopic resection of EGC are contradictory to each other.

Well-designed studies which evaluated the effect of *H. pylori* eradication in the patients who underwent surgery for gastric cancer are rare. In addition, because the biochemical and microbiological composition of gastric juice after gastrectomy dramatically change as along with the change of anatomical structures, to independently evaluate the protective effect of *H. pylori* eradication for metachronous gastric cancer becomes more difficult [16]. Recently, the results of Korean study which randomly allocated 190 patients who underwent subtotal gastrectomy for gastric cancer into *H. pylori* eradication group and placebo-administrated group and followed them up were published [17]. At 36-month follow-up, the incidence of metachronous gastric cancer was not different between the two groups and according to the final *H. pylori* infection status of the patient. However, because there is a possibility that bile reflux could act as carcinogen in these patients [18], further studies are required to make a conclusion.

53.3 Meta-analyses

In 2009, Fuccio et al. published the first meta-analysis which evaluated randomized controlled trials which compared the incidence of gastric cancer between *H. pylori* eradication group and *H. pylori* non-eradication group [19]. In this meta-analysis, a total of seven studies were included. Gastric cancer developed in 37 (1.1 %) of 3,388 patients who underwent *H. pylori* eradication and in 56 (1.7 %) of 3,307 patients who did not treat *H. pylori* infection. When the individual studies in which the follow-up duration was 4–10 years were taken together, The RR of gastric cancer development in the patients who underwent *H. pylori* eradication was 0.65 (95 % CI, 0.43–0.98). However, this meta-analysis separately evaluated two studies in which the follow-up duration was different but actually the same data were used. In addition, there was a criticism that when analysis was performed considering the duplicated two studies as one data, the statistical significance disappeared [20]. Therefore, new meta-analysis was performed for six randomized controlled studies which divided *H. pylori*-infected healthy people into *H. pylori* eradication group and control group and followed them up at least for 2 years [21]. Gastric cancer developed in 51 (1.6 %) of 3,294 people who underwent *H. pylori* eradication and in 76 (2.4 %) of 3,203 people who did not treat *H. pylori*. In the group in which *H. pylori* was successfully eradicated, the RR for gastric cancer development was 0.66 (95 % CI, 0.46–0.95). There was no heterogeneity among included studies. When subgroup analyses were performed according to the presence or absence of associated premalignant lesions, the incidence of gastric cancer after *H. pylori* eradication was not different. When the subjects were followed up during 5–14.7 years, 24 (1.1 %) of 2,242 in *H. pylori* eradication group died and 36 (1.6 %) of 2,233 controls died; the gastric cancer-related mortality rate was also significantly different between the two groups (RR in *H. pylori* eradication group 0.67, 95 % CI, 0.40–0.11). However, 192 (7.3 %) of 2,639 *H. pylori* eradication group died and 175 (6.7 %) of 2,614 controls died; the overall mortality rate was not significantly different (RR, 1.09;

95 % CI, 0.86–1.38). The number need to treat for gastric cancer development was mean 124. In the subgroup analyses, it was lowest in Chinese male (number need to treat = 15) and highest in American female (number need to treat = 245). The authors claimed that there is evidence that *H. pylori* eradication is effective for gastric cancer prevention in asymptomatic healthy people in Asia where the risk of gastric cancer is high. However, this meta-analysis also has several limitations. First, only three studies among included six studies have low risk of bias. In addition, because some studies did not have factorial design which can test interaction among the factors, it is difficult to judge whether prevention of gastric cancer development after *H. pylori* eradication is caused by *H. pylori* eradication itself. Second, all studies except just one study were performed in East Asia. Therefore, it is very difficult to conclude the effect of *H. pylori* eradication in non-Asian countries. Third, the result that the reduced risk of gastric cancer development after *H. pylori* eradication is not related with the presence or absence of precancerous lesions is somewhat difficult to accept. Therefore, it is a thesis to be demonstrated by large randomized controlled studies in the future. Finally, because the confidential interval of the results was wide, there is a possibility that the combined results may be affected by specific single study among included studies.

Recently, the results of meta-analysis which evaluated the effect of *H. pylori* eradication on the development of metachronous gastric cancer in the subjects who underwent endoscopic resection of EGC were reported [22]. The authors analyzed the data about 13 studies including 6,237 subjects. They reported that *H. pylori* eradication in the patients who underwent endoscopic resection of EGC decreased the incidence of metachronous gastric cancer approximately by half (odds ratio 0.42; 95 % CI, 0.32–0.56). However, there are some limitations in that all included studies were performed in Korea and Japan and randomized controlled studies were just two. In addition, because some studies included dysplasia as well as gastric cancer into the definition of metachronous neoplasm, the results should be interpreted with caution.

53.4 Guidelines

In Asia-Pacific area, both consensus guidelines for prevention of gastric cancer and management of *H. pylori* infection state that *H. pylori* eradication is an effective strategy for gastric cancer prevention in countries where incidence of gastric cancer is high [23, 24]. However, these guidelines qualify that preventive effect of *H. pylori* eradication for gastric cancer is more evident in the cases without precancerous lesions at the time of *H. pylori* eradication and gastric cancer can develop even in the patients who received successful *H. pylori* eradication [23]. The guidelines of Korea, Japan, and China which are representative countries where the incidence of gastric cancer is high also recommend *H. pylori* eradication for gastric cancer prevention [25–27]. In addition, a study to prevent gastric cancer in Japan suggests that appropriate timing of *H. pylori* eradication is younger age before the development of AG or IM [28]. This is because that *H. pylori* eradication decreased the prevalence of gastric cancer only in patients without premalignant lesions such as AG and IM [6, 7]. Similarly, in a Korean cohort study, the eradication of *H. pylori* could not reduce gastric cancer development in patients with IM [29].

Looking at the guidelines of Western countries in the management of *Helicobacter pylori* infection, the Maastricht IV/Florence Consensus Report also recommends *H. pylori* eradication for gastric cancer prevention [30]. Another European multicenter guideline about management of precancerous lesions also recommends *H. pylori* eradication after endoscopic or surgical treatment of gastric neoplasm [31]. Although it is a somewhat dated guideline, US guideline also considers endoscopic resection of EGC as established indication for *H. pylori* eradication [32].

Conclusions

Because gastric cancer develops only in a very small portion of *H. pylori*-infected population and it takes a very long time from *H. pylori* infection to gastric carcinogenesis, it is very difficult to prospectively demonstrate the effect of *H. pylori* eradication on the incidence

of gastric cancer. Nevertheless, the individual studies and meta-analyses to date mostly suggest that eradication of *H. pylori* is an effective strategy for prevention of gastric cancer. However, there are still controversies regarding when the optimal timing for *H. pylori* eradication is and whether we have to treat *H. pylori* infection in all asymptomatic healthy population to prevent gastric cancer.

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Part IX

The Effect of *H. pylori* Infection on the Gastric Microbiota

The Effect of *H. pylori* Infection on the Gastric Microbiota

54

Hyun Jin Jo

Abstract

Along with development of culture-independent analysis methods such as next-generation sequencing, it is known that there are a variety of bacteria in the stomach besides *Helicobacter pylori* (*H. pylori*). The composition of gastric microbiota is affected by various factors like a type of sample, dietary habits, disease status, pH, and *H. pylori* infection. The chronic infection of *H. pylori* induces gastric atrophy and elevation of gastric pH, which predisposes to increase gastric microbial diversity. It has been reported that the composition of *Streptococcus*, most commonly found in healthy stomach, increased in the gastritis or gastric cancer group compared to the control group.

Keywords

Helicobacter pylori • Microbiota • Pyrosequencing

54.1 Introduction

The discovery of *Helicobacter pylori* (*H. pylori*) in the 1980s brought about a change in the conventional concept that the stomach is a sterile organ. Thereafter, the interest in microbial ecosystem of the stomach has increased, but the actual researches were mainly focused on *H. pylori* [1].

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The state of chronic infection of *H. pylori* has proven to cause the inflammation of the gastric mucosa, atrophy, intestinal metaplasia, and further premalignant changes like dysplasia. Thus, it is clear that *H. pylori* plays an important role in the development of gastric cancer. However, it is not clear that *H. pylori* eradication has the effect of suppressing the occurrence of gastric cancer in the state of atrophic gastritis. Recently, the development of culture-independent molecular technologies such as next-generation sequencing (NGS) has increased an attention to the research about gastric microbiota. In this respect, the role of bacteria than *H. pylori* has received recent attention. In this chapter, we will find out the research

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about gastric microbiota and investigate the effect of *H. pylori* infection on the gastric microbiota.

54.2 The Development of Analysis Methods of Gastric Microbiota

The previous method for the analysis of gastric microbiota was mainly culture-based study using gastric fluid or gastric mucosal tissue. However, in 1980s, along with development of molecular biologic analysis technique like DGGE (denaturing gradient gel electrophoresis), T-RFLP (terminal restriction fragment length polymorphism), and microarray, it has been known that a diverse microbiota other than *H. pylori* exists in the stomach [1]. Eighty percent of bacteria known to exist in the stomach were not observed in the culture-based study.

Recently, a new analysis technique has been developed such as NGS, which sequences the genes of bacteria and finds out the species stage. After Bik et al. in the 2006 firstly published the results using 16S rRNA (small-subunit ribosomal RNA) analysis, recently several studies have been announced about the gastric microbiota [2–5]. Although NGS molecular approach is powerful and provides a complete view of the overall microbiota, it is unable to distinguish between living and dead bacteria. This is because bacterial genes may be present, even if bacteria are killed by antibiotics. However, it is an advantage that NGS shorten the time required for analysis and can analyze simultaneously the large amount of samples [4, 6].

54.3 Various Conditions Affecting the Gastric Microbiota

The most representative phyla of gastric microbiota are *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, and *Bacteroidetes* (Fig. 54.1). The composition of *Firmicutes* and *Proteobacteria* is major in the gastric mucosa, but, in the gastric fluid, *Actinobacteria*, *Bacteroidetes*, and *Firmicutes* relatively accounted more proportion [2]. *H. pylori* belonging to the *Proteobacteria* is found less in the gastric fluid. Actually, in the pyrosequencing study of our laboratory, *H. pylori* was accounted an average of 66.5 % in the gastric mucosa, but only 3.3 % in gastric fluid [8] (Fig. 54.2). This is because a suitable place that *H. pylori* live together is not the gastric fluid but gastric epithelial cells and the mucus adjacent to the mucosa [9].

Since the parietal cells that secrete gastric acid are mainly distributed to the gastric body than the antrum, the acidity of the gastric body is normally higher than that of the antrum.

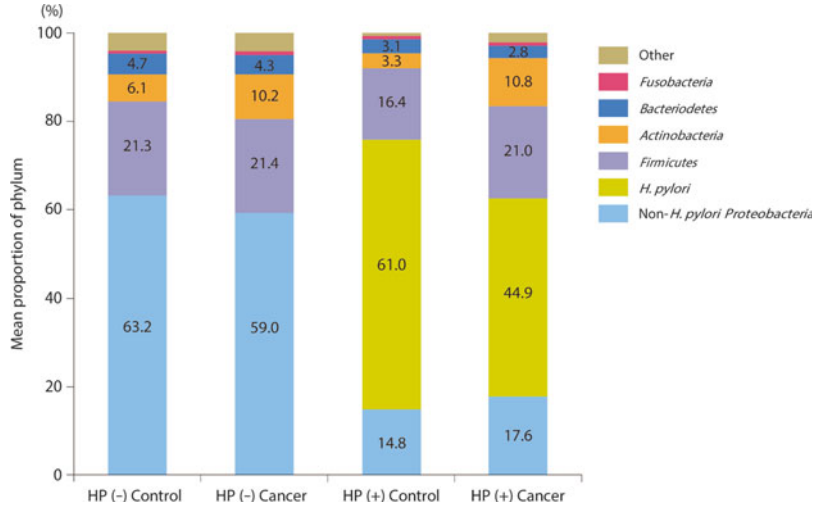

Fig. 54.1 The mean proportion of phylum. The most representative phylum was *Proteobacteria*, followed by *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Fusobacteria*. *HP H. pylori* (Adapted from Jo et al. [7])

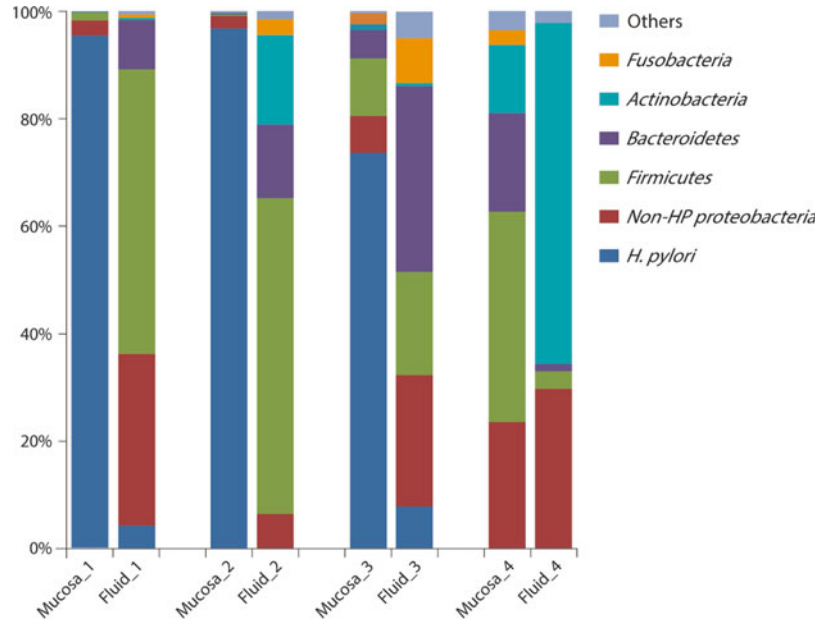

Fig. 54.2 The composition of phylum in the gastric mucosa and gastric fluid samples. The phylum composition was different between gastric mucosa samples and gastric fluid samples. The proportion of *H. pylori* was higher in the gastric mucosa samples (66.5 %) compared with that in gastric fluid samples (3.3 %). *HP H. pylori*

Therefore, it would be able to predict that the distribution of bacteria shows the difference depending on the site of the stomach. In the previous studies, there was no significant difference of gastric microbiota depending on the site of the harvested tissue [10, 11]. Our study using 17 body-antrum paired samples did not show the significant differences of gastric microbiota [7].

In addition to the type of sample, factors affecting gastric microbiota are various. It is affected by the dietary habits, disease status, medication like antibiotics or gastric acid secretion inhibitor, and *H. pylori* infection.

54.4 The Effect of *H. pylori* Infection on the Gastric Microbiota

Under the normal acidic condition of a healthy stomach, which was not infected with *H. pylori*, *Veillonella* spp., *Lactobacillus* spp., *Clostridium* spp., and *Corynebacterium* spp. were mainly cultured [12]. In one study, based on 16S rRNA analysis, it was shown that *Streptococcus*, *Prevotella*, *Neisseria*, *Haemophilus*, and *Porphyromonas* genera accounted for about 71 % of gastric microbiota. Especially, *Streptococcus* and *Prevotella* genera were equivalent to 41 % of the total bacteria [4]. Thus, *Streptococcus* is a bacterium that is most commonly found in healthy stomach. *Streptococcus mitis* is known as aciduric bacterium that can propagate under the acidic condition [13].

When the stomach is infected with *H. pylori*, *H. pylori* occupies the most of gastric microbiota. Therefore, microbial diversity is reduced under the *H. pylori*-infected stomach compared to healthy stomach [2, 3]. However, in the state of chronic infection of *H. pylori*, gastric microbial diversity increases again. There are possible explanations for this. The first, *H. pylori*-induced inflammation causes gastric atrophy and reduces the parietal cell mass, elevating gastric pH, which predisposes to colonization by environmental microbiota that cannot survive in the normal gastric pH [14]. There are other plausible reasons for *H. pylori*-induced changes in the microflora. *H. pylori* produces ammonia and bicarbonate from urea that can be used as substrates by other bacteria [15], and *H. pylori* infection is correlated with slower migrating motor complex (MMC) phase III activity, which contributes to the clearing of adherent bacteria from the antral mucosal compartment [11].

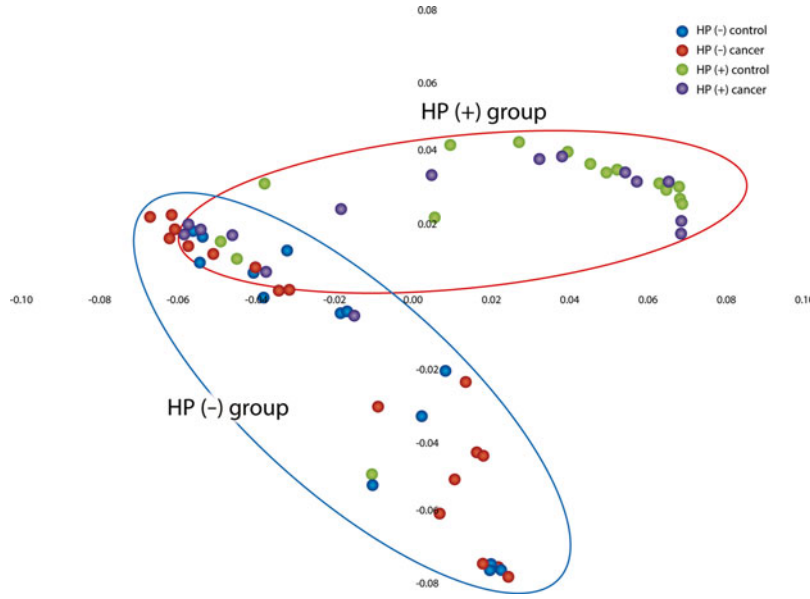

Fig. 54.3 Unweighted UniFrac-based principal coordinates analysis of gastric antral microbial communities. This analysis is based on taxa clustered at 97 % sequence 16S rRNA gene identity. The unweighted UniFrac analysis indicated that there was very little separation between control and cancer groups under the identical HP infection status. Samples obtained from patients of HP (−) control, HP (−) cancer, HP (+) control, and HP (+) cancer are represented by *blue*, *red*, *green*, and *purple circles*, respectively. *HP H. pylori* (Adapted from Jo et al. [7])

Several previous studies did not show that chronic *H. pylori* infection significantly alters the gastric microbiota [2, 16]. However, in the recent study that use pyrosequencing, the diversity of gastric microbiota increased with progression from chronic gastritis and intestinal metaplasia to gastric cancer, and this change was prominent in *H. pylori*-positive group [5]. Our study also showed the difference of gastric microbial communities according to *H. pylori* infection or not [7] (Fig. 54.3).

54.5 Altered Microbiota Composition Related with Disease State

Eun et al. [5] showed that, under the *H. pylori*-positive state, the gastric cancer group showed a significant decrease in the *Epsilonproteobacteria* class and *Helicobacteraceae* family compared with the chronic gastritis and intestinal metaplasia group, while the *Bacilli* class and *Streptococcaceae* family increased significantly [5]. In gastric cancer, the number of *Bifidobacte rial/Lactobacilli*, *Veillonella*, and *Streptococci* is increased as determined by culture [13]. Previous study for the microbial composition of human stomach by Dicksved et al. [17] using T-RFLP with bacterial sequencing method demonstrated that there was no significant difference in microbial composition of phylum between patients with gastric cancer and controls, while genera *Streptococcus*, *Lactobacillus*, *Veillonella*, and *Prevotella* were predominant in patients with gastric cancer. Among the *Streptococcus* a group including *S. mitis* and *Streptococcus parasanguinis* was shown to dominate [17].

Li et al. [10] showed that, even in the case of *H. pylori*-negative patients, *Streptococcus* was significantly higher in the gastritis group compared to the control group. As *Streptococcus* was cultured in a tissue sample subjected to rigorous washing steps, *Streptococcus* is likely to act as pathogen [10].

54.6 Influence of Change of pH on the Gastric Microbiota

It has been known that increased gastric pH by long-term use of proton pump inhibitor or H_2 receptor blocker bring the bacterial overgrowth and the change of composition of gastric microbiota. These changes were prominent under the longer administration of acid lowering drug and the infected state with *H. pylori*. And the bacterial overgrowth consists mainly of oral bacteria [18]. In the condition of achlorhydria related with aplastic anemia, coliform was found in the stomach [13].

Conclusions

It was known that various bacteria that have not been cultured exist in the stomach as the molecular biological technologies develop. Though, after that, various studies have been conducted, the research designs are different from each other. Therefore, it is not easy to compare uniformly. In addition, research about the impact on disease development of gastric microbiota is still in the early stage.

The protein analysis study, proteomics, should be done to uncover about the actual function of the detected bacteria. A better understanding of the gastric microbial communities in health and disease would shed light on the pathogenesis, diagnosis, treatment, and prevention of gastric illnesses.

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Part X

Animal Model

Animal Models of *H. pylori* Infection

55

Ju Yup Lee

Abstract

The best suited animal models of *Helicobacter pylori* (*H. pylori*) identified so far are rodents. *Helicobacter felis* infection provokes severe inflammation and induces well sequential carcinogenic events leading to dysplasia and gastric adenocarcinoma. *H. pylori* SS1 is the most useful strain identified so far. It expresses cytotoxin-associated gene A (*cagA*) and vacuolating cytotoxin (*vacA*) and can easily colonize the stomach of mice and induce humanlike gastric pathological changes. However, a long-term colonization of the stomach does not always induce dysplasia or adenocarcinoma. Unlike *H. pylori* SS1, however, it lacks the *cag* pathogenicity island (PAI) and does not adhere well to gastric epithelial cells. Individuals colonized by the same strain show different responses. This is ascribable to T cell immune responses, whereby the balance between the immune responses of Th1 and Th2 plays an important role. A variety of transgenic or knockout mice have been developed and used. Such animal models have greatly contributed to identifying immunophysiology associated with *H. pylori*.

Keywords

Animal • *Helicobacter felis* • *Helicobacter pylori* SS1

55.1 Introduction

For the past decades, animal infection models have been efficiently used for identifying the pathophysiology of *H. pylori*-related diseases. The following summarizes the achievements made to date using *H. pylori* animal models [1]. First, gastric acid has been found to play an important role in the creation of favorable habitat conditions for *H. pylori*. This explains why

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different population groups tend to develop different diseases and why gastric ulcers affect specific regions. Second, studies with different mouse species have led to the finding that individual factors play an important role in the colonization process. Third, pathophysiologic mechanisms of a variety of bacterial virulence factors, such as urease, have been identified. Fourth, the immuno-pathophysiological mechanisms have been identified through the knockout mice, and vaccination against *H. pylori* is possible. Lastly, antimicrobial research has gained momentum.

The foci of this chapter are the representative strains used for the animal models of *H. pylori* infection, animal species, predominantly rodents that are most commonly used at present and a variety of transgenic or knockout mice.

55.2 Selection of Animal Models

To date, many different animal species have been used in *H. pylori* infection research, ranging from primates to rodents. Big animals, such as monkeys, pigs, and dogs, were mostly used in earlier phases of research using animal models. Such models have been found to be easily colonized by *Helicobacter* strains [2–4]. While they have gastric physiology similar to that of humans, they are usually costly and have low values for practical use. Moreover, while lymphocytic gastritis could be induced in these animal models, severer conditions than that, such as gastric ulcer or adenocarcinoma, did not occur. Above all, their major disadvantage is the limitation in long-term utilization and the colonization of endemic *Helicobacter* strains, such as *H. heilmannii* for primates and *H. suis* for pigs [5].

Small animals such as ferrets, Mongolian gerbils, rats, and mice are more convenient to handle than big animals and have many additional advantages. The first report of carcinogenesis by *Helicobacter* strains was made on ferret models, whereby gastritis, dysplasia, and gastric adenocarcinoma were induced in ferret stomachs colonized by *H. mustelae* [6]. Moreover, ferrets are the first animal model in which gastric ulcers were verified [7]. However, ferrets are not easily available and the strain used for them, *H. mustelae*, has different properties compared to *H. pylori* that colonizes the stomach of humans.

Mongolian gerbils are easily colonized by *H. pylori* and once they are infected, they show the sequential steps of chronic active gastritis, peptic ulcers, intestinal metaplasia, and gastric cancer similar to those of humans [8]. G1.1, TN2, and B128 are some of the *H. pylori* strains that were verified to induce gastric adenocarcinoma by colonizing Mongolian gerbils. Additionally, in relation to many reports on the increased incidences of gastric cancer in the Mongolian gerbil group administered first chemical gastric carcinogenic agents such as MNU and MNNG (*N*-methyl-*N*'-nitro-*N*-nitrosoguanidine) and additionally infected with *H. pylori* when compared to the MNU or MNNG single administration group, there are also views that *H. pylori* plays the catalytic role for the gastric carcinogenesis in Mongolian gerbils rather than being single carcinogenic factor [9, 10]. While Mongolian gerbils are excellent as an animal model of *H. pylori* infection and induce inflammatory responses similar to humans, they are costly and lack transgenic or knockout species and thus compare unfavorably to mice in terms of practical use.

Tables 55.1 and 55.2 outlines the advantages and disadvantages about the animal models developed thus far [1, 4]. The conditions for an ideal animal model are cost-effectiveness, high reproducibility, short observation period, and pathogenesis leading to lesions close to human histological changes, and up to now, rodents have been found to meet these conditions better than any other animals [8]. Mice, in particular, have additional advantage of easy genetic manipulation and are being used most widely for *H. pylori* research.

55.3 Selection of Strains

With mice showing resistance to the colonization of various *H. pylori* strains, research for developing suitable strains for mouse models has been

Table 55.1 Advantages and disadvantages of *Helicobacter*-infected animal models

Animal	Colonized by	Advantage	Disadvantage
Primates	*H. pylori*	Closest animal species to human, endoscopy possible, gastric physiology similar to human	Expensive, colonized by endemic strains, presence of HHLO bacteria
Gnotobiotic piglets	*H. pylori*	Colonization pattern similar to human, gastric physiology similar to human, ulcers observed	Chronic gastritis only, expensive short-term experiments only
Ferrets	*H. mustelae*	Natural infection, useful for vaccine studies, gastric physiology similar to human	Pattern of colonization varies from human, predominantly chronic gastritis only
Cats and dogs	*H. pylori* *H. felis*	Gastric physiology similar to human	Gnotobiotic and SPF animals expensive, HHLO present in normal cats
Guinea pig	*H. pylori*	IL-8 homologue, active chronic gastritis	Limited used, data limited
Mouse	*H. pylori* *H. felis* *H. heilmannii*	Economical good for testing vaccines/antimicrobials, good colonizing strains of *H. pylori* (SS1) available	Dose not mimic human pathology
Gerbils	*H. pylori*	Chronic/active antral gastritis, gastric ulcers, adenocarcinoma induced with infection alone	Lack of immunological reagents, lack of transgenic/knockout strains

Adapted from Lee [1]
SPF specific pathogen free, *IL-8* interleukin 8, *HHLO Helicobacter heilmannii*-like bacteria

Table 55.2 Factors to consider in selecting *Helicobacter*-infected animal models

	Most	Favorable	Least
Cost and availability	Mice-rats	Ferrets/cats	Pigs, primates
Reproduces human disease	Primates-pigs-gerbils-ferrets		Mouse models
Availability of reagents	Mice-primates		Ferrets-gerbils

Adapted from Nedrud [4]

underway. Of the strains hitherto identified, *Helicobacter felis* (*H. felis*) and *H. pylori* Sydney strain 1 (SS1) are known to be the most promising strains [11].

55.3.1 Characteristic and Morphologic Differences Between *H. felis* and *H. pylori* SS1

H. felis is a *Helicobacter* species isolated from cat gastric mucosa. It has a structure very similar to that of *H. pylori* and grows well in the stomach of mice. In the 1990s, Lee et al. [12] made the first report on acute and chronic gastritis in germ-free Swiss Webster mice infected with *H. felis* similar to inflammatory responses in humans. In subsequent studies, numerous *H. felis* colonized the stomach of mice in large numbers, inducing severe gastritis and atrophic gastritis [13], and long-term colonization models developed intestinal metaplasia, dysplasia, and invasive gastric cancer over time [14, 15]. However, different from *H. pylori* colonizing human gastric mucosa, *H. felis* shows noticeably different pathological features from the pathophysiological changes occurring in human gastric mucosa [8]. Moreover, *H. felis* lacks the cytotoxin-associated gene pathogenicity island (*cag* PAI) and does not adhere well to gastric epithelial cells, floating in the gastric mucosa (Table 55.3). Therefore, using *H. felis* for animal models would serve the purpose in studies investigating host factors, but may

Table 55.3 Differences between *H. felis* and *H. pylori* SS1

H. felis	*H. pylori* SS1
Isolated from cat gastric mucosa	Isolated from human gastric mucosa
Lacks the *cag* pathogenicity island (PAI)	*cagA*(+), *vacA*(+)
Colonizes the mucus layer	Adheres directly to epithelial cells
Chronic active gastritis progressing to severe hyperplasia, atrophy, dysplasia, and carcinoma	Chronic active gastritis progressing to severe hyperplasia and atrophy
	Rarely induced dysplasia or carcinoma

have limitations in studies investigating virulence factors [16].

In an attempt to overcome these limitations, many studies have focused on *Helicobacter* strains that can grow well on the gastric mucosa of mice and have many virulence factors. In 1997, Lee et al. [17] succeeded in isolating *H. pylori* in the gastric mucosa of a 42-year-old who had suffered from gastric ulcer for 10 years and developed an animal model using this strain, which they named *H. pylori* Sydney strain 1 (SS1). Known to be the most useful strain identified so far, it expresses *cagA* and vacuolating cytotoxin (*vacA*), easily infects mice, and is reported to induce pathological changes similar to human gastric diseases [17]. Unlike *H. felis* that has weak adhesion or looks as if floating in the mucus when observed with an electron microscope, *H. pylori* SS1 is characterized by tight adhesion to the epithelial cells via fine projections with adherence pedestals [17] (Table 55.3). Another particular feature of *H. pylori* SS1 is that it colonizes BALB/c, DBA/2, and C3H/He mice in lower numbers while developing colonies in large numbers in C57BL/6 mice [11, 17].

In 2004, another strain (Sydney strain 2000, SS2000) that has virulence factors suitable for mice was isolated by Thompson et al., and this strain has been used for various virulence-related research models [18]. On the other hand, *H. pylori* ATCC43504 strain reported by Hirayama et al. [19] in Japan in 1996 showed 100% infection rate when Mongolian gerbils were colonized and induced gastritis pathologically similar to human gastritis. Along with *H. pylori* SS1, it is being widely used for various mouse models.

55.3.2 Pathological Difference Between *H. felis* and *H. pylori* SS1

Unlike *H. pylori*-related gastritis in humans, in which both neutrophils and monocytes are deposited on mucous membranes [20], neutrophils are less deposited in mouse models, as observed in both *H. felis* and *H. pylori* infections. While a chronic colonization of the mouse stomach by *H. felis* and *H. pylori* SS1 leads to chronic gastritis and premalignant lesions, there is an important difference between the two. Almost all *H. felis* infections in the C57BL/6 mice develop into gastric adenocarcinoma via sequential steps from intestinal metaplasia and dysplasia [21, 22], which is very similar to the human gastric carcinogenesis. In contrast, *H. pylori* SS1 failed to induce gastric adenocarcinoma in most mouse species [11]. Kim et al. [23] observed *H. pylori* SS1-infected C57BL/6 mice for 80 weeks and reported that while hyperplastic gastritis or chronic atrophic gastritis was easily induced, no incidence of dysplasia or gastric adenocarcinoma was observed and explained the reason to be the balance between cell proliferation and apoptosis. *H. felis* infection in the C57BL/6 mouse induces severe inflammation whereby the degree of early-phase inflammation shows higher level compared to *H. pylori* SS1 infection. Lee et al. [24] observed *H. pylori* SS1-infected C57BL/6 mice for 4 weeks and verified that inflammatory responses and hyperplastic gastritis are induced well. The same mice were then infected with *H. felis* and observed for 4, 24, and 52 weeks. They showed severer inflammatory responses, with significantly higher level of myeloperoxidase, a marker of inflammation, during the same period of 4 weeks compared to the *H. pylori* SS1 infection group [25]. Furthermore, from the fact *H. felis* infection led to significant increase in interleukin (IL)-1β and no increase in tumor necrosis factor (TNF)-α, it can be inferred that IL-1β is involved

in the immunological mechanism of the infected host [25]. Accordingly, the different characters of *H. pylori* and *H. felis* strains should be considered when establishing animal models of *Helicobacter* infection.

55.4 Differences in Individual Responses

Individual genetic factors play an important role in inflammatory responses or carcinogenesis of individual animals. When a mouse species was infected with a *Helicobacter* species, each mouse showed noticeably different responses. For example, histological changes observed 1, 2, and 6 months after infecting different mice with the same *Helicobacter* species *H. felis* yielded different results in different mouse models. While SJL, C3H/He, DBA/2, and C57BL/6 mice developed moderate-to-severe chronic active gastritis in the corpus, which got exacerbated over time, resulting in atrophy, BALB/c and CBA mice showed only mild antral gastritis, and no change was observed in the corpus, nor did atrophy occur [13]. Based on these results, it was proposed to classify the animal models into responder strains and nonresponder strains depending on the degree of individual inflammatory responses after *H. pylori* infection [13]. According to this classification, C57BL/6 and C3H/He can be defined as responder strains and BALB/c and CBA as nonresponder strains. However, when the nonresponder strain BALB/c mouse underwent long-term infection with *H. felis*, mucosa-associated lymphoid tissue (MALT) lymphoma-like pathology was observed [26]. In other words, as a result of observing BALB/c mice during 22 months of *H. felis* infection, it was found that 38 % BALB/c mice developed MALT lymphoma, whereas the uninfected control group did not develop it [26].

The interindividual differences in inflammatory responses among the mice infected with the same strain can be explained by the differences in T cells. RAG-$1^{-/-}$ mice, which lack both B and T cells, and TCR$\beta\delta^{-/-}$ mice lacking T cells only did not show pathological abnormalities despite a high degree of *H. felis* colonization. In contrast, the B-cell-deficient μMT mice infected with *H. felis* exhibited severe pathological changes in the stomach such as mucosal hyperplasia and intestinal metaplasia, which was similar to the normal-state immune responses of C57BL/6 mice [27]. T cells plays the most important role in the immune responses to *H. pylori* infection [28], and the balance of Th1/Th2 immune responses are essential in the pathogenesis of *H. pylori*-associated disease and host defense mechanisms (Fig. 55.1). In other words, while the predominance of Th1 immune responses greatly contributes to tissue damage, Th2 immune responses are known to exhibit a defense mechanism against the inflammatory responses of the gastric mucosa [29]. C57BL/6 mice primarily show proinflammatory Th1-dominant immune responses, which leads to epithelial cell damage and compensatory hyperplasia even in a small number of colonies [30]. On the other hand, BALB/c mice primarily exhibit noninflammatory Th2-dominant immune

Fig. 55.1 Balance between Th1 (cell-mediated) and Th2 (humoral) immune responses. *IL* interleukin, *IFN-γ* interferon gamma, *Th1* T helper 1, *Th2* T helper 2

response, which is characterized by a large number of colonies but a low proportion of epithelial lesions [18]. Various roles played by Th1 cytokine were proved by studies using gene-deficient mice. Among others, interferon (INF)-γ [31], TNF-α [32], and IL-7 [32] are known to be involved in the incidence of atrophic gastritis. Moreover, C57BL/6 mice with anti-inflammatory Th2 cytokine IL-10 deficiency developed severe hyperplastic gastritis even after short-term *H. felis* colonization (4 weeks) [33].

Furthermore, C57BL/6 mice are colonized by a large number of colonies in the antrum, whereas a small number of colonies were observed in BALB/c mice in the antrum-corpus transitional zone [17]. This reflects the fact that local gastric acid secretion is determinant for the position of *Helicobacter* strains within the stomach [34], which is ascribable to the interspecies differences in local gastric acid secretion or the difference in sensitivity to gastric acid of *H. pylori* or *H. felis* [17].

Moreover, phospholipase A_2 (PLA_2) was reduced in *H. felis*-infected C57BL/6 mice and increased in BALB/c mice according to research results [14, 35], whereas a high level of proinflammatory cytokine such as INF-γ was measured in C57BL/6 mice in a study. Studies with immunodeficient mouse models or mice with deficiency in cytokines such as IFN-γ, IL-10, IL-4, and IL-7 prove well the importance of individual immune system in the occurrence of severe gastritis and accompanying changes in the mucous membrane [27, 32, 33, 36–41].

55.5 Transgenic or Knockout Mouse Models

Research efforts using a large variety of genetically manipulated mice have shed light on the immune mechanism of *Helicobacter* infection and the association of *H. pylori* with gastric cancer. Most genetically manipulated mice are based on C57BL/6 mice. The following presents the representative mice used for clarifying pathophysiological mechanisms [30].

55.5.1 INS-GAS Mice

Transgenic INS-GAS mice overexpress human gastrin gene and thus develops spontaneous atrophic gastritis and gastric adenocarcinoma, and *Helicobacter* infection accelerates this process. The main contribution of this mouse model is the discovery of synergistic interaction between hypergastrinemia and *Helicobacter* infection in developing gastric adenocarcinoma [42].

55.5.2 IFN-γ and TNF-α Knockout Mice

From the observation of decrease in the degree of gastritis and change of epithelial cells when an IFN-γ-deficient mouse is infected with *H. felis* or *H. pylori*, it was found that IFN-γ plays an important role in the occurrence of *Helicobacter*-associated gastritis and epithelial cell damage [31, 43]. There is no consensus on the mechanisms associated with TNF-α. Hasegawa et al. [43] reported that TNF-α contributed to the carcinogenicity of *H. felis*-associated gastritis, whereas Yamamoto et al. [44] reported that gastric inflammatory responses did not decrease even in the absence of TNF-α. Lee et al. [25] also reported that long-term *H. felis* infection (52 weeks) did not yield any significant increase in the TNF-α level. Further research appears necessary to clarify this aspect.

55.5.3 IL-1β Transgenic Mice

IL-1β is a proinflammatory cytokine inhibiting gastric acid secretion, involved in the occurrence of atrophic gastritis and gastric adenocarcinoma [45]. It contributes to developing gastric adenocarcinoma in synergistic interaction with *Helicobacter* infection [46].

55.5.4 IL-10 Knockout Mice

IL-10 is known to be an anti-inflammatory and immunoregulatory cytokine. In IL-10-deficient mice with short-term (4 weeks) *H. felis* infection, severe hyperplastic gastritis and epithelial cell proliferation could be observed [33]. This arose from the Th1 immune responses and is indicative of the inhibitory mechanism of IL-10 in regulating immunity and inflammation.

55.5.5 Fas Antigen Transgenic Mice

Fas is a transmembrane protein that belongs to the TNF family and induces apoptosis. Apoptosis of epithelial cells is one of the most important responses of gastric mucosal membrane to *H. pylori* infection [47]. The Fas antigen transgenic mouse model contributed to gaining the knowledge that Fas-inducing signaling system plays a role of preventing *Helicobacter* infection-associated carcinogenicity of chronic gastritis by inducing apoptosis [48].

55.5.6 p27-Deficient Mice

The cyclin-dependent kinase inhibitor p27 is a tumor-inhibiting protein, and its loss is thus a common clinical feature associated with poor prognosis [49]. Additionally, p27-deficient mice easily developed tumors when exposed to environmental carcinogenic agents [50]. In a 60-week observational sturdy, p27-deficient mice infected with *H. pylori* SS1 developed metaplasia, dysplasia, and gastric cancer [51].

55.5.7 *cagA*-Transgenic Mice

Since *H. felis* lacks *cagA*, the *H. pylori* virulence factor, infection research with rodent models is often useless in clarifying the function of *cagA*. Moreover, even though *cagA* exists in *H. pylori* SS1, it often shows functional insufficiency for *cagA* translocation. To overcome this problem, *cagA*-transgenic mouse model was developed, and *cagA* expression was reported to increase in the mouse stomach, resulting in gastrointestinal cancer, although the frequency was low [52].

55.6 Limitations of Mouse Models of Infection

In general, lab animals are exposed to high levels of stress due to artificial inbred and growing conditions in restricted spaces, poor hygienic state, excessive temperature, humidity, and light changes, which can affect the experimental results and even yield completely different results in severe stress situations. In case of lab mice as well, it should be always taken into consideration that sex, diet, habitat, and genetic environment can affect inflammatory and genetic manipulation-related responses. Furthermore, concurrent infection with an enterohepatic *Helicobacter* species with the experimentally induced infection with a *Helicobacter* strain in a mouse model can exert adverse influence on the results [53, 54].

The following issues can be pointed out as the limitations of mouse models [11]. First, there are only a limited number of *H. pylori* strains (especially *cagA*-positive strains) that can colonize the stomach of mice. Second, only long-term colonization induces tumors. Third, while high-severity dysplasia or early gastric cancer occurs frequently, the occurrence rate of progressive or invasive gastric cancer is very low and the occurrence rate of metastatic gastric cancer is zero. Fourth, there are anatomical differences between human and rodent stomachs. The mouse stomach can be largely divided into the nongrandular forestomach, glandular corpus, and antrum [55, 56] (Fig. 55.2). Although several reports have been made on forestomach squamous cell carcinoma in mouse models [57, 58], the human stomach does not have the forestomach, and gastric squamous cell carcinoma is a very rare disease in human. Thus, such reports have little usability. Additionally, no reports have yet been made on a mouse model that can mimic human gastric cardia cancer.

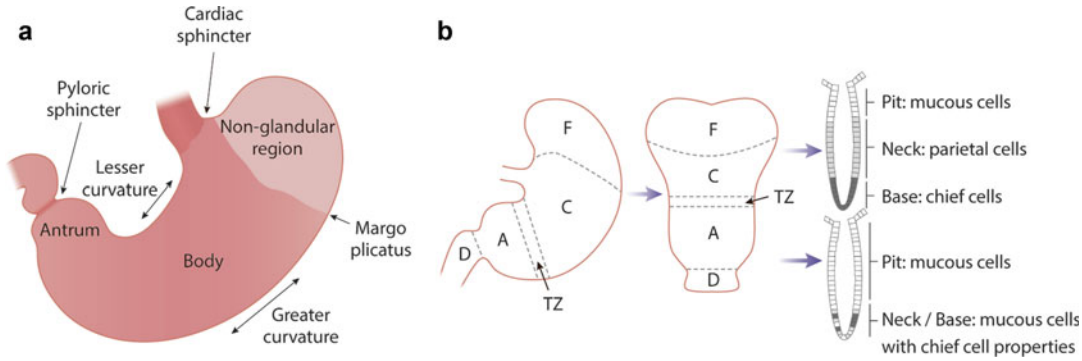

Fig. 55.2 Anatomical structure or mouse stomach. Mouse stomach is largely divided into glandular stomach, nonglandular forestomach, and the margo plicatus between these two parts. The glandular stomach and nonglandular forestomach consist of simple columnar epithelium containing the gastric gland and stratified squamous epithelium, respectively (**a**). The stomach corpus and antrum consist of different glandular cells and are separated by the corpus-antrum transition zone (**b**). *F* forestomach, *C* corpus, *TZ* transition zone, *A* antrum, *D* duodenum (Adapted from Ferrero et al. [55] and Rolig et al. [56])

Conclusions

Research on *H. pylori* animal models is still underway, focusing on the mouse species considered best suited for *H. pylori* infection, especially a variety of transgenic or knockout mice. Despite various limitations as described above, such animal models greatly contribute to understanding the pathophysiology of human *H. pylori* infection. These studies are expected to make their due contributions to developing future vaccine, overcoming antibiotic resistance, and preventing cancer through carcinogenesis research. As such, research on animal models should be continued for the prevention of various *H. pylori*-associated gastric diseases.

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