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ORIGINAL ARTICLE

A report of nonexistence of the non-*Helicobacter pylori Helicobacter* species in Iranian patients suffering from inflammatory bowel disease

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Abstract

Inflammatory bowel disease is a chronic, relapsing–remitting gastrointestinal disorder which has become a serious global concern, and it imposes a great degree of health and economic burdens on communities worldwide. Although the presence of non-*Helicobacter pylori Helicobacter* (NHPH) microorganisms has been reported in various gastrointestinal disorders, their putative role in the pathogenesis of IBD has been a matter of controversy. The present study aimed to investigate the existence of gastric and enterohepatic NHPHs and their probable coinfection with *H. pylori* in IBD. Totally, 168 clinical specimens including 70 colonic biopsies and 98 fecal specimens were obtained from IBD patients. Genomic DNA was extracted from all samples, and its quality and concentration were assessed by β-globin PCR and spectrophotometry. The *Helicobacter* genus-specific PCR was performed using 16S rRNA gene. All samples were also tested for *H. pylori* infection by PCR of *ureC* gene fragment (*glmM*). The presence of NHPH was examined by using species-specific PCR assays. Based on PCR results, *H. pylori* was detected in 12.9% and 3.1% of colonic biopsies and fecal specimens, respectively. However, no statistically significant correlation was observed (P value > 0.05). We failed to find NHPH in both colonic biopsies and fecal specimens from IBD patients. Despite the fact that none of the IBD patients harbored the NHPH in the current work, further cohorts with larger sample size are required to determine the possible relationship between NHPH infection and IBD pathogenesis.

Keywords Inflammatory bowel disease · Non-*H. pylori Helicobacter* · *H. pylori* · NHPH · IBD · Iran

Introduction

Inflammatory bowel disease (IBD) is a chronic, relapsing–remitting gastrointestinal disorder. It is connected to the various extents of intestinal damage and development of local and extraintestinal complications. Its two primary subtypes are as follows: ulcerative colitis (UC) and Crohn's disease (CD) (Lo Presti et al. 2019). In fact, a complex interplay of intestinal immune dysregulation, genetically susceptible host, and environmental exposure can promote IBD (Singh et al. 2017). Despite an unknown etiology, IBD has become a serious global concern and it imposes a great degree of health and economic burdens on communities worldwide, and the patient's overall quality of life is reduced as a result of this inflammatory condition (Alatab et al. 2020). IBD is one of the five most prevalent gastrointestinal disease burdens in the USA, while emerging trend of IBD in other countries and particularly Asian countries cannot be overlooked (M'Koma 2014; Singh et al. 2017). Given the dramatically increased

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prevalence of IBD in the world, it appears commensal enteric bacteria and more to the point, gut microbiota have a pivotal role in triggering intestinal inflammation in IBD (Zuo and Ng 2018; Wu et al. 2015). Since *Helicobacter* organisms have a strong association with colitic disease in monkeys which is similar to human UC, they are considered as potential pathogens in IBD as well (Hansen et al. 2011). To be more specific, members of the mucus-associated *Helicobacter* species are capable of colonizing various ecological niches in the gastrointestinal tract (Bohr et al. 2004).

Helicobacter species are Gram-negative and spiral-shaped bacteria. According to the preferential site of colonization, the *Helicobacter* genus splits into gastric *Helicobacter* and enterohepatic (non-gastric) *Helicobacter*. While the former colonizes the stomach, the latter has a preference of colonizing the intestinal or hepatobiliary system (Thomson et al. 2011). Since the inception of research on the *Helicobacter* genus, it has been reported that a considerable number of non-*H. pylori Helicobacter* (NHPH) can colonize in the human beings, mammals, and birds (Cao et al. 2016). Apart from *H. pylori*, whose role in various human gastric and extragastric diseases has been verified, there are established proofs that NHPHs contribute to various gastrointestinal disorders, particularly mucosa-associated lymphoid tissue (MALT) lymphoma (Peng et al. 2017). Based on *Helicobacter*-species classification, *H. acinonychis*, *H. baculiformis*, *H. bizzozeronnii*, *H. cetorum*, *H. cynogastricus*, *H. felis*, *H. heilmannii*, *H. muridarum*, *H. mustelae*, *H. salomonis*, *H. suis*, *H. suncus*, and also *Wolinella succinogenes* are known to be the members of gastric *Helicobacter* species, while enterohepatic *Helicobacters* are as follows: *H. bilis*, *H. canis*, *H. cholecystus*, *H. cinaedi*, *H. equorum*, *H. fennelliae*, *H. hepaticus*, *H. mesocricetorum*, *H. pametensis*, *H. pullorum*, *H. rodentium*, *H. trogontum*, *H. typhlonicus*, and *Flexispira rappini* (Yadegar et al. 2014a, b).

It is hypothesized that *Helicobacter* spp. is improbable to play a central role in the pathogenesis of IBD, but this view has been refuted by a number of investigators who claim family of *Helicobacteraceae* was detected in 92% of an IBD cohort study (Hansen et al. 2011). Although a considerable amount of attention has been paid to *H. pylori* colonization and its clinical sequelae, the putative role of NHPH in human IBD research is still a matter of controversy. Therefore, the present study aimed to investigate the existence of gastric and enterohepatic NHPH in clinical specimens including colonic biopsies and fecal samples in Iranian patients with IBD. We also investigated the probable coinfection of NHPH with *H. pylori* among these patients.

Materials and methods

Study design and patients

One hundred sixty-eight patients suffering from IBD who referred for the colonoscopy procedure in Research Institute for Gastroenterology and Liver Diseases (RIGLD) at Taleghani hospital in Tehran during September 2011 to May 2012 enrolled in this study. Inclusion criteria were as follows: diarrhea, cramping pains in the abdomen, weariness and fatigue, feeling generally unhealthy, loss of appetite and resultant loss of weight, while those who received antibiotics 4 weeks prior to sample collection were excluded. The definite diagnosis of IBD was made based on a combination of clinical symptoms, colonoscopic and pathologic reports. Clinical and demographic data such as age, gender, IBD subtype, duration of hospital stay, and frequency of defecation were recorded for all patients through a questionnaire on the day of admission. The study was approved by the Institutional Ethical Review Committee of RIGLD at Shahid Beheshti University of Medical Sciences (Project No. IR.SBMU.RIGLD.REC.1396.188). All the patients were asked to sign a written informed consent prior to their participation in the study.

Clinical specimens

Seventy colonic biopsies and 98 fecal specimens were obtained from all IBD patients participated in the study. The colonic biopsies and fecal specimens were collected and immediately transported to the *Helicobacter* laboratory of the Foodborne and Waterborne Diseases Research Center for further processing. The clinical specimens were stored at − 80 °C until used for PCR analysis.

Bacterial reference strains

The bacterial reference strains used in the present study were provided from Gastrointestinal Bacteria Reference Unit, Public Health England, UK, and they were as follows: *H. felis* (NCTC 12,436), *H. hepaticus* (NCTC 12,886), *H. muridarum* (NCTC 12,714), *H. pullorum* (NCTC 12,824), and *H. pylori* J99 (CCUG 47,164). The purified DNA sample of *Campylobacter* sp. (NCTC 12,222) was used as negative control.

DNA extraction and internal control PCR

The colonic biopsy samples were completely dissected and homogenized. Then, genomic DNA was extracted by the QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany) as per the manufacturer's instructions (Yadegar et al. 2019). To prepare DNA from fecal specimens, the samples were centrifuged at 14,000 rpm for 5 min. Then, the DNA was extracted from the supernatant using QIAamp DNA Stool Mini Kit (Qiagene, Hilden, Germany) according to manufacturer's instructions. PCR amplification of the human β-globin gene by using PC03-F, 5′-ACACAACTGTGTTCA CTAGC-3′, and PC04-R, 5′-CAACTTCATCCACGTTCA

CC-3′, specific primers was carried out as an internal control for DNA extraction. The expected product size of the amplicon was 110 bp (Greer et al. 1994). Besides, the concentration of DNA was assessed using Nanodrop (NanoDrop™ 2000/c Spectrophotometers, Thermo Fisher Scientific, USA). The extracted DNA samples were stored at − 20 °C until further molecular analysis.

Helicobacter genus- and species-specific PCR assays

To confirm the presence of *Helicobacter* DNA in both clinical specimens, the *Helicobacter* genus- and species-specific PCR reactions were performed using specific primers for the 16S rRNA and target genes for each *Helicobacter* species, respectively (Yadegar et al. 2014a, b). First, colonic biopsies and fecal samples were analyzed by 16S rRNA gene to identify *Helicobacter* genus. The expected PCR product for 16S rRNA was 764 bp. Then, specific primers were used to distinguish and confirm the strains of non-*H. pylori Helicobacter*. Additionally, molecular analysis for the presence of *H. pylori* in clinical specimens was carried out through detection of *glmM* gene. All primers used in this study are listed in Table 1.

Sanger sequencing

The PCR products were sequenced by the Sanger sequencing method using an automated sequencer (Macrogen, Seoul, Korea). Chromas Lite version 2.5.1 (Technelysium Pty Ltd, Australia) was used to edit the partial DNA sequences. Accordingly, sequence analysis was performed using BioEdit software version 7.2.5. The DNA sequences were aligned by BLAST sequence analysis available in NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Farzi et al. 2019). Finally, the nucleic acid sequence information of colonic biopsies and fecal specimens was deposited to GenBank database under accession numbers MT160750-MT160753.

Statistical analysis

Data were analyzed using SPSS software version 23.0 (SPSS Inc., Chicago, IL, USA). The non-parametric chi-square test was used to examine the statistical significance of differences between categorical variables. The relationship was considered statistically significant when a P value was less than 0.05.

Results

Patients and specimens

Totally, 168 specimens were obtained from patients underwent colonoscopy or clinical surveillance. Of these samples, 70 colonic biopsies (41.7%) were taken from patients underwent colonoscopy at Taleghani hospital. Besides, 98 fecal specimens (58.3%) were acquired from those patients who were not satisfied with colonoscopy procedure. Endoscopic diagnosis and clinical estimations showed that 131 patients had UC, 8 had CD, and 29 had disease other than IBD (control). These non-IBD patients were as follows: liver failure, dysentery, cancer, malnutrition. The IBD patients consisted of 68 (40.5%) men and 71 (42.2%) women. Besides, the control group contained 16 (9.5%) men and 13 (7.7%) women. The age range of participants was 1–96 years for IBD patients and 2–86 years for non-IBD patients. Patients with UC were among the most affected participants, while the maximum hospitalization days were assigned to those patients with CD. Regarding the disease phases, 123 and 16 patients revealed symptoms of flare-up and remission, respectively. The participants received different types of medications including antibiotics, anti-acid, or any other drugs used to alleviate their disease. Noticeably, the UC patients consumed a higher percentage of medications in comparison to CD and non-IBD patients. Tables 2 and 3 show the demographic data and clinical characteristics of IBD and non-IBD patients.

Presence of NHPH and coinfection

Fecal samples and colon biopsies from patients with IBD were examined for the presence of *H. pylori* and NHPH. The extracted DNA samples were checked by β-globin gene, which can evaluate the quality of human DNA present in the samples. Then, PCR of 16S ribosomal RNA was performed for all samples owing to ensure all the DNA samples belong to *Helicobacter* genus. As a matter of fact, we failed to find any evidence of NHPH species in colonic biopsies and fecal specimens.

By taking a more attentive look at the molecular results, it can be inferred that nine (12.9%) colonic biopsies and three (3.1%) fecal specimens were assigned to *Helicobacter* genus, but not recognized as NHPH as shown in Fig. 1. These samples were examined for the presence of *glmM* gene and identified as *H. pylori.*

When it comes to infection with *H. pylori*, the statistical test revealed that there is no significant correlation between *H. pylori* infection and IBD disease in patients who partook in endoscopic procedure (P values = 0.62) and those who were asked for stool samples (P values = 0.056).

Discussion

IBD is categorized as chronic inflammation of the gastrointestinal tract which contains alternating active and dormant phases and finally leads to an upward trend of global burden (Wu et al. 2015). Since several members of the *Helicobacter*

Table 1 Primers used for amplification of the different DNA targets in present study

Helicobacter spp.	Primer designation	Sequences (5′–3′)	Amplicon size (bp)	Ref
Helicobacteraceae 16S rRNA gene	C97-20	GGCTATGACGGGTATCCGGC	764	(Bohr et al. 2007)
	H3A-20	GCCGTGCAGCACCTGTTTTC		
H. pylori	GlmM2-F	GGATAAGCTTTTAGGGGTGTTAGGGG	296	(Kauser et al. 2005)
	GlmM1-R	GCTTACTTTCTAACACTAACGCGC		
H. bilis	C62	AGAACTGCATTTGAAACTACTTT	640	(Shomer et al. 1997)
	C12	GGTATTGCATCTCTTTGTATGT		
H. bilis	F2-cdtB-bilis	CGAATCTATTATCCGGGCTTG	151	(Rocha et al. 2005)
	R2-cdtB-bilis	GCCAAGCGAGTTCTATCATTAG		
H. bizzozeronii	Bi1F	AACCAA**Y**AGCCCCAGCAGCC	373	(Van den Bulck et al. 2005)
	Bi2R	TGGTTTTAAGGTTCCAGCGC		
H. felis	HfF	GTGAAGCGACTAAAGATAAACAAT	241	(Germani et al. 1997)
	HfR	GCACCAAATCTAATTCATAAGAGC		
H. felis	Fe1F	TTTGGTGCTCACTAACGCCCTC	436	(Van den Bulck et al. 2005)
	Fe3R	TTCAATCTGATCGCGTAAAG		
Candidatus *H. heilmannii*	HeilF	AAGTCGAACGATGAAGCCTA	112	(Bell et al. 2003)
	HeilR	GGTAATATTTGGTATTAATCAC		
H. hepaticus	B38	GCATTTGAAACTGTTACTCTG	417	(Foltz et al. 1998)
	B39	CTGTTTTCAAGCTCCCC		
H. marmotae	G70	GCGGGTAATTAAGTCAGATG	465	(Fox et al. 2002)
	G69	TGTTTTCAAGCTCCCCAAAG		
H. muridarum	HmF	GAAACTATTAGTCTA	409	(Goto et al. 2000)
	HmR	TTTCAAGCTCCACAGAAGTG		
H. pullorum	F1-cdtB-pullorum	GTCTTTTGAGTGGATTGGATTCT	148	(Rocha et al. 2005)
	R2-cdtB-pullorum	CACTCCGGGTGCTTGTGTAT		
H. pullorum	HpuF	ATGAATGCTAGTTGTTGTGAG	447	(Rocha et al. 2005)
	HpuR	GATTGGCTCCACTTCACA		
H. rappini	F1-ureB-rappini	GATGATTAGGGCGACACAGC	101	(Rocha et al. 2005)
	R2-ureB-rappini	CCCCAGATTCTATCTGCTTACTC		
H. rodentium	HrF	TTGCGAGGCTTGTCCTTG	324	(Goto et al. 2000)
	HrR	TTAGAGTGCTCTACCGAATA		
Candidatus *H. suis*	V832f	TTGGGAGGCTTTGTCTTTCCA	433	(De Groote et al. 2005)
	V1261r	GATTAGCTCTGCCTCGCGGCT		
H. felis, *H. bizzozeronii*, *H. salmonis*	GenusF	AACGATGAAGCTTCTAGCTTGCTAG	399	(Van den Bulck et al. 2005)
	GenusR	GTGCTTATTC**S**T**N**AGATACCGTCAT		
H. felis, *H. bizzozeronii*, *H. salmonis*	CAR577f	TGCGTAGGCGGGGTTGTAAG	78	(Van den Bulck et al. 2005)
	CAR636r	CAGAGTTGTAGTTTCAAATGC		

The nucleotides in bold type represent: Y, C or T; S, G or C; N, A or G or C or T

genus are considered as potential and animal pathogens, identification of their species has become a focal point of research in recent years (Yadegar et al. 2014a, b). Regarding epidemiological studies, the prevalence of NHPH in the community has been varied from 0.1 to 6.2%. Generally speaking, *H. bizzozeronii*, *H. felis*, *H. heilmannii* sensu stricto, *H. salomonis*, and *H. suis* have been discovered from human stomach (Øverby et al. 2017). More compelling evidence has been derived from human studies where *H. suis* is considered as the most prevalent gastric non-*pylori* *Helicobacter* species, whose prevalence varies between 13.9 and 30.9% in human infections. This is in agreement with Van den Bulck et al. (Powers-Fletcher and Couturier 2015) study that reported *H. suis* was found in 36.6% of isolates, while *H. salomonis*, *H. felis*, and *H. bizzozeronii* were less reported. On the contrary, several studies support the notion that *H. cinaedi* is the most commonly reported enterohepatic *Helicobacter* species in human populations and it has been usually recovered from patients suffering from diarrhea, bacteremia and other inflammatory conditions (García

Table 2 Demographic data and clinical characteristics of IBD patients who underwent NHPH analysis in their fecal specimen

Variables	IBD subtype/Control (No., %)		
	UC ($n=81$)	CD ($n=3$)	Control ($n=14$)
Female	38 (46.9%)	2 (66.7%)	7 (50%)
Male	43 (53.1%)	1 (33.3%)	7 (50%)
Age range (years)	1–96	24–45	2–86
Mean age (years)	36.89 ± 2	35.67 ± 6.1	44.93 ± 6.7
Hospital stay (day)	8–10	10–18	3–5
Frequency of defecation (per day)	3–5 (41, 50.6%)	3–5 (2, 66.7%)	0–1 (8, 57.1%)
	5–8 (23, 28.4%)	5–8 (1, 33.3%)	1–2 (6, 42.9%)
	8–10 (2, 2.5%)	-	-
	> 10 (15, 18.5%)	-	-
Medication	Consumed (54, 66.7%)	Consumed (3, 100%)	Consumed (9, 64.3%)
	Unconsumed (27, 33.3%)	-	Unconsumed (5, 35.7%)
Disease activity	Flare-up	Flare-up	-

NHPH non-*Helicobacter pylori Helicobacter*, *UC* ulcerative colitis, *CD* Crohn's disease

et al. 2006). It is often argued that *H. cinaedi* and *H. fennelliae* are isolated most frequently from human colonic samples, despite the fact they are considered as risk factors in immunocompromised patients as well. Supposedly, regular surveillance of these NHPHs could prevent nosocomial infections in immunodeficient patients (O'rourke et al. 2001; Rimbara et al. 2013).

Notwithstanding, it has been attractively hypothesized that *Helicobacter* infection possibly trigger IBD, the scientific evidence remains opposing (Veijola et al. 2007). Needless to say, (Hansen et al. 2011) were the first investigators who connected *Helicobacter* infection with IBD (Hansen et al. 2011). Accordingly, the *Helicobacter* organism was isolated from the rectal swab of homosexual men with proctitis. Thereafter, there appears to be an acceleration in the growth of *Helicobacter* genus during the past decade. The experimental data are rather controversial, and there is no general agreement about the prevalence of NHPH and their role in IBD. Previous findings have discovered the presence of NHPH in 83% and 87% of CD and UC patients, respectively. In the meantime, NHPH was also found in 40% of control group (Hansen et al. 2011; Thomson et al. 2011; Fox 2002) which is in harmony with Bohr et al., Zhang et al. and Laharie et al. studies in both IBD and controls (Bohr et al. 2004; Zhang et al. 2006; Laharie et al. 2009). Nevertheless, our findings support Bell et al. and Grehan et al. studies (Bell et al. 2003; Grehan et al. 2004) which concluded that no NHPH was found among clinical samples. Besides, it came to the light that *Helicobacter* spp. have not constantly been isolated from IBD patients (O'rourke et al. 2001). However, further investigations are required to explore the precise role of NHPH species, as the potential infectious triggers, in development and pathogenesis of IBD. Moreover, such understanding may subsequently improve the accuracy of

Table 3 Demographic data and clinical characteristics of IBD patients who underwent NHPH analysis in their colonic biopsy specimens

Variables	IBD subtype/control (no., %)		
	UC ($n=50$)	CD ($n=5$)	Control ($n=15$)
Female	28 (56%)	3 (60%)	6 (40%)
Male	22 (44%)	2 (40%)	9 (60%)
Age range	18–65	17–63	23–49
Mean age (years)	35.18 ± 12.47	34.6 ± 19.04	29.8 ± 8.1
Hospital stay (day)	5–9	10–18	2–5
Frequency of defecation (per day)	2–5 (39, 78%)	2–5 (4, 80%)	0–1 (9, 60%)
	5–8 (7, 14%)	-	1–2 (6, 40%)
	> 10 (4, 8%)	> 10 (1, 20%)	-
Medication	Consumed (45, 90%)	Consumed (5, 100%)	Consumed (15, 100%)
	Unconsumed (5, 10%)	-	-
Disease activity	Flare-up (36, 72%)	Flare-up (3, 60%)	-
	Remission (14, 28%)	Remission (2, 40%)	

NHPH non-*Helicobacter pylori Helicobacter*, *UC* ulcerative colitis, *CD* Crohn's disease

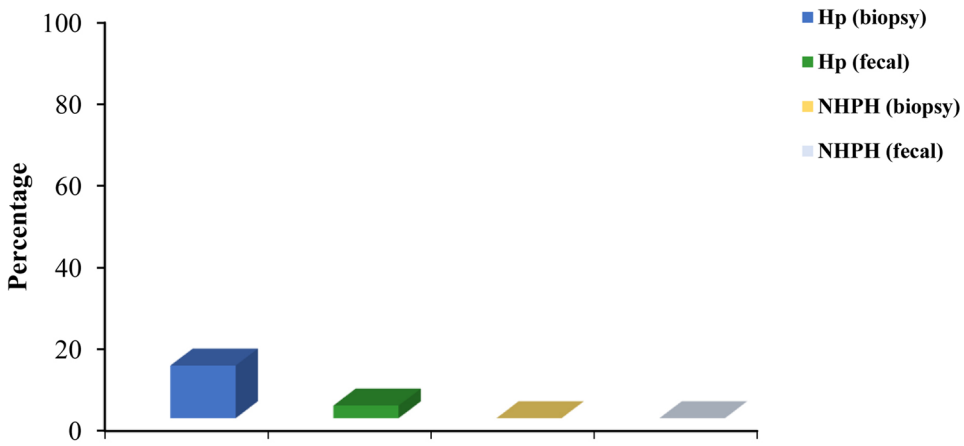

Fig. 1 The percentage of *H. pylori* and non-*Helicobacter pylori Helicobacter* (NHPH) in colonic and fecal specimens among patients with IBD

IBD research, and unravel its impact on current and new IBD therapeutic targets including perhaps immunization against potential pathogenic triggers, and targeted antibiotics- or probiotics-based therapies that may be used for treatment and clinical practice of IBD patients (Hansen et al. 2011). In addition, long-term exposures to immunomodulators and immunosuppressant drugs may predispose the IBD patients to retain more of the *Helicobacter* species as part of their gastric or colonic microbiota (Laharie et al. 2009). However, the role of past or concurrent exposure to IBD medications on the occurrence of infection with NHPH species needs to be investigated using both animal studies and human trials.

Broadly speaking, there is an increasing risk for IBD patients to develop high-grade dysplasia and colorectal cancer. Regarding animal IBD models, it came to the attention that *H. bilis* triggers severe inflammation and hyperplasia in a short period of time in mice, while *H. hepaticus* is able to stave off the development of IBD (Maggio-Price et al. 2005). Interestingly, several NHPHs including *H. bilis*, *F. rappini*, *H. pullorum* are isolated from the bile and gallbladder of patients; revealing their involvement in various biliary diseases, mainly cancer (Vafaeimanesh et al. 2012). It has been believed that analyzing the fecal specimen is considered a relatively better and noninvasive method to detect *Helicobacter* spp. in comparison with the biopsy which requires endoscopy procedure (Man et al. 2008). On the contrary, we found higher rate of *H. pylori* in colonic biopsies than fecal specimens. However, the molecular tests in the current work have failed to show any correlation between *Helicobacter* spp. and IBDs. Our results could be possibly explained by the reports of Amorim et al. (Amorim et al. 2015) who stated the frequent isolation of gastric *Helicobacter* spp. in pet animals. The prevalence of these species varies between 67–86% in clinically healthy dogs and 61–100% in animals presenting chronic vomiting, while all the laboratory Beagle dogs and dogs from local shelters carry *Helicobacter* spp. Regarding NHPH in pet animal, *H. bizzozeronii*, *H. felis*, and *H. heilmannii* sensu stricto were the predominant species found so far . Accordingly, no NHPH was found in present study since the majority of Iranian people do not keep pet animals in their home. *H. pylori* has also been identified in the intestinal mucosa of IBD patients, but studies have failed to associate *H. pylori* with pathogenesis of IBD (Wu et al. 2015). Although there was no NHPH in present study to report, but we identified 12.9% and 3.1% *H. pylori* in colonic and fecal specimens, respectively.

A substantial body of reports revealed the protective effect of *H. pylori* infection on IBD (Papamichael et al. 2014; Yu et al. 2018, 2015; Sayar et al. 2019). It is well established that *H. pylori* infection is associated with larger family size, poor hygiene, inadequate sanitation conditions, and lower socioeconomic status mostly in developing countries (Hooi et al. 2017). On the other hand, the prevalence of *H. pylori* infection has fallen in countries undergoing "westernization" where the incidence of developing autoimmune diseases including IBD has instead increased (Castaño-Rodríguez et al. 2017; Burisch and Jess 2019). There are two theories behind this inverse relationship. First, the various antibiotics and medications frequently used to treat IBD may possibly lead to *H. pylori* eradication. Secondly, it can be the alterations of IBD-associated mucosa which may cause inhibition of the *H. pylori* colonization in the harsh gastric environment. Others argue that the protective effect of *H. pylori* can be attributed to the *H. pylori*-induced systematic immune tolerance and the suppression of inflammatory response (Yu et al. 2018). Moreover, there is also accumulating evidence of the possibility of harnessing the immunomodulatory properties of *H. pylori* for the immune system, such as by increasing the *H. pylori*-induced dendritic cells (DCs) with the tolerogenic phenotype and immunosuppressive regulatory T cells (Tregs) (Arnold et al. 2012). However, it remains uncertain as to whether it is the *H. pylori*-associated treatments per se, or the eradication of the pathogen resulting from the treatment regimens, that has a deleterious impact on the development of IBD.

There are some earlier reports that suggest the presence of non-*H. pylori* gastritis as a common and independent condition from *H. pylori*-associated gastritis (Peura et al. 2010; Genta and Sonnenberg 2015; Kunihiko and Murase 2016). In a recent cross-sectional study performed by Shiota et al. *H. pylori*-negative gastritis was present in approximately 18% of patients with gastritis (Shiota et al. 2017). More importantly, Genta et al. (Genta and Sonnenberg 2012) showed that both non-*H. pylori* gastritis and duodenitis would be more common among paediatric IBD patients than among non-IBD controls, as well as among adult IBD patients. These preliminary data support the concept that occurrence of inflammatory infiltrates into the upper gastrointestinal mucosa in IBD is possibly a true condition most likely unrelated to current or past *H. pylori* infection. Accordingly, a large number of non-*H. pylori* acid-resistant mucosa-associated microbiota has been identified in the human stomach, many of which are derived from transient flora in the mouth, including *Neisseria*, *Streptococcus*, and *Lactobacillus* strains (Li et al. 2009). It is suggested that these mucosa-associated microbiota and their metabolites can directly affect the host physiology and immune status, and they have the potential to be associated with clinical outcomes. However, the risk factors, clinical course and pathogenesis of non-*H. pylori* gastritis remains unclear in patients with gastritis or IBD. Taken together, identifying these factors is important for providing a new avenue to study the pathophysiology of IBD patients suffering from non-*H. pylori* gastritis.

Conclusions

In conclusion, the present study has some limitations. The main limitation of this study was the small number of sample size. Since the etiology of IBD is still indeterminate, larger population-based cohort studies are required. Additionally, it would be better to obtain the colonic biopsies and fecal specimens from each IBD patient, simultaneously. By taking all the evidence in account, *H. pylori*-induced gastrointestinal disorders in humans has drawn all the attentions as might be expected, while other member of *Helicobacter* genus are important as *H. pylori* in human disease. Thus, it is important to identify and detect various species of *Helicobacter*, and aware the clinicians about the importance of these strains in developing gastrointestinal disease and probable IBD in each region.

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Author contribution SP and MA performed the microbiological and molecular tests. AY and NM reviewed the literature and prepared the manuscript draft. AY designed the study, analyzed the data, and revised the manuscript. JYK, HAA, and MRZ provided clinical advice and critically revised the manuscript. All authors approved the final version of the manuscript and the authorship list.

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Declarations

Ethical approval The study was approved by the Institutional Ethical Review Committee of RIGLD at Shahid Beheshti University of Medical Sciences (Project No. IR.SBMU.RIGLD.REC.1396.188).

Informed consent All the patients were asked to sign a written informed consent prior to their participation in the study.

Conflict of interest The authors declare no competing interests.

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