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Does *Helicobacter pylori*-induced inflammation of gastric mucosa determine the severity of symptoms in functional dyspepsia?

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Background. Inflammation induces some structural and biochemical alterations and oxidative damage in gastric tissue. In this study, we aimed to investigate the relationship among the severity of symptoms, oxidative stress, and grading scales of *Helicobacter pylori*-induced gastric inflammation in functional dyspepsia. ***Methods.*** Thirty-five patients with functional dyspepsia were enrolled in the study. The severity of dyspepsia within the last 6 months was evaluated by Glasgow Dyspepsia Severity Score. In biopsy specimens of gastric mucosa, severity of gastritis was estimated by the revised Sydney Classification System, and oxidative stress parameters were studied. ***Results.*** Although there was no statistically significant relationship between symptom scores and degree of chronic inflammation, a tendency for symptoms to be more severe has been observed in low levels of gastritis. Levels of sulfhydryl groups were lower in subjects with high levels of chronic inflammation, and *Helicobacter pylori* intensity ($P < 0.001$ and $P = 0.02$, respectively). Levels of malondialdehyde were higher in subjects with high levels of chronic inflammation ($P = 0.04$). There was a statistically significant but a weak positive correlation between symptom scores and sulfhydryl levels ($P < 0.001$, $r = 0.323$). ***Conclusions.*** In conclusion, there may be an inverse relation between severity of symptoms and level of *Helicobacter pylori*-induced gastric inflammation or oxidative stress in patients with functional dyspepsia.

Key words: oxidative stress, chronic gastritis, symptom scores

Introduction

Although the pathogenesis of functional dyspepsia remains uncertain, different pathophysiological mechanisms such as gastroduodenal motor dysfunction,[1–4] abnormal visceral sensory perception,[5] vagal dysfunction,[6] *Helicobacter pylori* infection,[7] and physicosocial factors[8] have been suggested to play a role in development of functional dyspepsia. However, no structural or biochemical cause has been established to explain the symptoms.

H. pylori infection is the major cause of chronic gastritis. Therefore, chronic gastric inflammation may have a role in the development of symptoms experienced by functional dyspepsia patients with *H. pylori* infection. Different investigators have shown several structural and biochemical changes that seem to be related to the inflammation of gastric tissue. The severity of inflammation may cause changes in the numbers of endocrine cells regulating acid secretion.[9] There may be a relationship between serum pepsinogen levels and severity or activity of inflammation.[10] The severity of inflammation affects proximal gastric motility, and the activity of inflammation determines the severity of duodenitis.[11,12] In experimental models, gastric inflammation has been shown to change both motility and visceral sensory perception.[13]

Another change caused by *H. pylori*-associated inflammation is the release of free oxygen radicals by inflammatory cells. Free oxygen radicals may have a role in pathogenesis of gastritis and peptic ulcer by damaging the integrity of biological tissues.[14–16] However, it is unclear that mediators or oxidative stress secondary to inflammation explain symptoms in patients with functional dyspepsia.

In this study, we investigated the possible relationships among the severity of symptoms, oxidative stress, and grading scales of gastritis caused by *H. pylori* infection in patients with functional dyspepsia.

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Methods

Thirty-five consecutive patients, more than 18 years of age, who were diagnosed with functional dyspepsia by the Rome II Diagnostic Criteria[17] were enrolled into the study. Organic disease was ruled out by means of endoscopy of the upper gastrointestinal tract, abdominal ultrasonography, and routine biochemistry. Patients with peptic ulcer, erosive gastroduodenitis, gastroesophageal reflux disease, gastric cancer, pancreatobiliary disease, irritable bowel syndrome, diabetes mellitus, organ failure, and long-standing nonsteroidal anti-inflammatory drug usage were excluded. The study protocol was approved by the ethics committee of Afyon Kocatepe University, and informed consent of all patients was obtained.

The severity of dyspepsia symptoms of patients within the last 6 months has been evaluated by Glasgow Dyspepsia Severity Scoring, shown to be a valid and reliable measure. This measure is a verbal questionnaire including 8 parameters, and score ranges between 0 and 20;[18] high scores indicate more severe dyspepsia.

Two biopsy specimens were obtained from the antrum and corpus. For histopathological examination, the antral specimens were obtained from 2 cm proximal to the pylorus and the corpus specimens from the middle along the greater curvature. Biopsy specimens were examined according to revised Sydney Classification and chronic inflammation (mononuclear cell infiltration), activity (polymorphonuclear neutrophil infiltr ation), glandular atrophy, intestinal metaplasia, and *H. pylori* density were graded on a scale from 0 to 3 (0, absent; 1, mild; 2, moderate; 3, marked).[19]

In addition, two biopsy specimens were obtained from the antrum for analysis of oxidative stress markers. A portion of each specimen was homogenized for all assays. Homogenization was performed in 1:10 (w/v) 0.1 M potassium phosphate buffer (pH = 7.4) with an Ultra Turrax homogenizer (IKA T18 basic; Wilmington, NC, USA). After centrifuging, the homogenates were centrifuged at 9500 × g, +4°C for 10 min; the supernatants were removed and subjected to analysis. In these specimens, reduced glutathione (GSH), malondialdehyde (MDA), and protein sulfhydryl (SH) levels were determined by using a modified method of Beutler,[20] Ohkawa et al.,[21] and Levine et al.,[22] respectively.

Continuous variables are expressed as mean ± SD; categorical variables are presented as frequency and percentage. Comparisons of continuous variables among subjects were made using the Kruskal–Wallis *H* test or Mann–Whitney *U* test. Comparisons of categorical variables were made by the χ^2 test. Correlation between the mean value of SH and symptom scores was calculated using the Spearman correlation test. The SPSS 10.0 for Windows statistical program was applied for all statistical analyses with 95% confidence interval; $P < 0.05$ was considered statistically significant.

Results

Thirty-five patients with functional dyspepsia were studied, 24 women and 11 men (mean age, 43 ± 2 years; range, 21–70 years). Based on the results of the biopsy specimens obtained from the antrum and corpus, all 35 patients had histologically chronic gastritis. In only 3 patients (8.6%) was *H. pylori* negative in both biopsy specimens. There were no cases found to have atrophy. Results of the biopsy specimens obtained from the antrum and corpus are shown in Tables 1 and 2, respectively. The symptom scores of patients and the levels of oxidative stress parameters in antral specimens are shown in Table 3.

The symptom scores were higher in patients with mild or moderate chronic inflammation in the corpus

Table 1. Frequency of patients in relationship to severity of histological features in biopsy specimens obtained from the antrum

	Histological scores			
	0	1	2	3
Chronic inflammation (*n*)	0	0	8	27
Neutrophil activity (*n*)	11	6	11	6
Intestinal metaplasia (*n*)	33	2	0	0
Helicobacter pylori density (*n*)	4	7	19	5

Table 2. Frequency of patients in relationship to severity of histological features in biopsy specimens obtained from the corpus

	Histological scores			
	0	1	2	3
Chronic inflammation (*n*)	0	5	2	28
Neutrophil activity (*n*)	11	10	5	9
Intestinal metaplasia (*n*)	31	3	0	1
H. pylori density (*n*)	7	6	19	3

Table 3. Dyspepsia scores and tissue sulfhydryl, glutathione, and malondialdehyde levels in patients

	Mean ± SD (range)
Glasgow Dyspepsia Severity Scores	10.9 ± 3.3 (5–18)
Sulfhydryl (nmol/mg protein)	31.1 ± 11.2 (14.7–67.4)
Glutathione (nmol/mg protein)	19.0 ± 4.6 (8.8–30.2)
Malondialdehyde (nmol/mg protein)	0.4 ± 0.1 (0.2–1.0)

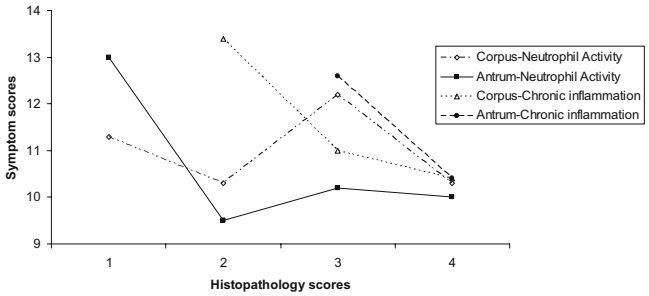

Fig. 1. Symptom scores in relationship to chronic inflammation and neutrophil activity in the antrum and corpus. *$P < 0.05$

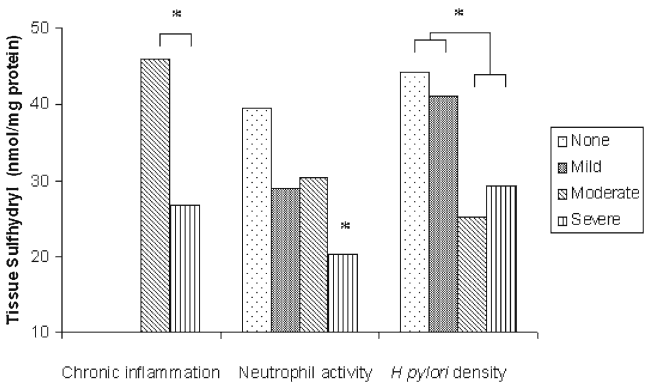

Fig. 2. Tissue sulfhydryl levels in relationship to the severity of chronic inflammation, neutrophil activity, and *Helicobacter pylori* density. *$P < 0.05$

and the antrum than in patients with severe chronic inflammation, although this did not reach significance. Furthermore, the symptom scores were higher in patients without neutrophil activity in the antrum than in patients with any degrees of neutrophil activity, although this difference was also not significant (Fig. 1). No statistically significant relationship was found between the density of *H. pylori* and the symptom scores.

There were no significant differences in MDA and GSH levels with respect to *H. pylori* density, but mean SH levels were significantly higher in patients with mild or no *H. pylori* density than in patients with moderate or severe *H. pylori* density ($P = 0.02$). As the severity of inflammation increased, a significant decrease in SH levels and a significant increase in MDA levels were observed ($P < 0.001$ and $P = 0.04$, respectively). Furthermore, in patients with severe degrees of neutrophil activity, SH levels in antral tissue were found to be significantly lower ($P = 0.004$) (Fig. 2). There was a weak but significant positive correlation between the symptom scores and SH levels ($P < 0.001$, $r = 0.323$).

Discussion

In our study, we found no relationship between symptom scores and the severity of histological gastritis. The relationship between gastric inflammation and dyspeptic symptoms has been investigated in studies of *H. pylori* gastritis. In these studies, the role of *H. pylori* in the development of symptoms was studied by determining the symptomatic response after *H. pylori* eradication therapy.[23–26] Eradication therapy was shown to elicit a significant but low level of symptomatic relief in patients with functional dyspepsia.[27] However, long-term follow-up studies confirmed that chronic gastric inflammation persisted for several years, even if *H. pylori* was successfully eradicated.[28–30] One of the explanations for symptom failures may be ongoing inflammation. Therefore, we investigated the association between the severity of gastric inflammation and clinical symptoms of patients with functional dyspepsia.

In a few studies, investigating the relationship between the severity of histological gastritis and the severity of symptoms showed different results. In studies by Joshi et al.[31] and Pereira-Lima et al.,[32] no relationship was found between the severity of histological gastritis and the severity of symptoms. Czinn et al.[33] found a relationship between epigastric pain and the severity of inflammation. Similarly, van der Schaar et al.[12] also found an indirect relationship between the severity of symptoms and the severity of inflammation in the corpus. In our study, although we found no relationship between histological gastritis and the severity of symptoms, the symptom scores tended to be higher both in patients with mild or moderate chronic inflammation and in those without neutrophil activity. These findings suggested that the degree of *H. pylori*-associated inflammation may modify the sensory gain of afferent neurons by changing the functional and structural features of the visceral afferent receptors. These receptors can be normally sensitized under conditions of inflammation, which may cause visceral hypersensitivity.[13,34] However, it is not known whether visceral hypersensitivity is associated with the degree of inflammation. Marked inflammation may contribute to desensitization of visceral afferent receptors, and this may explain the present inverse relationship between the severity of inflammation and dyspeptic symptoms. Previously, it has been reported that gastric inflammation associated with alkaline reflux was partly associated with desensitization of capsaicin-sensitive afferent neurons by bile.[35] Additionally, capsaicin, a component of red pepper, has been found to be efficacious in patients with functional dyspepsia, probably through a desensitization of afferent neurons.[36] In one study, evaluation of jejunal specimens of patients with irritable bowel syndrome showed preganglionic inflammation and neuronal degeneration in

the myenteric plexus.[37] Furthermore, visceral hypersensitivity was found to be more frequent in patients with functional dyspepsia than in patients with organic dyspepsia, although the frequency of psychological symptoms was similar.[5] By contrast, Kadouchi et al.[38] have recently reported that gastric atrophy was correlated with clinical symptoms such as indigestion in patients with functional dyspepsia. In their study, Kadouchi et al. speculated that the possible mechanisms of this relationship include premature movement of undigested food to the intestine, as well as diminished mucosal defenses caused by decreased gastric acid secretion. In fact, we found no glandular atrophy in histological examination of our cases, which can explain the discrepancy, especially in view of the relationship between histological features and clinical symptoms.

In studies investigating the role of oxidative stress in the development of ulcer and gastritis, it was shown that oxidative stress markers have increased and antioxidant parameters have decreased in gastric tissue.[14,16] However, the association of these parameters with the severity of chronic inflammation, activity of inflammation, density of *H. pylori*, or dyspeptic symptoms has not been investigated. In our study, MDA level, one of the oxidative stress markers, has shown an increase parallel to the severity of chronic inflammation. SH levels decreased with an increase in the density of *H. pylori* and severity of inflammation; this may be related to irreversible oxidation of SH groups at higher amounts with marked inflammation. A positive correlation was found between symptom scores and SH levels, possibly suggesting that there was an inverse relationship between oxidant injury and clinical symptoms. These findings were parallel with a relationship between severity of histological gastritis and symptom scores, although this did not reach significance. In contrast to SH levels, we did not find any relationship between GSH and symptom scores; this suggested that this compound may have a different metabolism and clearance in tissue level.

Our limitations include the small numbers of the study population; consequently, the study population included no patient with glandular atrophy, and a very small number of functional dyspepsia patients without *H. pylori* infection were included. Because atrophic gastritis is a multifocal disease, one of the reasons for absence of glandular atrophy is the possibility of sampling error. Furthermore, the absence of significance between clinical symptoms and histological gastritis weakens our suggestions.

In conclusion, symptoms may have an inverse relationship with severity of *H. pylori*-associated inflammation and oxidative damage in patients with functional dyspepsia. Further studies are needed to disclose the relationship between gastric inflammation and dyspeptic symptoms and the mechanisms involved in the genesis of symptoms in patients with *H. pylori*-associated gastritis.

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