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Eosinophil infiltration, gastric juice and serum eosinophil cationic protein levels in *Helicobacter pylori*-associated chronic gastritis and gastric ulcer

Selim Aydemir[1,CA], Isak Ozel Tekin[2], Gamze Numanoglu[3], Ali Borazan[4] and Yucel Ustundag[1]

[1]Department of Gastroenterology, [2]Department of Immunology, [3]Department of Pathology, and [4]Department of Internal Medicine, Zonguldak Karaelmas University, Faculty of Medicine, Iç Hastaliklari ABD, Gastroenteroloji BD, 67800 Zonguldak, Turkey

[CA]Corresponding Author
Tel: +90 372 2576169
Fax: +90 372 2610155
E-mail: selimaydemir@hotmail.com

***Introduction*: *Helicobacter pylori* is one of the main causes of gastroduodenal diseases, such as chronic gastritis and peptic ulcer. It has been shown that eosinophils increase in the stomach in *H. pylori* infection. Eosinophilic cationic protein (ECP) is a cytotoxic molecule secreted by the activated eosinophils. However, there are no sufficient data about the role of ECP in *H. pylori* infection and its effect on ulcer development. In this study we investigated the gastric eosinophilic infiltration, gastric juice and serum ECP levels in patients with chronic gastritis and gastric ulcer associated with *H. pylori*.**

***Materials and methods*: Forty-four *H. pylori*-positive and 20 *H. pylori*-negative patients who underwent upper gastrointestinal system endoscopy after admitting with dyspeptic complaints were enrolled in the study. Twenty-one of the *H. Pylori*-positive patients had gastric ulcer while 23 patients had none. During endoscopy, multiple gastric biopsies and juices were taken. In gastric biopsies, *H. pylori* and eosinophilic infiltration were assessed. Additionally, gastric juice and serum ECP levels were measured.**

***Results*: Eosinophil infiltration, gastric juice ECP levels, and gastric juice/serum ECP ratios in the *H. pylori*-positive group were greater than in the *H. pylori*-negative group ($p < 0.01$). There was no statistically significant difference regarding serum ECP levels between the two groups ($p > 0.05$). When *H. pylori*-positive patients were compared with regard to gastric ulcer presence, however, there was no significant difference in gastric eosinophil infiltration, gastric juice ECP levels, serum ECP levels, and gastric juice/serum ECP ratios ($p > 0.05$).**

***Conclusion*: The results of this study suggest that eosinophils and eosinophil-released ECP may contribute to inflammatory changes seen in chronic gastritis, whereas there is no proof that they play a role in ulcer development.**

Key words: *Helicobacter pylori*, Gastric ulcer, Eosinophil, Eosinophil cationic protein, Gastric juice, Serum

Introduction

Helicobacter pylori is one of the main causes of gastroduodenal diseases, such as chronic gastritis and peptic ulcer. In addition, it is closely associated with gastric carcinoma and gastric B-cell mucosa-associated lymphoid tissue lyphoma.[1–4] *H. pylori* causes chronic gastritis in all patients whereas only a small proportion of patients infected with this microorganism develop a peptic ulcer.[5] Inflammatory response is pivotal in the epithelial dysfunction and mucosal injury caused by *H. pylori*. *H. pylori* leads to mucosal increases in many proinflammatory and immunoregulatory cytokines.[6–8]

Eosinophils are involved in a broad range of diseases such as allergic, inflammatory, and malignant disorders.[9–11] Several recent studies focused on the function of eosinophils in gastrointestinal disease.[10,12,13] They are present only in small amounts in healthy gut mucosa.[14,15] Their presence has been considered to be a protective mechanism against bacteria and parasites.[11] The specific granules of the eosinophils contain a number of highly cationic proteins.[16,17] One of the very important of these is the eosinophil cationic protein (ECP). They are markedly cationic proteins with cytotoxic capacities probably leading to tissue destruction as well as modulators of the immune response.[18,19] There is increasing evidence that eosinophils are functionally involved in the pathophysiology of various inflammatory disorders of the gut.[12,20]

It has been showed that eosinophils increase in the stomach in *H. pylori* infection.[21–23] However, there are not sufficient studies about the role of ECP

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in *H. pylori* infection and its effect on ulcer development. We investigated in this study gastric eosinophil infiltration, gastric juice and serum ECP levels in patients with chronic gastritis and gastric ulcer associated with *H. pylori*.

Research design and methods

Patients

In total, 44 *H. pylori*-positive patients above the age of 20 years who underwent endoscopy in the gastroenterology laboratory because of dyspeptic complaints (group 1) were enrolled into the study. The patients were divided into two subgroups according to the presence of a gastric ulcer (group 1a, ulcer present; group 1b, ulcer absent). As the control group we selected 20 *H. pylori*-negative patients admitted with dyspeptic complaints who were gastric ulcer negative (group 2). Exlusion criteria were prior eradication therapy for *H. pylori*, anti-ulcer drug use within the past 1 month, gastrointestinal system and other organ malignancies, inflamatuar and infectious diseases, and prior gastric surgery.

Endoscopic procedure

Gastroduodenal endoscopic examination was performed following overnight fasting using a Pentax EG2930K (Asahi Optical, Tokyo, Japan). In each patient, 5 cm^3 of gastric juice, obtained during endoscopy through a sterilised tube fitted in the operation channel of the gastroscope, were collected. For the histopathological examination, multiple biopsies from the antrum and the corpus were taken. The biopsy samples were sent to the pathology laboratory for examination in 10% buffered formalin.

Assessment of the state of *H. pylori* infection

The state of *H. pylori* infection was assessed histologically. The biopsy specimens were fixed in 10% formalin and embedded in paraffin. The precense of *H. pylori* was determined by Giemsa staining as the presence of typical gently spiralled or curved bacteria. When at least one of the biopsies of each patient yielded a positive result, the patient was considered positive for *H. pylori*.

Histological assesment eosinophil infiltration

Sections were also stained with hematoxylin and eosin. For each section, five superficial fields ($\times 400$ magnification) were randomly selected; eosinophils were individually counted by the same investigator and a mean score between five fields was obtained from each patient.[21] The hematoxylin and eosin-stained specimen was given one overall score from 0 to 3 for eosinphil infiltration (0 = no eosinophil infiltration, 1 = mild or patchhy eosinphil infiltration, 2 = moderate eosinphil infiltration, 3 = marked, confluent eosinphil infiltration).

Serum and gastric juice ECP measurement

Sera were obtained in a fasting state before endoscopy. Serum and gastric juice ECP levels were measured with a fluoroenzymeimmunoassay kit and a UNICAP device (Pharmacia&Upjohn, Uppsala, Sweden).

Statistical analysis

Results are expressed as the mean $\pm$ standard deviation. In the comparison between groups, statistically significant differences were assessed by the Student *t*-test and the Mann–Whitney U-test. $P < 0.05$ was considered statistically significant.

Results

When the patients were divided into two groups according to *H. pylori* presence, group 1 had a total of 44 patients (25 males and 19 females). The mean age of the patients was 43.2 ± 11.3 years. Group 2 included a total of 20 patients (11 males and nine females). In this group the mean age was 42.5 ± 10.1 years. The age and sex distribution had an insignificant difference in both groups ($p > 0.05$). Demographic characteristics, mean eosinophil scores, gastric juice ECP levels, serum ECP levels, and gastric juice/serum ECP ratios for both groups are presented in Table 1. Eosinophil scores, gastric juice ECP levels, and gastric juice/serum ECP ratios were apparently greater in group 1 than group 2 ($p < 0.01$). Serum ECP levels were higher in the *H. pylori*-positive group than the *H. pylori*-negative group, although a statistically significant difference was not present ($p > 0.05$).

The age, sex, mean eosinophil scores, gastric juice ECP levels, serum ECP levels, gastric juice/serum ECP ratios of group 1a and group 1b are presented in Table 2. Both the age and sex distribution had an insignificant difference between the two groups ($p > 0.05$). There was no significant difference in gastric eosinophil scores, gastric juice ECP levels, serum ECP levels, and gastric/serum ECP ratios between group 1a and group 1b ($p > 0.05$).

In a comparison of group 1a and group 1b with group 2, gastric eosinophil scores, gastric juice ECP levels, gastric juice/serum ECP ratios were greater in both group 1 subgroups than in group 2 ($p < 0.01$). There was no significant difference between groups according to the serum ECP levels ($p > 0.05$).

Table 1. Demographic characteristics, mean eosinophil score, gastric juice ECP level, serum ECP level and gastric juice/serum ECP ratio in *H. pylori* presence

Parameter	Group 1	Group 2	*p* value
Subjects (*n*)	44	20	
Mean age (years)	43.2±11.3 (20–60)	42.5±10.1 (22–54)	Not significant
Sex (male/female)	25/19	11/9	Not significant
Eosinophil score	1.14±0.95	0.35±0.59	<0.01
Gastric juice ECP (μg/l)	54.8±51.6	19.1±18.6	<0.01
Serum ECP (μg/l)	19.7±20.1	14.1±8.1	Not significant
Gastric juice/serum ECP ratio	4.24±4.06	1.24±1.01	<0.01

Group 1 = *H. pylori* positive, Group 2 = *H. pylori* negative.

Discussion

H. pylori is the most common bacterial infection worldwide. It is estimated that 60% of the world's population is infected by this microorganism. *H. pylori* is generally acquired in childhood and causes lifelong chronic gastritis unless initial acute gastritis is adequately managed.[24,25] Approximately 20% of persons infected by *H. pylori* develop peptic ulcer disease in some period of their lifespan.[5] Antral biopsies of individuals infected with *H. pylori* show focal epithelial cell damage as well as an inflammatory infiltrate in the lamina propria. This infiltrate consists of polymorphonuclear leukocytes, eosinophils, and mononuclear cells.[7,24–29] Inflammatory response is pivotal in the epithelial dysfunction and mucosal injury caused by *H. pylori*. *H. pylori* stimulates the release of a variety of inflammatory mediators both directly by bacterial products such as vacuolating cytotoxin, lipopolysaccharide, neutrophil-activating factor and porins, and indirectly as a result of interaction with gastric epithelial cells.[6,8,28,30] The role of eosinophils in the pathogenesis of *H. pylori*-associated gastritis and ulcer is not clearly explained.

The present study provides some evidence of an association between gastric eosinophil infiltration, gastric juice ECP and serum ECP levels in *H. pylori* infection and gastric ulcer disease.

Eosinophils play a role in many disorders, such as allergic, immunologic, and malignant diseases.[9–11] Eosinophil granulocytes, predominantly tissue-dwelling cells, are normally present in low numbers in healthy gut mucosa.[14,15] A possible role of the eosinophil in several intestinal diseases has been suggested. Patients with celiac disease have been shown to have prominent infiltration of eosinophils in the lamina propria, and activation of eosinophils was suggested by the release of ECP in the tissue and lumen of the intestine.[31] The eosinophil may also be a major actor in the pathogenesis of inflammatory bowel disease because bowel biopsies from patients with inflammatory bowel disease have demonstrated an infiltration of eosinophils in the lamina propria and marked extracellular deposits of ECP.[10,14,18,32] Moreover, eosinophilic gastrointestinal tract infiltration is encountered in food allergies and eosinophilic gastroenteritis.[12,14,15]

The presence of eosinophils is assumed as a protective mechanism of unspecific mucosal immunity response against bacteria and parasites.[11,15] Since eosinophil granules contain many proinflammatory and cytotoxic mediators, their protective role has become controversial.[14] Activation of eosinophils seems to contribute to the pathophysiology of several inflammatory conditions.[18,19,32] The role of the eosinophil granulocyte in the inflammatory reaction still remains obscure. One of the major constituents of the granule matrix is the eosinophil cationic protein. It is not only a strong helminthotoxic mediator, but it can also turn its destructive mechanisms against the host by cytotoxicity toward a variety of target cells including epithelial cells, smooth muscle cells, and nerve cells.[10,14,17]

We found that gastric mucosal eosinophil infiltration and the gastric juice ECP level were apparently greater in *H. pylori*-infected subjects. According to

Table 2. Demographic characteristics, mean eosinophil scores, gastric juice ECP level, serum ECP level and gastric juice/serum ECP ratio in *H. pylori*-positive patients according to the presence or absence of gastric ulcer

Parameter	Group 1a	Group 1b	*p* value
Subjects (*n*)	21	23	
Mean age (years)	41.1±10.7 (20–60)	45.2±11.7 (29–59)	Not significant
Sex (male/female)	12/9	13/10	Not significant
Eosinophil score	1.29±0.85	1.02±1.4	Not significant
Gastric juice ECP (μg/l)	56.51±47.1	53.3±56.6	Not significant
Serum ECP (μg/l)	20.5±19.3	19.1±21.1	Not significant
Gastric juice/serum ECP ratio	4.64±4.54	3.88±3.63	Not significant

Group 1a = *H. pylori* positive, gastric ulcer present.
Group 1b = *H. pylori* positive, gastric ulcer absent.

the serum ECP level, however, no significant difference between two groups was found. On the other hand, gastric juice/serum ECP ratios were greater in patients infected by *H. pylori*. We failed to find any significant difference in regard to gastric mucosal eosinophil infiltration, gastric juice ECP levels, serum ECP levels and gastric juice/serum ECP ratios between those patients having gastric ulcer disease and those having not.

There are inadequate data in the literature on the role of eosinophil-associated ECP on diseases caused by *H. pylori*. In a study by McGovern *et al.*,[21] eosinophilic infiltration and degranulation in gastric mucosa were investigated histopathologically in *H. pylori* infection, and eosinophilic infiltration and degranulation in gastric mucosa were greater in patients with *H. pylori* gastritis. Berstad *et al*.[33] in their study revealed that gastric juice ECP concentration was apparently greater in *H. pylori*-positive patients than the negative ones. We also found in accordance with the aforementioned studies that eosinophil infiltration in gastric mucosa and gastric juice ECP levels increased in *H. pylori*-positive patients. Ojetti *et al.*[34] assessed serum/gastric juice ECP levels and gastric mucosal eosinophil infiltration in idiopathic chronic urticaria patients infected or not with *H. pylori* and evaluated the modification after bacterium eradication. However, they found that *H. pylori* infection affects gastric juice ECP and eosinophil infiltration of only idiopathic chronic urticaria patients.

As a result, it has been demonstrated that eosinophil infiltration in gastric mucosa and ECP levels in the gastric juice are increased in *H. pylori*-positive patients whereas serum ECP levels do not change significantly. No apparent difference was found in eosinophilic infiltration in gastric mucosa, gastric juice and serum ECP levels between patients with and without gastric ulcer. This result, although it supports the role of eosinophils and eosinophil-derived ECP in inflammatory changes in chronic gastritis, suggests that these two factors do not contribute to gastric ulcer disease development.

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