Turk J Gastroenterol 2002; 13 (2): 98-102

The relationship between Helicobacter pylori intensity and histopathological findings in cases with chronic gastritis and duodenal ulcer

Kronik gastrit ve duodenal ülserli vakalarda Helicobacter pylori yoğunluğu ve histopatolojik bulgular arasındaki ilişki

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Background/aims: *Helicobacter pylori infection affects many people in developing countries. The inflammation it induces can cause malignant gastric lymphoma and also gastric carcinoma, depending on the intensity of inflammatory reaction, specific properties related to the strain and the host response.* ***Methods:*** *Fifty patients (35 cases with gastritis and 15 with duodenal ulcer) were included in the study. Hematoxylin-eosin paraffin sections were prepared from their endoscopic biopsies and Helicobacter pylori presence, activity of the infection, lymphoplasmacytoid cell infiltration and the degree of atrophy were determined semiquantitatively. Toludin O stain was applied to determine Helicobacter pylori intensity.* ***Results:*** *We found a statistically significant positive correlation of Helicobacter pylori intensity and activity of infection, lymphoplasmacytoid cell infiltration and atrophy in the 35 cases with gastritis and the relationship between Helicobacter pylori intensity and inflammatory activity was statistically significant in the 15 cases with duodenal ulcer.* ***Conclusion:*** *As morphological characteristics are crucial for the early diagnosis and treatment of gastric malignities; histopathological confirmation of helicobacter intensity is important.*

Key words: Helicobacter pylori intensity, chronic gastritis, duodenal ulcer, gastric carcinoma.

Amaç: *Helicobacter pylori gelişmekte olan ülkelerdeki pek çok bireyi etkilemektedir. Helicobacter pylori'nin indüklediği inflamasyon; inflamatuar reaksiyonun şiddeti, suşa ait spesifik özellikler ve konak cevabına bağlı olarak malign gastrik lenfoma ve gastrik karsinomaya neden olabilir.* ***Yöntem:*** *Çalışmamıza 50 vaka (35 gastritli, 15 duodenal ulkuslu) dahil edildi. Bu hastaların endoskopik biyopsilerinden hematoksilen-eozin kesitler elde edildi. Semikantitatif olarak Helicobacter pylori varlığı, infeksiyonun aktivasyonu, lenfoplazmositer hücre infiltrasyonu ve atrofi derecesi değerlendirildi. Helicobacter pylori şiddetini belirlemek için toluidin-O boyası kullanıldı.* ***Bulgular:*** *Gastritli 35 vakada Helicobacter pylori'nin şiddeti ile enfeksiyonun aktivasyonu, lenfoplazmositer hücre infiltrasyonu ve atrofi arasında istatistiksel açıdan anlamlı pozitif bir ilişki saptanırken, duodenal ulcuslu 15 vakada iltihabi aktivasyon ile Helicobacter pylori yoğunluğu arasındaki ilişki istatistiksel olarak anlamlı idi.* ***Sonuç:*** *Gastrik kanserlerin erken tanı ve tedavisinde morfolojik özellikler önemli olduğundan; Helicobacter pylori yoğunluğunun histopatolojik olarak gösterilmesi gereklidir.*

Anahtar kelimeler: Helicobacter pylori şiddeti, kronik gastrit, duodenal ülser, gastrik karsinom.

INTRODUCTION

Studies about the role of Helicobacter pylori (H. pylori), a gram negative spiral shaped bacteria, in the etiology of upper gastrointestinal disease have become popular since Warren and Marshall first identified it in antral gastric mucosa in 1983 (1). In gastroduodenal mucosa it stimulates pro-inflammatory and immunoregulatory cytokines, chemotactic agents, specific T and B cell response and lymphoid follicle development (2-5).

In the intestinal system, H. pylori is especially found in the gastric antrum where the mucus layer is thicker and within areas of gastric metaplasia. The nature of the inflammatory reaction induced by H. pylroi changes according to the bacterial factors specific to the strain, the abundance of the bacteria and the response of the host (6). This response varies from limited inflammatory reaction restricted to the foveolar area to diffuse

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Manuscript received: 10.1.2002 **Accepted:** 11.6.2002

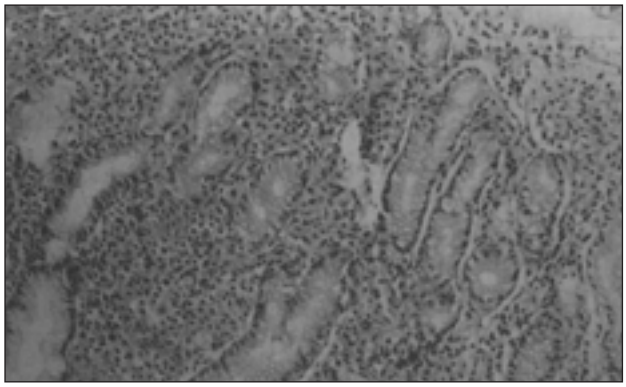

Figure 1. Severe mucosal inflammation in chronic active gastritis (HE)x125.

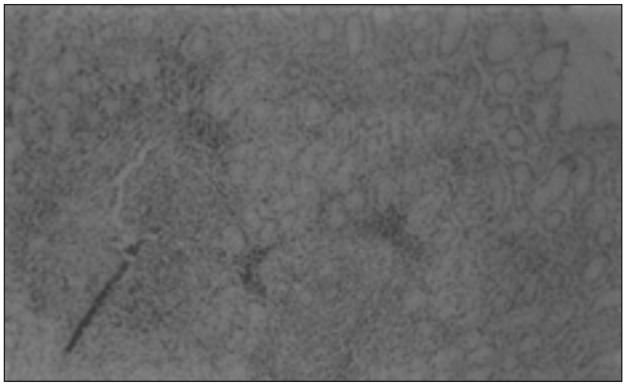

Figure 2. Lymphoid follicle presence in chronic active gastritis (HE)x 62.5.

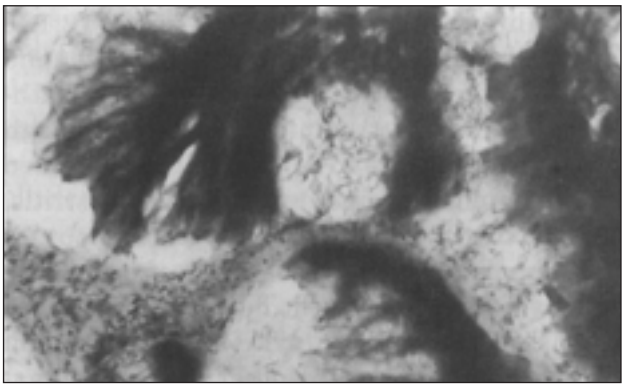

Figure 3. H. pylori localization within mucosa of a case with active inflammation and lymphoid follicle presence (Toluidin-Ox440)

inflammatory reaction confined to all parts of the mucosa which can result in atrophy (7). While one part of the inflammatory response is characterized by leucocyte infiltration, the other is a due to chronic response characterized by lymphoid follicle formation at the gastric mucosa. These different morphological characteristics are important in the diagnosis, treatment and follow up of the disease for gastric carcinoma and lymphoma, which may occur later due to these changes (5,6).

In this study, we evaluated the relationship between Helicobacter pylori abundance and morphological characteristics (inflammatory activation, lymphoplasmacytoid cell infiltration (LMN) density and the degree of atrophy).

MATERIALS AND METHODS

Fifty patients who had undergone endoscopy for dyspeptic complaints at Cumhuriyet University Gastroenterology Clinic were included in the study. Each of their endoscopic gastric biopsies (two antrum, two corpus) were evaluated semi-quantitatively for H. pylori presence, activity, degree of infection, LMN degree and intensity of atrophy.

Routine hematoxylin-eosin (HE) stain and Toluidin O stain were applied to five mm paraffin sections prepared from patient biopsies. H. pylori intensity was scored as follows

1. H. pylori was present in less than 1/3 of the surface epithelium

2. H. pylori was present in area between one third-two thirds of the epithelium

3. It covered more than two thirds of the epithelium forming clusters (8).

In order to find out the degree of activitiy in routine HE sections, it was accepted to be 0 when very rare leucocytes were present in the lamina propria (LP) and epithelium. It was classified as 1 when neutrophils were seen in a few areas of LP forming small foci, 2 when there was infiltration of glands and to a lesser degree, accompanied the occupation of LP by leucocyte clusters and 3 when infiltration was very dense and widespread in both LP and glands (8).

Atrophy was determined to be the loss of glandular tissue. The degree of gland loss in LP was

graded semiquantitatively from 1 to 3.

The classification of LMN infiltration degree was 0 when two-three lymphocytes were present in mucosa between foveolas, 1 when there were more than three deformed lymphocytes, 2 when lymphocytes formed small aggregates, and 3 when they formed follicles (9).

The tampons for Toluidin O stain were as follows (10):

1. 9.465g m/15 dibasic sodium phosphate (Na2HPO4) was dissolved in one liter of distilled water.

2. 9.08g m/15 potassium acid phosphate (KH2PO4) was dissolved in one liter of distilled water.

After this 25 mls were taken from each of the two solutions and mixed in 1 ml of the toludin blue stain in order to make the pH 6.8.

Spearmans' correlation analysis was used in order to evaluate the relationship between H. pylori degree and activity, LMN infiltration and atrophy.

RESULTS

There were 35 (26 female, nine male) gastritis cases with an age range of 18 to 78 years (mean 48.2±16.3) and 15 (10 female, five male) duodenal ulcer cases with an age range of 18-74 years (mean 46.7±18.1).

Helicobacter pylori could not be demonstrated by toluidin O stain in six (17%) of the cases with gastritis and in three (20%) of the cases with duodenal ulcer.

When the degree of H. pylori colonization was compared with activity, LMN infiltration and atrophy, a significant positive correlation was found in the group with gastritis. ($r=0,78; p<0.01$; $r=0.55$, $p<0.01$, $r=0.62; p<0.01$ respectively)

While a significant correlation was found between H. pylori intensity and activity in cases with duodenal ulcer ($r=0.72$, $p<0.01$) , the relationship between LMN infiltration and the degree of atrophy was not significant ($r=-0.16$, $p>0.05$) in this group.

Although we found intestinal metaplasia in two of the gastritis and six of the duodenal ulcer cases, no statistical comparison was possible due to the limited number of our cases.

DISCUSSION

The correct diagnosis and effective treatment of H. pylori infection is an important issue due to its high prevalence worldwide, and its endemicity from an early age in developing countries such as Turkey. The bacteria, which is ingested, penetrates along the gastric mucus layer and begins to multiply by adhering to the epithelial cells lining the gastric mucosa, frequently causing different degrees of inflammation (11). The mucosal destructive mechanism of H. pylori is not precisely known. The bacteria destroys the gastric mucus which is glycoprotein in structure, by forming protease and causes acid in gastric juıce to irritate the mucosa by passing the mucus bicarbonate layer (6,7,10,12). The bacteria increase ammonia production in the gastric lumen by their urease activity. Ammonia causes cell destruction by its direct toxic effect and also disintegrates cell permeability and active transport (12,13). Additionally, H. pylori secretes cytokines and chemotactic factors which have a direct cytopathic effect or initiate the inflammation. Although neutrophil infiltration in LP is generally accepted to be the unanimous finding of H. pylori gastritis, there are conflicting results in studies about H. pylori abundance and the intensity of inflammatory activity. While Petross and Prıce reported no relationship between H. pylori and activity in their investigations (14,15), other authors emphasize the presence of a relationship (16,17).

In this study, a significant relationship was observed between the intensity of colonization and activity in not only cases with duodenal ulcer but also in cases with gastritis.

The response of surface epithelium to H. pylori infection is mucus depletion, desquamation of the epithelium and regenerative change. In one study a significant positive correlation was found between H. pylori infection and PNL infiltrate, lymphocytic infiltrate and epithelial regenerative activity in 200 patients with chronic gastritis (18). Some investigators suggest that leucocyte infiltration of the gastric mucosa, which is accepted to be the definitive finding of H. pylori infection, could be induced by other etiological agents. Disintegration of the apical mucus in the form of epithelial pits unrelated to inflammatory infiltrates and erosions with overt ulcerations are suggested to be more specific findings of H. pylori infection (7). Likewise, mucus loss on the surface of the epithelium and the presence of pits in

epithelial cells were observed in this study. It is believed that there are no lymphoid follicle in normal gastric mucosa, but lymphoid folicule presence in gastric mucosa with chronic active gastritis due to H. pylori infection has been demonstrated in various studies. Wyatt and Rathbone have shown lymphoid follicle presence in 27% of 415 cases with H. pylori gastritis (17) while Stolte and Eidt found a rate of 54% in 1297 cases. These investigators observed a negative correlation between lymphoid follicles and leucocyte infiltration and suggested that evident lymphocytic response occurs as chronicity develops (19). Other studies claim that lymphoid folicules are more common and wide spread in cases with gastric and duoedonal ulcer than in cases with gastiritis (6,17,20). However, it should not be forgotten that lymphoid follicles may be present regardless of any H. pylori infection in certain ulcer cases when biopsies are taken from areas adjacent to the ulcer and H. pylori presence in areas other than the ulcer should therefore be confirmed by histochemical stains (20). In our study we also found LMN infiltration in the form of two or more lymphoid follicles localized in the antrum, in five out of 15 cases with duodenal ulcus. Moreover, this infiltration was not related with the intensity of the H. pylori colonization. This finding was not in concordance with the literature.

Lymphoid follicles, which are accepted to be an important finding in H. pylori gastritis, are also said to be responsible for the development of low grade gastric MALT lymphoma. According to some authors, serial sections should be prepared when lymphoid aggregates with irregular borders are observed at biopsies and lymphoid follicle presence should be confirmed, with the number, diameter and shape of the follicles being noted at patient follow-up (5,21).

Atrophy, which is known to be the loss of the glands in gastric mucosa, is defined as the presence of two or less glands in antral sections. As the glands become distant due to chronic inflammation and fibrosis, the diagnosis of atrophy becomes difficult. Corporal atrophy, induced by free O_2 radicals released from neutrophils, is closely related to the suppression of acid secretion and intestinal metaplasia and also increases the risk of gastric carcinoma development. While corporal atrophy is more related to the host response, antral atrophy depends on the intensity of H. pylori activity (6).

The possible role of H. pylori in the pathogenesis of gastric carcinoma pathogenesis has recently aroused much interest because H. pylori infection causes chronic active gastritis, a lesion which is accepted to be a precursor in the development of gastric carcinoma. Martinez-Madrigal et al found a relationship between the intensity of H. pylori colonization and chronic atrophic gastritis and also an association with atypical regeneration and dysplasia (22). Other factors (genetic, diet, age of onset and environmental) are thought to have role in the pathogenesis of carcinoma because gastric carcinoma cases form a minor group in the large number of cases with H. pylori gastritis.

It is concluded that determination of the degree of morphological change accompanying H. pylori infection is important in follow up to determine the risk of malignant lymphoma or gastric carcinoma development. However this relationship should be further evaluated in prospective studies with larger numbers of patients. Also, the development of stains for use in the pathology laboratory, (such as toulidin 0, giemsa and gram) to make an early histopathological confirmation of H. pylori presence should be developed to control the rate of malignancy development.

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