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Assessment of COX-2 expression presence and severity by immunohistochemical method in patients with chronic active gastritis and intestinal metaplasia

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Background/aims: *The risk of gastric cancer is increased in patients with intestinal metaplasia. Cyclooxygenase-2 activity is crucial for gastric cancer cell survival and proliferation. We aimed to assess cyclooxygenase-2 expression in patients with intestinal metaplasia or chronic active gastritis and in patients with or without a family history of gastric cancer, i.e. a first-degree relative with gastric cancer.* ***Materials and Methods:*** *One hundred and six patients with histologically proven intestinal metaplasia, chronic active gastritis or normal gastric mucosa were included. Immunohistochemical staining was performed using the immunoperoxidase method.* ***Results:*** *Cyclooxygenase-2 expression was detected in 23.1% of normal gastric mucosa, 70.6% of chronic active gastritis, and 90.5% of intestinal metaplasia patients. Cyclooxygenase-2 expression was significantly higher in intestinal metaplasia than in chronic active gastritis (p=0.018). Cyclooxygenase-2 expression was significantly more severe in the intestinal metaplasia group when compared to the chronic active gastritis group (p=0.017). Severe cyclooxygenase-2 expression (>60% of cells) was more frequent in the intestinal metaplasia group. Cyclooxygenase-2 expression was higher in the Helicobacter pylori-positive group when compared to the Helicobacter pylori-negative group (80.3% vs 57.1%, respectively; p=0.012). Cyclooxygenase-2 expression did not significantly differ according to presence of a first-degree relative with gastric cancer.* ***Conclusions:*** *Patients with intestinal metaplasia demonstrated increased presence and severity of cyclooxygenase-2 expression. Our findings suggest that cyclooxygenase-2 plays an important role in the stepwise process that eventually leads to gastric cancer. There was no statistically significant difference between the patients with and without a first-degree relative with a history of gastric cancer in terms of cyclooxygenase-2 expression.*

Key words: Intestinal metaplasia, cyclooxygenase-2 expression, *Helicobacter pylori*

Kronik aktif gastrit ve intestinal metaplazili hastalarda COX-2 ekspresyon varlığı ve ciddiyetinin immunohistokimyasal yöntemle değerlendirilmesi

Amaç: *İntestinal metaplazili hastalarda mide kanseri riski artmıştır. Siklooksijenaz-2 mide kanseri hücrelerinin proliferasyonu için kritik öneme sahiptir. İntestinal metaplazili hastalarda ve birinci derece akrabalarında mide kanseri öyküsü olan ve olmayan hastalarda siklooksijenaz-2 ekspresyonunu değerlendirmeyi amaçladık.* ***Gereç ve Yöntem:*** *Histolojik olarak intestinal metaplazi, kronik aktif gastrit ya da normal gastrik mukoza tanısı almış olan 106 hasta çalışmaya alındı. İmmünhistokimyasal boyama, immünoperoksidaz yöntemiyle yapıldı.* ***Bulgular:*** *İntestinal metaplazili hastaların %90.5'inde, kronik aktif gastritli hastaların %70,6'sında, normal gastrik mukozası olanların %23,1'inde siklooksijenaz-2 ekspresyonu saptandı. Siklooksijenaz-2 ekspresyonu, intestinal metaplazi grubunda kronik aktif gastrit grubuna göre anlamlı derecede yüksekti (p=0.018). İntestinal metaplazi ve kronik aktif gastrit grupları arasında siklooksijenaz-2 ekspresyonunun şiddeti açısından da anlamlı fark saptandı (p=0.017). Şiddetli siklooksijenaz-2 ekspresyonu (hücrelerin %60'ından fazlasında ekspresyon), intestinal metaplazi grubunda daha sıktı. Helikobakter pilori pozitif grupta, negatif gruba göre siklooksijenaz-2 ekspresyonu daha fazlaydı (%80.3'e karşılık %57.1, p=0.012). Siklooksijenaz-2 ekspresyonu, birinci dereceden bir akrabada mide kanseri öyküsü olup olmamasına göre değişkenlik göstermedi.* ***Sonuç:*** *İntestinal metaplazili hastalarda siklooksijenaz-2 ekspresyonunun sıklığı ve şiddeti artmıştır. Bulgularımız, mide kanserine kadar ilerleyen süreçte siklooksijenaz-2'nin önemli bir rol oynayabileceğini düşündürmektedir. Birinci derece akrabalarında gastrik kanser öyküsü olan ve olmayan hastalar arasında siklooksijenaz-2 ekspresyonu açısından fark saptanmamıştır.*

Anahtar kelimeler: İntestinal metaplazi, siklooksijenaz-2 ekspresyonu, *Helikobakter pilori*

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INTRODUCTION

Gastric cancer is the fourth most common cancer type across the world, and it is the second leading cause of mortality associated with cancer. Each year, 1,000,000 individuals are diagnosed with gastric cancer and 850,000 of those die in the same year (1). Generally, gastric cancer is believed to follow a sequence of developmental stages known as Correa's cascade, which is comprised of chronic gastritis, atrophic gastritis, intestinal metaplasia (IM), and dysplasia (2). Usually, *Helicobacter pylori (H. pylori)* infection is the triggering event of this cascade. While gastric cancers are categorized as diffuse and intestinal types according to the Lauren classification (3), atrophic gastritis and IM are regarded as precursor lesions particularly for intestinal-type gastric cancers (4).

Intestinal metaplasia (IM) is the replacement of gastric mucosa with an epithelium similar to the small bowel mucosa. IM originates from gastric stem cells, which instead of transforming into cells specific to the stomach, differentiate into intestinal cell types such as absorptive cells, goblet cells and Paneth cells. IM is usually triggered by the continuous irritation of the gastric mucosa, and *H. pylori* infection is known to be one of the most important triggering factors (4). Nonetheless, gastric cancer is not encountered in all patients with IM, and factors involved in the development of cancer are not fully understood (4).

H. pylori, the main cause of chronic gastric disorders, has been defined as a Class I gastric carcinogen (5). The diseases associated with *H. pylori* are duodenal ulcer, gastric ulcer, gastric cancer, and B-cell mucosa-associated lymphoid tissue lymphomas (6).

Atrophic gastritis, regarded as the first important step towards gastric cancer, develops as a result of the long-standing gastric inflammation induced by *H. pylori.* Chronic atrophic gastritis often presents itself in combination with IM, and both lesions are closely associated with *H. pylori* (4).

Moreover, cellular kinetics is thought to have a role in the progression towards gastric cancer. Inhibition of apoptosis and/or increasing proliferation may be involved in cancer development as well. *H. pylori* infection stimulates apoptosis and proliferation in the normal gastric epithelium (7,8).

Topal et al. (9) reported that *H. pylori,* which is known to induce apoptosis, paradoxically increases the expression of bcl-2, an anti-apoptotic gene. They also observed more prominent bcl-2 positivity in IM than that in atrophy.

Cyclooxygenase-2 (COX-2) is the key enzyme that allows synthesis of prostaglandins and other eicosanoids from arachidonic acid. COX-2 expression can be stimulated by mitogens, hormones, cytokines, and growth factors (10). Elevated COX-2 expression is believed to play an important role in the early stages of gastric cancer development (11) through apoptosis inhibition (12) and increased proliferation (13).

The aims of this study were: 1) to compare patients with IM, chronic active gastritis (CAG) and normal gastric mucosa (NGM) with regard to presence of COX-2 expression, 2) to assess the severity of COX-2 expression between patients with IM, CAG and NGM, and 3) to determine whether or not COX-2 expression is different between patients with versus without a history of gastric cancer in first-degree relatives.

MATERIALS AND METHODS

This study was conducted on 106 patients (13 with normal upper gastrointestinal endoscopy and 93 with antral predominant gastritis) who had previously undergone upper gastrointestinal endoscopy due to dyspeptic symptoms and referred to our outpatient clinics with their biopsy results. The patients with antral predominant gastritis did not have any additional gastric lesions. The biopsies had been obtained in order to detect the presence of *H. pylori* and any histopathological changes. Four biopsy specimens had been obtained from each patient: two from the corpus and two from the antrum.

The histopathological results of the whole study population were consistent with IM, CAG or NGM. Informed consents were obtained from each patient, and the study was approved by the university ethical committee. Patients who had been on H2 receptor antagonists or proton pump inhibitors over the last four weeks, patients with a history of *H. pylori* eradication treatment, gastric surgery, non-steroid anti-inflammatory drug use, COX-2 inhibitor or acetylsalicylic acid use, antibiotic use during the previous month, or exposure to corrosive substances, and those with malignancy and vasculitis were excluded from the study.

Family history of gastric cancer was questioned in all patients. The study population was divided in-

to two groups as patients with (n: 18) or without (n: 88) a history of gastric cancer in first-degree relatives.

Histopathological Analysis

Histopathological sections of all patients were evaluated according to the Updated Sydney System Score (14) before undergoing immunohistochemical staining for the assessment of COX-2 expression.

Antral biopsies were immunohistochemically stained for COX-2 expression.

Immunohistochemical staining was performed using the indirect immunoperoxidase method with streptavidin and biotin-3. The antibody used for COX-2 expression was monoclonal and of IgG type. RM-9121-R7 antibody specific to the human cyclooxygenase enzyme was employed for the detection of COX-2 expression (Lab Vision/ NeoMarkers, Fremont, CA, USA). The sections with a thickness of a few microns were deparaffinized by being kept at 56C° for 12 hours (h) in a sterilizer, held in xylene for 30 minutes (min), and hydrated in descending alcohol solutions (100%, 95% and 90%). Following being washed with tap water, they were placed in 3% hydrogen peroxide for 10 min in order to inhibit endogenous peroxidase. The sections were washed with phosphate buffered saline (PBS) solution (pH: 7.6) for 5 min and processed in a microwave oven for 5 min in a 0.01M sodium citrate buffer (pH: 6.0). They were washed with distilled water three times. Then, the sections were placed in a non-immune serum for 20 min for protein block. Primary antibodies were applied so as to cover the sections and they were kept at room temperature for 2 h. They were washed with PBS for 5 min twice and dried. Incubation with secondary (binding) antibody (Multi-species Ultra streptavidin detection system-HRP; Signet, Massachusetts, USA) was performed at room temperature for 20 min. Then, sections were washed with PBS for 5 min twice. Streptavidin-biotin complex was applied and allowed to set for 30 min, then washed with PBS for 5 min twice. Diaminobenzidine tetrachloride (DAB, Novocastra, Newcastle-upon-Tyne, UK) incubation was performed for 10 min and sections were again washed with distilled water for 5 min. Background staining was carried out with hematoxylin by rapid staining technique. The sections were dehydrated in ascending alcohol solutions, 5 min each (90%, 95% and 100%). Finally, the sections were rendered more transparent by xylene and sealed with Entellan.

Colon carcinoma was used as the positive tissue control for COX-2. Cytoplasmic staining was recognized as positive for COX-2. Apart from the positive control, a negative control staining without any primary antibody was applied. The degree of staining was determined with a semiquantitative method.

COX-2 expression was evaluated by counting at least 1000 cells at randomly selected 10 fields under high magnification, and thus a “staining index” was calculated.

The severity of COX-2 expression was categorized as negative (0-4%), weak (5-29%), moderate (30-59%), and severe (>60%) as described by Sun et al. (15).

Statistical Analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) 12.0 software package (SPSS Inc., Chicago, IL, USA). In case of normal distribution, ANOVA was employed for comparison between three groups, and Student’s t test was employed for comparison between two groups. In case of abnormal distribution, comparison between three groups was made using the Kruskal-Wallis test, and comparison between two groups was made using the Mann-Whitney U test. For categorical data, Pearson’s chi-squared test and Fisher’s exact chi-squared test were used. Level of significance was assumed as 0.05; p value <0.05 represents a significant difference or correlation, whereas p value >0.05 is interpreted as indicating no significant difference or correlation.

RESULTS

Fifty of the patients were male and 56 were female; the age range was 18-81 years (mean age: 46.1±14.5). Among 106 patients, 42 (39.6%) had IM, 51 (48.1%) had CAG, and 13 (12.3%) had NGM according to histopathological examination (Table 1).

There was a significant difference between individuals with IM, CAG and NGM with regard to age ($p=0.002$) (Table 1). The mean age in the IM group was statistically higher than in the CAG and NGM groups.

There was no statistically significant difference between the IM, CAG and NGM cases in terms of gender ($p>0.05$) (Table 1).

Table 1. Demographic data of the study group

	IM	CAG	NGM	p
Number of the patients (n:106)	42 (39.6%)	51 (48.1%)	13 (12.3%)	
Age	52.02±13.74	43.04±14.21	39.23±11.94	p=0.002
Gender (Male/Female)	22/20	22/29	6/7	p>0.05

IM: Intestinal metaplasia. CAG: Chronic active gastritis. NGM: Normal gastric mucosa.

All of the patients with a normal histopathology were negative for *H. pylori*. Comparison of CAG and IM groups with regard to presence of *H. pylori* (76.5% vs 76.2%, respectively) revealed no statistically significant difference (p>0.05).

No statistically significant difference was determined between the individuals with IM, CAG and NGM with regard to history of gastric cancer in a first-degree relative (23.8% vs 11.8% vs 15.4 %, respectively; p>0.05).

There was a statistically significant difference between the patients with IM, CAG and NGM in terms of COX-2 expression (p=0.0001) (Table 2, Figure 1). COX-2 expression was detected in 23.1% of NGM, 70.6% of CAG and 90.5% of IM patients.

COX-2 expression was statistically significantly more frequent in the IM group than in the CAG group (90.5% vs 70.6%, respectively; p=0.018). The COX-2 expression was weak in patients with NGM.

There was a statistically significant difference between the CAG and IM groups with regard to the severity of COX-2 expression (p=0.017) (Table 3). Severe expression (>60% of cells) was more frequent in the IM group.

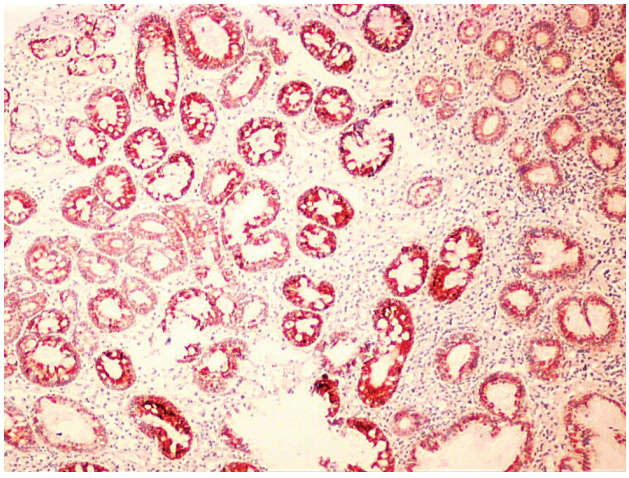

Figure 1. COX-2 expression in a case with intestinal metaplasia.

COX-2 expression was more frequent in the *H. pylori*-positive group when compared to the *H. pylori*-negative group (80.3% vs 57.1%, respectively) (p=0.012).

Comparison of the patients with and without history of gastric cancer in first-degree relatives showed no statistically significant difference with regard to presence of IM (55.6% vs 36.4%, respectively; p>0.05).

Comparison of the patients with and without history of gastric cancer in first-degree relatives showed no statistically significant difference with regard to COX-2 expression (77.8% vs 71.6%, respectively; p>0.05).

DISCUSSION

Intestinal metaplasia (IM) is the replacement of gastric mucosa with an epithelium similar to the small bowel mucosa (4). It is the most common metaplasia type seen in the stomach. Epidemiological studies show that significant IM can be observed very rarely in people with no history of *H. pylori* infection. Therefore, it is widely agreed that IM is one of the outcomes of chronic infection with *H. pylori* (16).

Cyclooxygenase-2 (COX-2) is a key enzyme that enables production of prostaglandins from arachidonic acid. While COX-1 isoform is structurally expressed in many tissues, COX-2 isoform is an inducible enzyme that is expressed particularly in areas with inflammation (10). Elevated COX-2 expression in the early stages is thought to have an important role in the development of gastric cancer (17) through inhibition of apoptosis (12) and increased proliferation (13). Gastritis caused by *H. pylori* is associated with elevated COX-2 expression (18).

Sun et al. (15) evaluated the relationship between *H. pylori* infection and COX-1/COX-2 expression in the gastric mucosa of chronic superficial gastritis, gastric glandular atrophy, gastric mucosal IM, moderate gastric epithelial dysplasia, and gastric

Table 2. COX-2 expression relative to histopathology

	IM (n: 42)	CAG (n: 51)	NGM (n: 13)	p
COX-2 expression positivity	90.5% (n: 38)	70.6% (n: 36)	23.1% (n: 3)	0.0001

IM: Intestinal metaplasia. CAG: Chronic active gastritis. NGM: Normal gastric mucosa.

Table 3. Assessment of COX-2 expression severity in CAG and IM groups

	COX-2 expression			
	Negative (0-4%)	Weak (5-29%)	Moderate (30-59%)	Severe (>60%)
CAG	29.4% (n:15)	27.5% (n:14)	29.4% (n:15)	13.7% (n:7)
IM	11.9% (n:5)	26.2% (n:11)	21.4% (n:9)	40.5% (n:17)

IM: Intestinal metaplasia. CAG: Chronic active gastritis.

cancer patients. In that study, the percentage of positive staining cells was graded semiquantitatively, and each sample was assigned to one of the categories below: - (negative, 0% - 4%); + (weak, 5% - 29%); ++ (moderate, 30% - 59%); or +++ (strong, more than 60%). An ascending percentage of COX-2 expression was observed from chronic superficial gastritis to gastric atrophy, IM, dysplasia, and finally to gastric cancer. *H. pylori*-positive patients with IM and dysplasia had a higher level of COX-2 expression when compared to *H. pylori*-negative patients with the same histopathological findings.

Yu et al. (19) evaluated COX-2 expression in paracancerous and tumor tissues after surgical resection in 30 patients. COX-2 expression was 66.7% in the tumoral tissue and 26.7% in the paracancerous tissue, and the difference between the two groups was statistically significant. Moreover, they found significantly elevated COX-2 expression in the areas with metaplasia and dysplasia compared with the paracancerous tissue. COX-2 expression was significantly raised in patients with *H. pylori* infection compared with those who did not have *H. pylori* infection. The authors noted that elevated COX-2 expression associated with *H. pylori* infection might play a role in the development of gastric cancer.

Recently Liu et al. (20) found that *H. pylori* infection may increase COX-2 expression in precancerous gastric lesions. The average optical density (AOD) values of COX-2 staining in chronic superficial gastritis, CAG, IM, and gastric mucosal dysplasia patients were compared, with significant differences among chronic superficial gastritis, CAG and IM patients in ascending order.

COX-2 expression is more prominent in intestinal-type gastric cancer when compared to the diffuse type (21,22). Ma et al. (23) reported that COX-2 expression is essential for the survival and proliferation of gastric cancer cells.

Zhang et al. (24) reported elevated COX-2 expression in the first-degree relatives of gastric cancer patients. Sheu et al. (25) demonstrated that gastric COX-2 expression is higher in *H. pylori* -infected relatives of gastric cancer patients. In our study, there was no statistically significant difference between the patients with and without first-degree relatives with gastric cancer in terms of COX-2 expression ($p>0.05$). As the sample sizes of these studies are relatively small, these contrasting findings may be only coincidental. On the other hand, as these studies were carried out in different ethnic groups, it may be postulated that the role of genetic factors might vary across different populations.

In our study, there was a statistically significant difference between the IM, CAG and NGM groups with regard to COX-2 expression ($p=0.0001$). COX-2 expression was present in 23.1% of NGM, 70.6% of CAG and 90.5% of IM cases. COX-2 expression was significantly more frequent in the IM than in the CAG patients ($p=0.018$). Moreover, frequency of severe (>60% of cells) COX-2 expression was statistically significantly higher in the IM group than in the CAG group ($p=0.017$).

While 80.3% of patients with *H. pylori* infection had COX-2 expression, this rate was 57.1% for the patients without *H. pylori* infection, and the difference between the two groups was statistically significant ($p=0.012$).

When patients were categorized as those with and without IM, 90.5% of the IM group and 60.9% of the non-IM group demonstrated COX-2 expression

(p=0.001). Moreover, 40.5% of the IM group and 10.9% of the non-IM group had severe COX-2 expression (p=0.001).

Currently, there is no effective treatment for gastric precancerous lesions. While *H. pylori* infection has a critical role in the initial developmental phase of gastric premalignant lesions, *H. pylori* eradication treatment fails to induce an improvement in the precancerous lesions (25-27). The reason behind this failure may be persistent or mildly reduced COX-2 expression despite *H. pylori* eradication. In these patients, COX-2 inhibition following *H. pylori* eradication may be considered (28). However, there is insufficient data in the literature that would support routine use of such a method. Therefore, there is increasing need for comprehensive studies investigating the preventive effect of COX-2 inhibition on development of gastric cancer in humans.

Our data appears to be consistent with the literature with one exception. Zhang et al. (24) reported elevated COX-2 expression in individuals who have a first-degree relative with a history of gastric cancer. Sheu et al. (25) demonstrated that gastric COX-2 expression is higher in *H. pylori*-infected relatives of gastric cancer patients. In our study, there was no statistically significant difference between the patients with and without a first-degree relative with a history of gastric cancer in terms of COX-2 expression.

The main limitation of our study was that the number of patients with a history of gastric cancer in a first-degree relative was relatively small. Further studies with larger patient samples are required to elucidate changes in COX-2 expression in individuals who have a first-degree relative with a history of gastric cancer.

COX-2 expression in the gastric mucosa bears great importance in the progression towards gastric cancer. In the present study, patients with IM, which is a precancerous lesion, demonstrated increased presence and severity of COX-2 expression. Moreover, COX-2 expression was determined to be statistically significantly higher in patients with *H. pylori* infection than in those without *H. pylori* infection. The exact role of COX-2 activity in the process that eventually progresses to gastric cancer remains to be elucidated.

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