Relation of cyclooxygenase-2 expression with premalignant gastric lesions

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Abstract

***Introduction* : We studied the relation between premalignant gastric lesions and cyclooxygenase-2 (COX-2) expression.**

***Methods* : The study included 254 patients, who were histologically diagnosed with chronic active gastritis, atrophy, dysplasia and metaplasia. Gastric biopsy specimens of the patients were histopathologically examined in terms of the presence of Helicobacter pylori (*H. pylori*) infection, atrophy The Operative Link for Gastritis Assessment ; (OLGA staging system), dysplasia (Vienna classification), and metaplasia (Sydney classification). COX-2 expression was investigated by immunohistochemical staining. COX-2 immunoreactivity score was calculated as the product of staining intensity and staining area. A score of >1 was defined as COX-2-positive expression.**

***Results* : Of these patients, 84 (33.1%) had negative COX-2 expression (Score 0 and Score 1) and 170 (66.9%) had positive COX-2 expression (Score 2 and Score 3). We found that in patients with a moderate-marked metaplasia, or with moderate-severe atrophy, a higher OLGA stage, or with dysplasia, the COX-2 expression was found to be higher than those with mild lesions. In 59.8% of the patients H. pylori was positive. While, the rate of severe atrophy was higher in H. pylori-positive patients ; no significant difference was determined between the H. pylori-positive and H. pylori-negative patients regarding age, smoking status, intestinal metaplasia grade, dysplasia, and COX-2 expression.**

***Conclusion* : We found a relation between the level of COX-2 expression and the grade of premalignant gastric lesions. COX-2 plays an important role in the gradual process resulting eventually in gastric cancer.** (Acta gastroenterol. belg., **2020**, 83, **249-254**).

Keywords : cyclooxygenase-2, atrophy, metaplasia, dysplasia**.**

Introduction

Gastric cancer (GC) is one of the most common and lethal malignant tumors worldwide (1). Its etiopathogenesis is complicated and various factors play a role in the development of gastric cancer, such as diet, infections, and genetic factors (2). The studies made so far show an association between *H. pylori* infection and the development of gastric cancer (3). In addition, in recent studies, the role of COX-2 in the development and progression of gastric cancer has been investigated, and it has been found that COX-2 expression increases in tissues with gastric cancer (4). Cyclooxygenase is an enzyme playing a critical role in prostaglandin synthesis and has two isoforms, namely COX-1 and COX-2. COX-1 is continuously expressed in large quantities in the gastrointestinal system and it plays a role in the protection of mucosal integrity via prostaglandin synthesis (5,6). The expression of COX-2, on the other hand, is low or undetectable in most tissues but can be highly induced in response to cell activation by hormones, proinflammatory cytokines, growth factors, and tumor promoters (7). It is thought that COX-2 overexpression plays an important role in the early stages of gastric cancer development by increasing the cell proliferation, inhibiting apoptosis, and contributing to angiogenesis (4) In some studies, it was suggested that *H. pylori* infection was significantly related to COX-2 expression (8).

H. pylori infection may increase the risk of gastric cancer by causing local inflammatory response, phenotypic change of epithelial cells, promotion of cell proliferation and inhibition of cell apoptosis (8). *H. pylori* infection is associated with cancer due to its atrophy, intestinal metaplasia (IM), and dysplasia, seen in chronic infection (5). Although recently there have been researches suggesting that COX-2 is highly expressed in gastric cancer, less is known about the relation between the premalignant lesions of gastric cancer, that is atrophic gastritis, intestinal metaplasia, and dysplasia, and the COX-2 expression. In this study, we researched the relation between *H. pylori* infection, atrophic gastritis, intestinal metaplasia, dysplasia and COX-2 protein expression.

Materials and Methods

A total of 254 patients were studied. Of these, 114 were males and 140 were females. The mean age was 55.08±12.02 years. (range, 25-78). Endoscopies with biopsy were performed in all patients. Patients who were histologically diagnosed with chronic active gastritis, atrophy, dysplasia, and metaplasia were included in the study. Patients who had undergone gastric surgery, on non-steroid anti-inflammatory drug use, COX-2 inhibitor or acetylsalicylic acid use, those with upper gastrointestinal malignancy or antibiotic use during the previous month were excluded from the study.

Informed consents were obtained from each patient, and this study was approved by the university ethical committee.

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Upper endoscopy examinations were conducted by one experienced gastroenterologist using video endoscopes. The gastric mucosa was examined, and five biopsies were obtained from standard sites of the stomach according to the Updated Sydney System two from the corpus, one from the incisura angularis and two from the antrum. All specimens were reviewed by one experienced pathologist. Each biopsy was investigated and a diagnosis was made based on the most severe histology. Later every patient was given a global diagnosis containing five biopsies based on the most severe histology.

Gastric biopsy specimens of the patients were histopathologically evaluated in terms of the presence of *H. pylori*, atrophy, dysplasia, and metaplasia. The degree of atrophy was assessed using the OLGA staging system by evaluating the extent of distal and proximal gastric atrophy between Stage 0 (none) and Stage IV (severe) (9). Presence of dysplasia was assessed by the Vienna classification as follows : Category 1, negative for dysplasia; Category 2, indefinite for dysplasia; Category 3, non-invasive low grade dysplasia; Category 4, non-invasive high grade dysplasia; Category 5, invasive neoplasia (10). Presence of intestinal metaplasia was assessed by the Sydney classification (11) as none, mild (+), moderate (++), and marked (+++). COX-2 expression was investigated by immunohistochemical staining. COX-2 immunoreactivity score was calculated as the product of staining intensity and staining area (12). The intensity of staining was graded as 0 (no staining of cells), 1 (weak staining), 2 (moderate staining), 3 (strong staining). The percentage of staining area was graded semiquantitatively as 0 (none or <5% cells), 1 (5-30% positive cells), 2 (30-60% positive cells), and 3 (≥60% positive cells) (Table 1). A score of above 1 was defined as COX-2-positive expression.

Table 1. — **Scoring for cyclooxygenase-2 immunoreactivity**

COX-2 score	Characteristics of immunohistochemical staining
Score 0	Severity: 0 or 1 Extent: 0 (<5%)
Score 1	Severity 1, Extent1 (>5%) Severity 2, Extent 0-1 (0-30%) Severity 3, Extent 0 (<5%)
Score 2	Severity. 2, Extent 2 (>30%) Severity 3, Extent 1-2 (5%-60%)
Score 3	Severity 3, Extent 3 (>60%)

Statistical Analysis

Data analyses were performed using the Predictive Analytics Software (PASW) 18.0 for Windows program. Descriptive statistics were expressed as number and percentage for categorical variables and as mean, standard deviation, median, minimum and maximum for numerical variables. Comparison of the categorical

Table 2. — **Characteristics of the patients with negative and positive cyclooxygenase-2 expression**

		COX-2 expression		
	Total N=254 (%)	**Negative N=84 (%)**	**Positive N=170 (%)**	**P**
Smoking status	134 (52.8)	43 (51.2)	91 (53.5)	0.655[a]
Atrophy-antrum				
None	100 (39.4)	39 (46.4)	61 (35.9)	**0.020[a]**
Mild	90 (35.4)	34 (40.5)	56 (32.9)	
Moderate	51 (20.1)	9 (10.7)	42 (24.7)	
Severe	13 (5.1)	2 (2.4)	11 (6.5)	
OLGA stage				
Stage 0	57 (22.4)	32 (38.1)	25 (14.7)	**<0.001[b]**
Stage 1	107 (42.1)	37 (44.0)	70 (41.2)	
Stage 2	68 (26.8)	12 (14.3)	56 (32.9)	
Stage 3	19 (7.5)	3 (3.6)	16 (9.4)	
Stage 4	3 (1.2)	0 (0)	3 (1.8)	
Dysplasia (Vienna Classification)				
No dysplasia	76 (29.9)	41 (48.8)	35 (20.6)	**<0.001[b]**
Indefinite dysplasia	116 (45.7)	33 (39.3)	83 (48.8)	
Low-grade adenoma/dysplasia	55 (21.7)	8 (9.5)	47 (27.6)	
High-grade dysplasia	7 (2.8)	2 (2.4)	5 (2.9)	
IM grade (Sydney Classification)				
None	17 (6.7)	17 (20.2)	0 (0)	**<0.001[a]**
Mild (+)	109 (42.9)	56 (66.7)	53 (31.2)	
Moderate (++)	103 (40.6)	11 (13.1)	92 (54.1)	
Marked (+++)	25 (9.8)	0 (0)	25 (14.7)	
Helicobacter pylori				
No	102 (40.2)	34 (40.5)	68 (40.0)	0.942[a]
Yes	152 (59.8)	50 (59.5)	102 (60.0)	

Data are expressed as number (%). IM, intestinal metaplasia ; COX-2, cyclooxygenase-2. [a]Chi-square ; [b]Fisher's Exact Test.

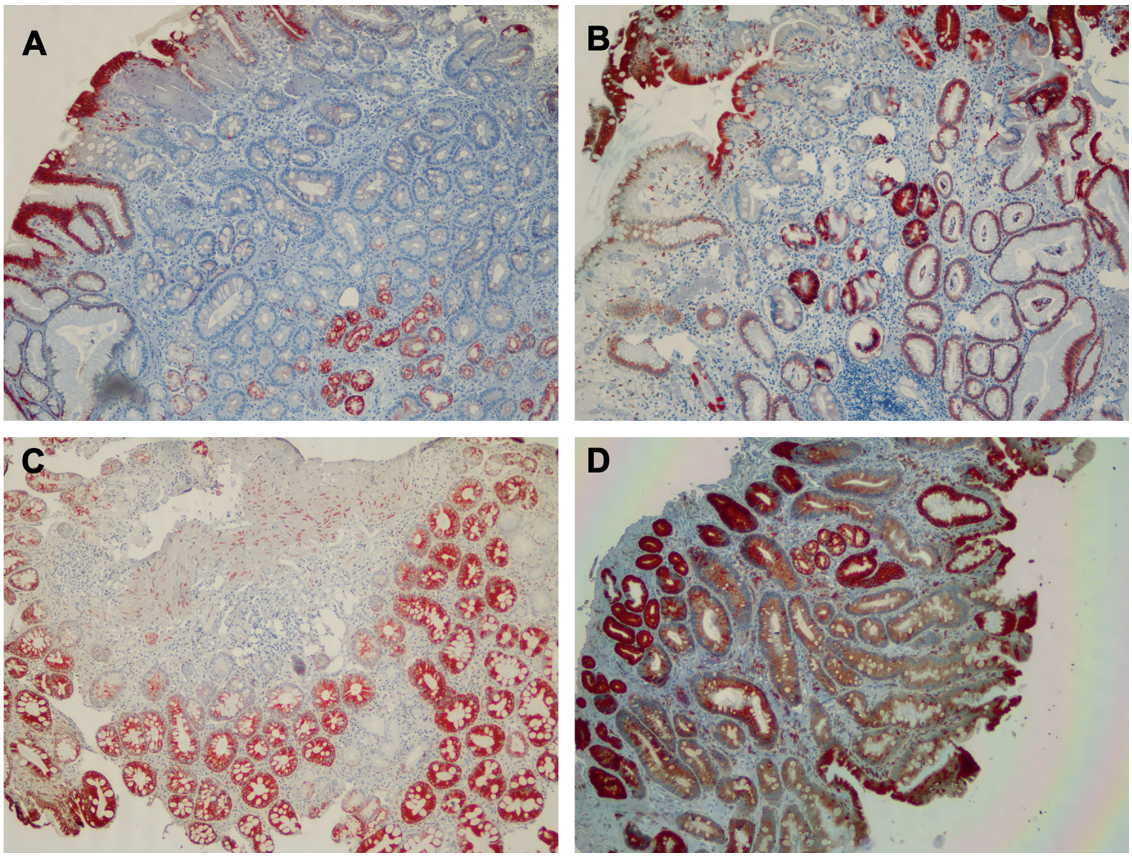

Figure 1. — Immunohistochemical staining of Cyclo-oxygenase-2 expression in the gastric mucosa with graded semiquantitatively score 0 (A), score 1 (B), score 2 (C), and score 3 (D). Magnification x100.

variables between the groups was performed by chi-square analysis and, if chi-square condition was not met, by Fisher's exact test. Two group comparisons of non-normally distributed numerical variables were performed using the Mann-Whitney U test. The level of statistical significance was accepted as $p<0.05$.

Results

The present study included 254 patients with a mean age of 55.08±12.02 years. The COX-2 expression levels were evaluated as Score 0 in 9.4% of the patients, as Score 1 in 23.6% of the patients, Score 2 in 58.3% of the patients, and Score 3 in 8.7% of the patients. Accordingly, the patients with Score 0 and 1 were evaluated as having a negative COX-2 expression, and the ones with Score 2 and 3 evaluated as a positive COX-2 expression. There were 84 (33.1%) patients with a negative COX-2 expression (mean age, 53.4 ± 12.9 years), and 170 (66.9%) with a positive COX-2 expression (mean age, 55.9 ± 11.4 years). There were no significant differences between the groups in terms of age. The characteristics of the patients with positive and negative COX-2 expression are summarized in Table 2. There were no significant differences between the two groups in terms of smoking status, and *H. pylori* positivity. The difference in atrophy, dysplasia and IM grades between COX-2 positive and COX-2 negative groups was statistically significant (respectively $p<0,001$, $p<0,001$, $p<0,001$). We found the moderate-severe atrophy, high OLGA stage in the positive COX-2 expression group higher than in the negative COX-2 expression group. In the positive COX-2 expression group, the number of patients with indefinite dysplasia, low-grade dysplasia and high-grade dysplasia was found to be higher than in the negative COX-2 expression group. At the same time, the number of patients with moderate-marked IM was higher in the COX-2 positive group (Table 2). Strong expression of COX-2 was also found on foveolar and glandular epithelium of IM (Figure 1).

H. pylori infection was positive in 59.8% of the patients. Comparison of *H. pylori*-positive and *H. pylori*-negative patients revealed that the rate of severe atrophy was higher in *H. pylori*-positive patients. There were no significant differences between the mean age of the patients with negative (mean age, 56.8 ± 12.1 years) and positive (mean age, 54.9 ± 12.0 years) *H. pylori* infection. Moreover, no significant difference was determined between the two groups in terms of smoking status, IM grade, dysplasia and COX-2 expression (Table 3).

Discussion

Gastric cancer is one of the most common malignant tumors worldwide, and more than 90% of this disease are adenocarcinomas, which are divided into intestinal and

Table 3. — **Characteristics of H. pylori-positive and H. pylori-negative patients**

		H. pylori		
	Total N=254 (%)	**Negative N=102 (%)**	**Positive N=152 (%)**	**P**
Smoking status	134 (52.8)	49 (48.0)	85 (55.9)	0.225[a]
Atrophy-antrum				
None	100 (39.4)	30 (29.4)	70 (46.1)	**0.006**[b]
Mild	90 (35.4)	48 (47.1)	42 (27.6)	
Moderate	51 (20.1)	21 (20.6)	30 (19.7)	
Severe	13 (5.1)	3 (2.9)	10 (6.6)	
OLGA stage				
Stage 0	57 (22.4)	19 (18.6)	38 (25.0)	**0.009**[a]
Stage 1	107 (42.1)	51 (50.0)	56 (36.8)	
Stage 2	68 (26.8)	30 (29.4)	38 (25.0)	
Stage 3	19 (7.5)	2 (2.0)	17 (11.2)	
Stage 4	3 (1.2)	0 (0)	3 (2.0)	
Dysplasia (Vienna Classification)				
No dysplasia	76 (29.9)	30 (29.4)	46 (30.3)	0.762[b]
Indefinite dysplasia	116 (45.7)	50 (49.0)	66 (43.4)	
Low-grade adenoma/dysplasia	55 (21.7)	20 (19.6)	35 (23.0)	
High-grade dysplasia	7 (2.8)	2 (2.0)	5 (3.3)	
IMgrade (Sydney Classification)				
None	17 (6.7)	3 (2.9)	14 (9.2)	0.268[a]
Mild (+)	109 (42.9)	46 (45.1)	63 (41.4)	
Moderate (++)	103 (40.6)	42 (41.2)	61 (40.1)	
Marked (+++)	25 (9.8)	11 (10.8)	14 (9.2)	
COX-2 expression score				
0	24 (9.4)	6 (5.9)	18 (11.8)	0.168[a]
1	60 (23.6)	28 (27.5)	32 (21.1)	
2	148 (58.3)	62 (60.8)	86 (56.6)	
3	22 (8.7)	6 (5.9)	16 (10.5)	

Data are expressed as number (%). IM, intestinal metaplasia ; COX-2, cyclooxygenase-2. [a]Chi-square ; [b]Fisher's exact test.

diffuse histological types. Intestinal-type gastric cancer is thought to result from precursor changes such as gastric atrophy, intestinal metaplasia, and dysplasia (13). Recent studies have found increased COX-2 expression in gastric cancer tissues (4). In this study, we demonstrated that COX-2 was also expressed in precursor lesions of gastric cancer such as atrophy, intestinal metaplasia, and dysplasia.

COX is a rate-limiting enzyme in the conversion of arachidonic acid to prostanoid. One of the two isoforms of it COX-2, which is involved in many pathological processes such as inflammation and carcinogenesis (14). Research has found that the expression of COX-2 increased in gastric cancer (15) and that this overexpression was associated with increased prostaglandin E2 (PGE2)biosynthesis and angiogenesis. The various effects of COX-2, such as the proliferation of gastric cancer cells, the inhibition of apoptosis, the contribution to angiogenesis and lymphatic metastasis, and the participation in cancer invasion and immunosuppression may cause the development and progression of gastric cancer, which are facilitated by PGE_2 (16). The correlation of COX-2 with the density of CD34-positive microvascular endothelial cells may show the association between COX-2 overexpression and angiogenesis in gastric cancer (15). Sun et al. (13) reported that COX-2 expression had an increasing tendency from superficial gastritis to atrophy, metaplasia, dysplasia and finally gastric cancer. In the same study, the level of COX-2 expression in IM and dysplasia was significantly higher in *H pylori*-positive than in *H pylori*-negative subjects. Similar outcomes have been reported also in the study by Yu et al. In patients operated due to gastric cancer, the COX-2 expression was higher in the gastric cancer tissues than in the paracancerous tissues. Moreover, COX-2 expression of intestinal metaplasia or dysplasia with positive *H pylori* was significantly higher than intestinal metaplasia or dysplasia with negative *H pylori*. Furthermore, they found significantly elevated COX-2 expression in the areas with metaplasia and dysplasia compared with the paracancerous tissue (17). Erkan et al. (18) detected COX-2 expression in 23.1% of the cases with normal gastric mucosa; this rate was found as 70.6% in the patients with chronic active gastritis and as 90.5% in the patients with IM. In addition, COX-2 expression was observed to be higher in *H. pylori*-positive group than in *H. pylori*-negative group. *H. pylori* has been shown to play a role in the pathogenesis of mucosal associated lymphoid tissue (MALT) lymphoma by triggering COX-2 overexpression (19).

In the present study, *H. pylori* positivity was detected in 59.8% of the patients and COX-2 expression positivity was detected in 66.9% of the patients. While in those positive for *H. pylori*, COX-2 expression was higher, the difference between *H. pylori*-positive and *H. pylori*-negative groups was not statistically significant. McCarthy et al. (20) showed that COX-2 expression in antral mucosa was reduced, but not eliminated in the epithelium after successful eradication of *H pylori*. Kimura et al (21) reported that immunoreactivity of COX-2 was observed in all cases of IM even after the cure of *H pylori* infection. For this reason, the cure of *H pylori* infection might decrease the risk of gastric carcinogenesis owing to COX-2-related compounds in gastric mucosa, yet this is not the case for those with IM. According to the researches, it is a fact that *H. pylori* infection causes inflammation, and that COX-2 is involved in inflammatory responses (13). However, in *H. pylori* associated gastritis, COX-2 protein mainly localizes to the lamina propria with variable levels in the epithelium (22,23); but in gastric cancer, COX-2 is most strongly expressed in the epithelium of malignant and dysplastic glands (22,14). Joseph et al also found that in the eradication of *H. pylori,* COX-2 expression in the gastric epithelium showed a modest decrease, whereas it has decreased markedly in the lamina propria. This fact revealed that COX-2 expression did not only result from inflammation, but that other factors in stomach, even after a successful eradication of *H. pylori,* might lead to the perpetuation of COX-2 expression. All in all, the eradication of *H. pylori* alone, may not be sufficient to reverse the gastric carcinogenesis process (22). In this study, in terms of COX-2 expression, the reason of the difference between *H. pylori*-positive and *H. pylori*-negative groups not being statistically significant is that the COX-2 expression continues in patients having received successful treatment for *H. pylori*. Another reason is that the disassociation in the COX-2 expression of the epithelium and lamina propria implies that COX-2 expression does not solely result from an inflammation, but because other gastric factors also contribute to the continuation of COX-2. In addition, a study by Zhao et al. demonstrated that COX-2 was expressed in almost all gastric cancers infected with *H. pylori*, but it was also expressed in <2/3 of gastric cancers without *H. pylori* infection. Besides, COX-2 was positively expressed in <1/6 of control subjects regardless of *H. pylori* infection (24).

Researches show that chronic atrophic gastritis induced by *H. pylori* leads to the synthesis of growth factors and cytokines, and also to hypergastrinemia. These trigger COX-2 overexpression, which induces angiogenesis and inhibits apoptosis. By doing so, it causes gastric precancerous lesions, such as atrophic gastritis, IM and dysplasia; and finally they results in gastric cancer. All these findings suggest that COX-2 plays a role in early gastric carcinogenesis (25,26). Apart from its effect through COX-2, gastrin, stimulated by chronic atrophic gastritis associated with *H. Pylori*, may trigger directly gastric precancerous lesions and finally may lead to gastric cancer.

In this study, in patients with a moderate-marked metaplasia, or with moderate-severe atrophy, a higher OLGA stage, or with dysplasia, the COX-2 expression was found to be higher than those with mild lesions**.**

A study showed that there is a correlation between the aggravation of gastric precancerous lesions and the severity of COX-2 expression and that COX-2 expression increases in the early stage of gastric carcinogenesis (2). Joseph et al. have shown that COX-2 is expressed in the epithelial lining of the stomach in all the stages of *H. pylori*-associated gastric carcinogenesis pathways, such as chronic active gastritis, gastric atrophy and IM, and finally in gastric cancer, which is suggestive that COX-2 might be involved in the early stages of gastric cancer development (22).

The overexpression of COX-2 protein is related to tumor size and advanced clinical stage, intestinal histological subtype, proximal location, depth of invasion and metastasis and lymph node involvement in gastric cancer, but not to the clinicopathological characteristics of gastric cancer patients (27,28). Even though the results are controversial between COX-2 and survival rate, most of the studies support the fact that COX-2 expression is a prognostic factor for gastric cancer. That is, in a study it has been found that there is no correlation between COX-2 expression and survival rate, and that COX-2 has little sense in predicting the prognosis of gastric cancer (29). In contrast, in another study it was found that the 5-year survival rate in COX-2 positive patients was lower than those of the negatively expressed patients, and this reveals that COX-2 is an independent prognostic factor for gastric cancer (30). In other studies, it has been found that the COX-2 overexpression in patients with gastric cancer leads to poor prognosis and is an independent prognostic factor for poor survival due to angiogenesis, cancer invasion and metastasis. In early-stage gastric cancer patients with high expression of COX-2, cancer-related death risk has been found to be higher than those with a low level of COX-2 expression (31). It has been found that COX-2 positive expression in gastric cancer tissues at the developing stage was significantly higher than that at early stage, the positive rate in gastric cancer with lymph node metastasis was significantly higher than that without lymph node metastasis (17).

Zhang et al. found a relationship between the level of COX-2 expression and risk of gastric lesions; and in patients with strong COX-2 expression, indefinite dysplasia and dysplasia were higher than those with mild lesions (12).

In this study, we found a relation between the level of COX-2 expression and the grade of premalignant gastric lesions. These results provided evidence that COX-2 might contribute to an early event in gastric carcinogenesis. Further large-scale studies with long-term follow-up periods would facilitate better understanding

of the role of COX-2 in cancer risk prediction, as well as the role of COX-2 inhibitors in cancer treatment.

Conflicting Interest

There is no conflict of interest of the author with the results of this study.

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