

Expression of Cox 2 in Gastric Mucosa of Patients with Gastric Carcinoma and Its Correlation with the Disease Progression

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Abstract: Gastric cancer is the second most common cause of cancer-related death in the world. It exists as two main histological types, diffuse and intestinal, and is thought to result from a combination of environmental factors and accumulation of specific genetic alterations, and consequently mainly affects older patients. Esophagogastro-duodenoscopy (EGD) is the diagnostic imaging procedure of choice in the work-up of gastric carcinoma. The two isoforms COX-1 and COX-2 regulate a key step in prostanooid (i.e. thromboxanes and PGs) synthesis. PGs regulate various pathophysiological processes such as inflammatory reaction, gastro-intestinal cytoprotection and ulceration, hemostasis and thrombosis, as well as renal haemodynamics. The present study was conducted on eighty (80) patients attending the out-patient clinics of El-Hussein and El- Sayed Galal University Hospitals during the period (from November 2010 to July 2013). According to the histopathological diagnosis, these cases were subdivided into 6 groups after doing the following (1-Medical history 2-Clinical evaluation 3-Laboratory investigations including (C.B.C, Liver function test, gastric biopsy, histological examination and immunohistochemical examination. The study revealed COX2 was not detected in normal cases and mean of cox2 compared with different gastric lesions showed that gradually increased from more pathological stage gastritis→metaplasia→dysplasia→adenoma→ and gastric carcinoma. There was a highly significant value of malignant group when compared to control group, gastritis group, metaplasia group and dysplasia group at $p < 0.01$. Conclusion: The study revealed that up-regulation of cox2 from normal gastric mucosa to different gastric lesion suggests that cox2 expression correlated with disease progression.

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1. Introduction

Gastric cancer is the second most common cause of cancer-related death in the world. It exists as two main histological types, diffuse and intestinal, and is thought to result from a combination of environmental factors and accumulation of specific genetic alterations, and consequently mainly affects older patients (Chen, *et al.*, 2013).

The steady decline in the incidence and mortality of stomach cancer in most affluent countries has been attributed to changes in dietary pattern, food storage, and control of *H. pylori* infection. The incidence of gastric cancer varies in different parts of the world with highest incidence rates documented in Eastern Asia, Eastern Europe, and South America, while North America and Africa show the lowest recorded rates (Schildberg *et al.*, 2013).

The pathogenesis of gastric cancer involves multiple risk factors including dietary, infectious, occupational, genetic and preneoplastic risk factors, most of which act on the gastric mucosal

microenvironment over a prolonged time period. The diagnosis of gastric cancer is often delayed by the lack of early symptoms, with early gastric cancer causing non-specific gastrointestinal complaints, such as dyspepsia, in only 50% of patients. Up to 90% of Western gastric cancer patients first present with advanced carcinomas, which have more serious symptoms such as abdominal pain, bleeding, vomiting, or severe weight loss. Endoscopic screening is considered to be the most sensitive and specific diagnostic test for gastric cancer Mrena *et al* (2005).

IHC is also widely used in basic research to understand the distribution and localization of biomarkers and differentially expressed proteins in different parts of a biological tissue. IHC assists the pathologists in areas of tumor classification, multilineage differentiation, molecular correlates, and infectious etiologies. Moreover, IHC is commonly used to detect markers, which in turn can provide information on the biological behaviour and prognosis of a tumor (Tseng, 2011).

Aim of Work:

This study aimed to evaluate expression of cox2 in patient with gastric carcinoma and its relation with the histopathological activity, laboratory and clinical parameters, to study its correlation with the disease progression and to study the role of H pylori in cox2 expression in different gastric lesions.

2. Patients and Methods:

The present study was conducted on eighty (80) patients attending the out-patient clinics of El-Hussein and El- Sayed Galal University Hospitals during the period (from November 2010 to July 2013). The selected patients were (48) males and (32) females, their ages ranged from (20-67years) with a mean of 43.5±4.5 years, as well as (10) subjects (normal individuals) serving as a control group. They were (4) males and (6) females, their ages ranged from (26-53 years) with a mean of 34±3.5 years. According to the histopathological diagnosis, these cases were subdivided into 6 groups:

- **Group I: 10 cases as normal control.**
- **Group II: 20 cases as chronic gastritis.**
- **Group III: 10 cases as metaplasia.**
- **Group IV: 10 cases as dysplasia.**
- **Group V: 10 cases as adenoma.**
- **Group VI: 20 cases as adenocarcinoma.**

The patients were included according to the following criteria:

Inclusion criteria:

- persistent upper gastrointestinal dyspepsia for more than 3 months
- Patients with *H pylori*.

Exclusion criteria:

- Patients receiving non-steroidal antiinflammatory drugs.

- Who had taken antibiotics, bismuth or proton pump inhibitors within the past 6 weeks had undergone previous gastric surgery.

- With predominant symptoms of irritable bowel syndrome.

- Children and old age more than 70 years.

- Patients with chronic liver diseases and having esophageal varices.

- Patients refused to be including in our study.

All the studied subjects were subjected to:

1- Complete history taking and clinical assessment.

2- Laboratory evaluation including:

Complete blood picture, Serum bilirubin (conjugated & un conjugated), aminotransferases (ALT&AST) and alkaline phosphatase, Serum albumin, Prothrombin time, Hepatitis markers and Renal function tests:

3- Abdominal ultrasonography.**4- Upper gastrointestinal endoscopy:**

Was performed using an Olympus XQ40 endoscope. Thorough endoscopic examination of the oesophagus, stomach and duodenum was performed, abnormalities were recorded and gastric biopsies were obtained from the apparent mucosal lesions.

(A) Histopathological Examination:

The fixed biopsy specimen was processed in ascending grades of ethyl alcohol, xylene and wax at 60°C. Paraffin sections 4µm thick were prepared on 3 aminopropyltriethoxy saline coated slides. Sections were then stained with hematoxylin and eosin for histological evaluation of the severity of gastritis and with Giemsa stain for *H. pylori* detection.

(B):Immunohistochemical staining**3.Results****Table (1): Results of cox2 expression in the studied groups**

Cox2	Normal		Mild		Moderate		Marked	
	N.	%	N.	%	N.	%	N.	%
Control(10)	10	100	0	0	0	0	0	0
Gastritis(20)	6	3	14	70	0	0	0	0
Metaplasia(10)	0	0	4	40	6	60	0	0
Dysplasia(10)	0	0	3	28.5	7	71.4	0	0
Adenoma(10)	0	0	2	20	6	60	2	20
Malignant(20)	0	0	6	30	6	30	8	40

This table show that normal gastric mucosa show no cox2 expression, while most cases in gastritis group 14/20 showed mild expression, in metaplasia group most cases 6/10 showed moderate expression while the

others show mild expression, in dysplasia group most cases 7/10 showed moderate expression, in adenoma group 2/10 mild expression while 6/10 moderate expression and 2/10 marked expression, the last group

malignant showed 6/20 mild expression while 6/20 moderate expression and 8/20 marked expression.

Table (2): correlation of h pylori and cox2 expression in the studied groups

Hp by immunohistochemistry		Cox ₂				Total
		Normal	Mild	Moderate	Marked	
Negative	number	15	18	19	9	61
	%	18.7%	24%	25.3%	11.2%	76.2%
Positive	number	3	9	4	3	19
	%	4%	12%	5%	4%	23.8%

*p=454 compared to negative group (N.S.)

the number of cases associated with h pylori showed non sig. expression of cox2 compared to cases without *H pylori* stained with IHC at (p= 454).

Table (3): Comparison of endoscopic findings among the studied groups:

Endoscopic findings	Group I (n=10)		Group II (n=20)		Group III (n=10)		Group IV (n=10)		Group V (n=10)		Group VI (n=20)		Total	
	No	%	No	%	no	%	no	%	no	%	No	%	No	%
Gastritis	0	0%	5	25%	2	20%	1	10%	7	70%	14	70%	29	36.2%
Gastric Erosion	0	0%	2	10%	3	30%	1	10%	6	60%	12	60%	24	30%
Benign ulcers	0	0%	0	0%	1	10%	3	30%	4	40%	8	40%	16	20%
Malignant Tumor (ulcers)	0	0%	0	0%	0	60%	0	50%	0	90%	16	80%	16	20%
Polyp	0	0%	0	0%	2	20%	2	20%	6	60%	13	65%	23	28.7%

As regard endoscopic findings, It revealed presence of gastritis in 29 patients, gastric erosion in 24 patients, benign ulcers in 16 patients, malignant ulcers in 16 patients and polyps in 23 patients.

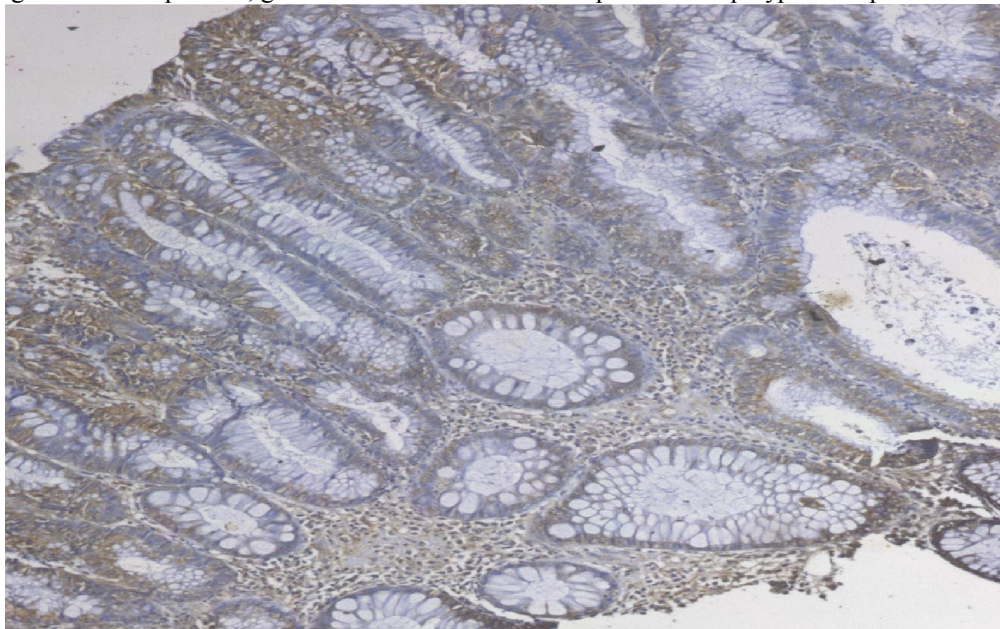


Figure 1: Chronic gastritis with moderate dysplasia showing moderate cytoplasmic Cox2 positivity in the cells lining the gastric glands. (Immunohistochemistry, DAB, x200).

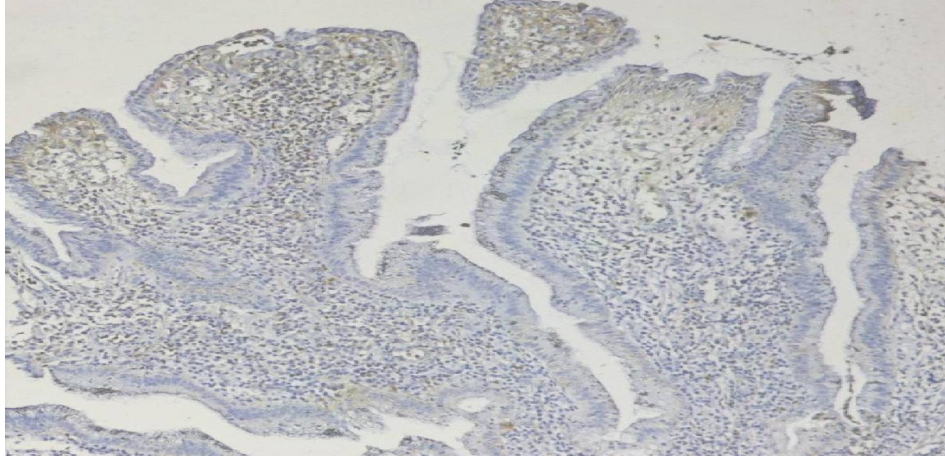


Figure 2: Tubulovillous adenoma without dysplasia showing moderate cytoplasmic Cox2 positivity in the cells lining the gastric glands and crypts. (Immunohistochemistry, DAB, x200).

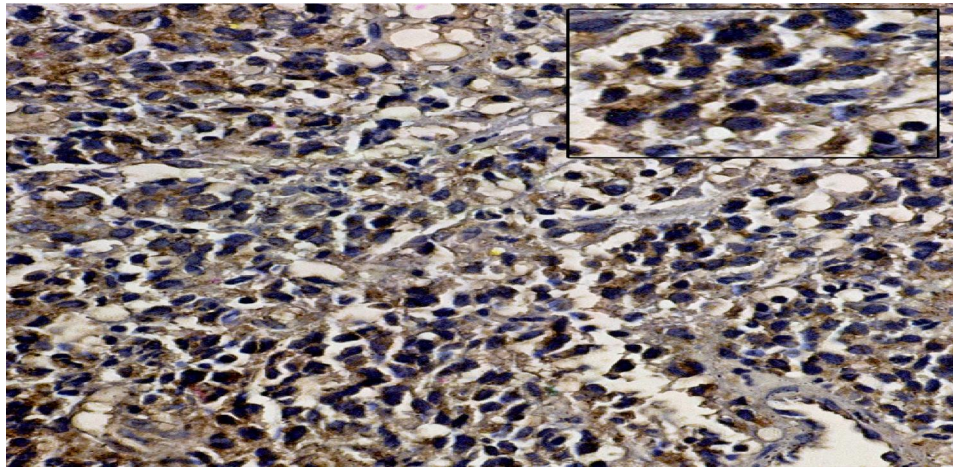


Figure 3: Moderately differentiated gastric adenocarcinoma showing marked positive cytoplasmic immunoreactivity for Cox2 within the malignant glands (Immunohistochemistry, DAB, x400).

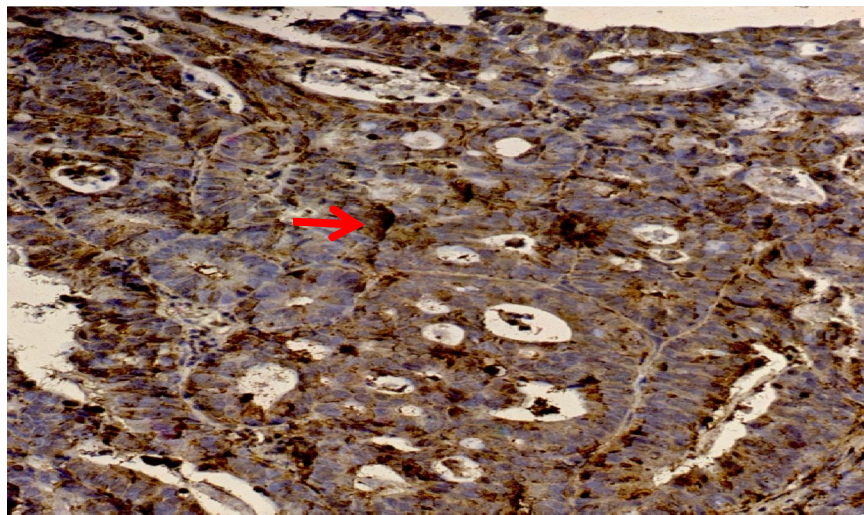


Figure 4: well differentiated gastric adenocarcinoma showing moderate positive cytoplasmic immunoreactivity for Cox2 within the malignant glands (arrow) (Immunohistochemistry, DAB, x400).

4. Discussion

Cyclooxygenase (COX) is one of the rate-limiting enzymes in metabolism of arachidonic acid that catalyzes the arachidonic acid into a series of products, such as prostaglandins and other eicosanoids. It has two isoforms in human, constitutive COX-1 and inducible COX-2. COX-2 maps to 1q25.2-q25.3, contains 11 exons and 10 introns, is 8.3 kb in size (Tseng, 2011). COX-1 is now known to be present in most tissues as the housekeeper enzyme, to maintain the normal physiological function. It maintains normal gastric mucosa and influences kidney function. COX-2 is considered “the immediate early gene”, it is composed when the cell is stimulated and it takes part in many pathophysiologic processes, such as carcinogenesis and inflammation (Mohammed *et al.*, 2004). The regulation of COX-2 expression is mainly on the level of transcription, in the other words, the signal transduction pathway leading to COX-2 protein expression is initiated when the cell is stimulated. It has been documented that COX-2 plays an important role in the development of human tumor (Singh *et al.*, 2005). The high expression of COX-2 is the early process of carcinogenesis in general (Yazawa *et al.*, 2005).

COX-2 is undetectable in most normal tissues (except for the central nervous system, kidneys, and seminal vesicles), but is induced by various inflammatory and mitogenic stimuli. Growth factors (EGF, PDGF), pro-inflammatory cytokines (IL1, IL2, TNF), tumor promoters, bile acids and UVB irradiation are all stimulators of COX-2 expression (Dempke *et al.*, 2001). Although the mechanism of COX-2 up-regulation is not fully understood, it could result from activation of Ras and the MAPK (mitogen-activated protein kinase) pathway (Shin *et al.*, 2012). It has been demonstrated that Protein kinase B (Akt/PKB) activity is implicated in K-Ras-induced expression of COX-2, and the stabilization of COX-2 mRNA partly depends on the activation of Akt/PKB (Sheng *et al.*, 2001). These pathways lead to the activation of regulatory factors that eventually bind the promoter region of the COX-2 gene. This area contains several transcription factor-response elements, including the NF-KB and AP1 response elements (Rodrigues *et al.*, 2004). The regulation of COX-2 expression is also ensured by the presence of multiple repeated sequences in the COX-2 mRNA that are responsible for its rapid degradation (Dannenberg *et al.*, 2005).

COX enzyme, is almost undetectable in the intact gastric mucosa (Schildberg *et al.*, 2013). In gastric cancer, several studies have shown enhanced expression of COX-2 in tumor tissues as compared with normal tissues, thus suggesting that COX-2 may

play an important role in gastric carcinogenesis (Shi *et al.*, 2003, Sun *et al.*, 2004, Tatsuguchi *et al.*, 2004).

In our study, 100% of patients of gastric carcinoma had positive COX-2 staining by IHC. ranging from mild 6(30%) to moderate 6(30%) and marked expression 8(40%).

In our study showed that COX-2 protein expressed at a high level in tumor tissues, which was significantly higher than that in precarcinoma stages ($P < 0.01$), normal gastric mucosa show no COX-2 expression, while most cases in gastritis group 14/20 showed mild expression, in metaplasia group most cases 5/10 showed moderate expression while the others show mild expression, in dysplasia group most cases 5/10 showed moderate expression, in adenoma group 2/10 mild expression while 6/10 moderate expression and 2/10 marked expression

Furthermore, our study demonstrated that overexpression of COX-2 is consistently observed in precancerous lesions such as gastritis, metaplastic and adenomatous cells as well as in cancer cells of the stomach. Overexpression of COX-2 observed in metaplastic and adenomatous cells and not in normal mucosa in our study suggests that COX-2 may contribute to an early event in the gastric tumor formation.

In addition, COX-2 was consistently up-regulated in Barrett's metaplastic tissues, a highly premalignant condition of the esophagus (Wilson *et al.*, 1998). The above results suggest that overexpression of COX-2 constitutes an early event in the gastrointestinal neoplastic transformation process. Ristima *et al.*, (2000) demonstrated that overexpression of COX-2 is one of the properties shared by gastric carcinoma of both intestinal and diffuse types, thus suggesting that COX-2 is connected to the early stages of carcinogenesis. also suggest that COX-2 overexpression plays an important role in the initiation of gastric carcinogenesis (Chen, *et al.*, 2013). These findings suggest the possibility that the use of selective COX-2 inhibitors may provide a chemopreventive strategy against gastric carcinogenesis.

So we conclude from that malignant cells may secrete chemical mediators that could induce COX-2 expression in stromal cells in a paracrine fashion. Stromal cells with positive COX-2 expression may be essential for survival of malignant cells and progression of tumor. This finding was similar to Tatsuguchi *et al.* (2004), Sun *et al.* (2004)

There still remains the question of what could trigger the induction of COX-2 activity in gastric cancer. An association between *H. pylori* infection and gastric cancer has been clearly demonstrated, resulting in the classification of *H. pylori* as a class I carcinogen for gastric cancer. However, the exact role of *H. pylori*

in the development of gastric cancer is not fully understood. It has been reported that *H.-pylori* directly induces COX-2 expression in a gastric cancer cell line (Masumeh *et al.*,2012).

Chen *et al.*(2009) reported a positive correlation between COX-2 expression and *H.-pylori* infection in patients with gastric cancer. also Kawabe, (2007) found that COX-2 expression in tissues with *H. pylori* positive intestinal metastasis or dysplasia was significantly higher than that in tissues with *H pylori* negative infection. *H pylori* could induce acute and chronic inflammation of gastric mucosa, and the production of cell factors such as IL-8 and IL-1 and the secondary high COX-2 expression which caused gastric mucosal lesions. *H pylori* infection could also induce gastric mucosal cell proliferation by COX-2 expression. COX- 2 gene expression was one of the related factors mediating the progress from gastritis with *H pylori* infection to pre-carcinoma lesions even gastric carcinoma.

However, Dannenberg *et al* (2005) found that COX-2 expression was not correlated with *H. pylori* infection in gastric cancer. Another study (Konturek *et al.*, 2000) suggested that *H.-pylori* infection may contribute to the development of gastric cancer via COX-2, which may account for the stimulation of tumor growth and angiogenesis.

In the light of these findings, we also investigated the effect of *H.pylori* infection on COX-2 expression levels. Similar to Dannenberg *et al* (2005). study, we found no association between COX-2 expression and *H.-pylori* infection. Therefore, we presume that *H.-pylori* may have only a minor role in COX-2 expression in patients with gastric cancer. However, this study includes a fairly small number of patients, and this may have an impact on the conclusions that we were able to draw. Further studies investigating higher number of patients can clarify these issues better.

In regards to age and sex of patients We compared the clinicopathologic features of patients with cox2 –positive and negative primary tumors. Although there p was no relationship between cox2 expression and age, sex, and other clinical and laboratory. which was in agreement with Sun *et al* (2005) did not emphasize a significant correlation between the COX-2 expression and the age, sex and tumor localization, the histological type and the tumor differentiation type.

However in opposite of our result Mrena *et al.*(2005) Found that male gender one of clinicopathological factors associated with increase of cox2 expressions

Conclusion

Overexpression of COX-2 is consistently observed in precancerous lesions such as gastritis,

metaplastic and adenomatous cells as well as in cancer cells of the stomach. But not observed in normal gastric mucosa suggests that COX-2 may contribute to an early event in the gastric tumor formation. *H.-pylori* may have no or only a minor role in COX-2 expression in patients with gastric cancer, that may be due to this study includes fairly small number of patients, and this may have an impact on the conclusions that we were able to draw. so Further studies investigating higher number of patients can clarify these issues better.

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