Immunohistochemical assessment of Cox-2, and ki-67 expression in gastric cancer

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Abstract:

Cell proliferation characteristics may reflect the aggressiveness of gastric cancer and their eventual prognosis. The aim of this study was to evaluate whether the proliferative activities determined by Ki-67 labeling index (LI) could be used as a predictor in patients with gastric cancer, and examine the relation between COX-2 and Ki-67 expression and gastric cancer. The expression of COX-2 and Ki-67 were detected in 40 patients with gastric cancer and 30 healthy individuals were assessed by immunohistochemistry. The biopsies were fixed in 10% buffered formaldehyde solution, embedded in paraffin and stained immunohistochemically with anti-human COX-2 and Ki-67 antibodies. Expression of Ki-67 andCOX-2was significantly higher in tumor compared with control samples.A significant correlation was found between COX-2 and Ki-67 protein expression. In conclusion,the expression of COX-2 and Ki-67 proteins was closely connected with gastric cancer.

Key word: COX-2, Ki-67, gastric cancer

Introduction:

Gastric cancer is one of the most common malignant gastrointestinal tumors; the incidence and mortality of gastric cancer show an upward tendency (1).The interaction of both environmental and gastric factors contributes to the etiology and pathogenesis of these aggressive cancers; mainly smoking, alcohol consumption, besides dietary habits and bacterial infection by Helicobacter pylori (2). The cyclooxygenase (COX) is a key enzyme in prostaglandin synthesis and two forms, COX-1 and COX-2 are involved in the process (3). COX-1 is a constitutive isoform expressed in various tissues and COX-2 is an inducible enzyme (4). Various epidemiological and experimental studies have suggested that non-steroidal anti-inflammatory drugs (NSAIDs) decrease the incidence of gastrointestinal carcinomas, especially colonic carcinomas (5). NSAIDs are responsible to blockage of prostaglandin production through the inhibition of COX. Prostaglandins have been incriminated to participate in resistance to apoptosis process (6, 7).

Cyclooxygenase is induced in numerous processes such as cellular growth, differentiation, inflammation and tumorigenesis (8). Overexpression of COX-2 proteins has been detected in carcinomas of the stomach, esophagus, breast, pancreas lung and endometrium (9). Moreover, it was reported that it's overexpression was associated with poor prognosis and reduced survival (10).

The mechanism by which COX-2 contributes to the carcinogenesis is not known until present. It seems that COX-2 enzyme stimulates cell proliferation inhibitsapoptosis and increases malignant cells (11).Regarding tumor cell proliferation, it is widely accepted that proliferative capacity may influence the clinical course, and hence patients prognosis (12).

A nuclear antigen (Ki-67) is expressed in all stages of the cell cycle except G0 and early G and is often used to indicate the proliferative activity of tumor (13). Ki-67 (also known MK167) is expressed in the nuclei of cells in G1, S, G2 and mitosis phases of cell cycle and associated with ribosomal RNA transcription (14). The aim of this study was to evaluate the expression of COX-2 and Ki-67 in gastric cancer and to examine the correlation between them.

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Material and Methods:

Patients: This study included 75 subjects from Baghdad Teaching Hospital, AL-Yarmook Teaching Hospital and Gastroenterology and Hepatology Teaching Hospital. This study was carried out on (40) patients with gastric cancer

(GC) (22 males and 18 females) with a mean of age 51.7 range between 20 and 81 years. The control group included (35) gastric normal tissue (GN) (20 males and 15 females) with a mean of age 49.2 and range between 20 and 75 years. For each patients and control included in this study; serial sections from paraffin embedded block were taken from the archive of department of pathology of these hospitals (mention above). Tissue sections cut into 4μm thickness, put on Fisherbrand positively charched slides.

Immunohistochemical analysis (IHC) for detection of COX-2and Ki-67 proteins expression in paraffin embedded sections:

UniversalDakoCytomation streptavidin- biotin system was purchased from DakoCytomation (USA) Immunohistochemistry detection kit. Mucosal biopsies were immunostained with polyclonal antibodies to COX-2 (polyclonal, J1602, 1:50dilution; Santa Cruz Biotechnology, Inc.) and Ki-67. Anti-mouse immunoglobulin G (Sigma, St. Louis, MO, U.S.A.)

The primary antibody reacts with antigen in the tissue, and then a biotin labeled secondary antibody (link antibody) binds to the primary antibody. When the conjugate is added, the biotinylated secondary anti-body will form a complex with the peroxidase-conjugated streptavidin and by adding the substrate, which contains 3,3□-diaminobenzidine (DAB) in a chromogen solution, a brown-colored precipitate will form at the antigen site. In the peroxidase secondary detection system, the presence of a brown reaction product at the site of the target antigen is indicative of positive reactivity. Counter stain will be pale to dark blue coloration of the cell.

Evaluation of the immunostaining was done with the assistance of a histopathologist. The observer was blinded to the clinical diagnosis of the tissues at the time of assessment, and tissues were independently assessed by two observers positive or negative cases, positive immunostaining gave nuclear and/or cytoplasmic dark brown granules. Counting the number of positive cells which gave brown cytoplasmic staining system under light microscope. The extent of the IHC signal was determined in 10 fields (X100magnification). In each field the total number of cells was counted and the extent of cytoplasmic staining cells was determined as a percent. The total staining score was divided by the number of whole cells per field in 10 fields, so the percentage of positively stained cells in the 10 fields was calculated for each case by taking the mean of the percentage of the positively stained cell in the 10 fields. COX-2 immunoreactivitywas assessed as being positive

Specimens in which less than 4% of the cancer cells were immunostained with COX-2 were classified as negative, and the rest were classified as positive (1). But cancer were regarded as Ki-67 positive when there immunoreactivity scores were ≥ 10 (12).

Statistical analysis: Student test (t-test) was used for the quantitative data. The relationship between the markers was measured qualitatively by using the correlation coefficient(r).

Results:

The COX-2 in gastric cancer patients and control group were measured by IHC. The mean percentage of COX-2 protein increase significantly ($p < 0.01$) in gastric cancer patients than control group (Table -1).

Table (1): Mean percentage of the expression of COX-2 among studied group.

Studied group	No.	Mean ± SE	P value
Control	35	9.4 ± 1.4	P< 0.001*
Gastric Cancer patients	40	70.3 ± 3.2	

*Highly significant differences.
Highly significant differences between Ki-67 expression in gastric cancer patients and control group ($p < 0.01$) (Table – 2).

Table (2): Mean percentage of the expression of Ki -67 among studied group.

Studied group	No.	Mean ± SE	P value
Control	35	25.2 ± 1.8	P< 0.001*
Gastric Cancer patients	40	63.3 ± 7.5	

*Highly significant differences.
Positive expression of COX-2 and Ki-67 immunoreaction has been observed in gastric carcinoma (31%) and (23%) respectively (Table – 3).

Table (3): Immunostaining of COX-2 and Ki-67 protein in patients with gastric cancer.
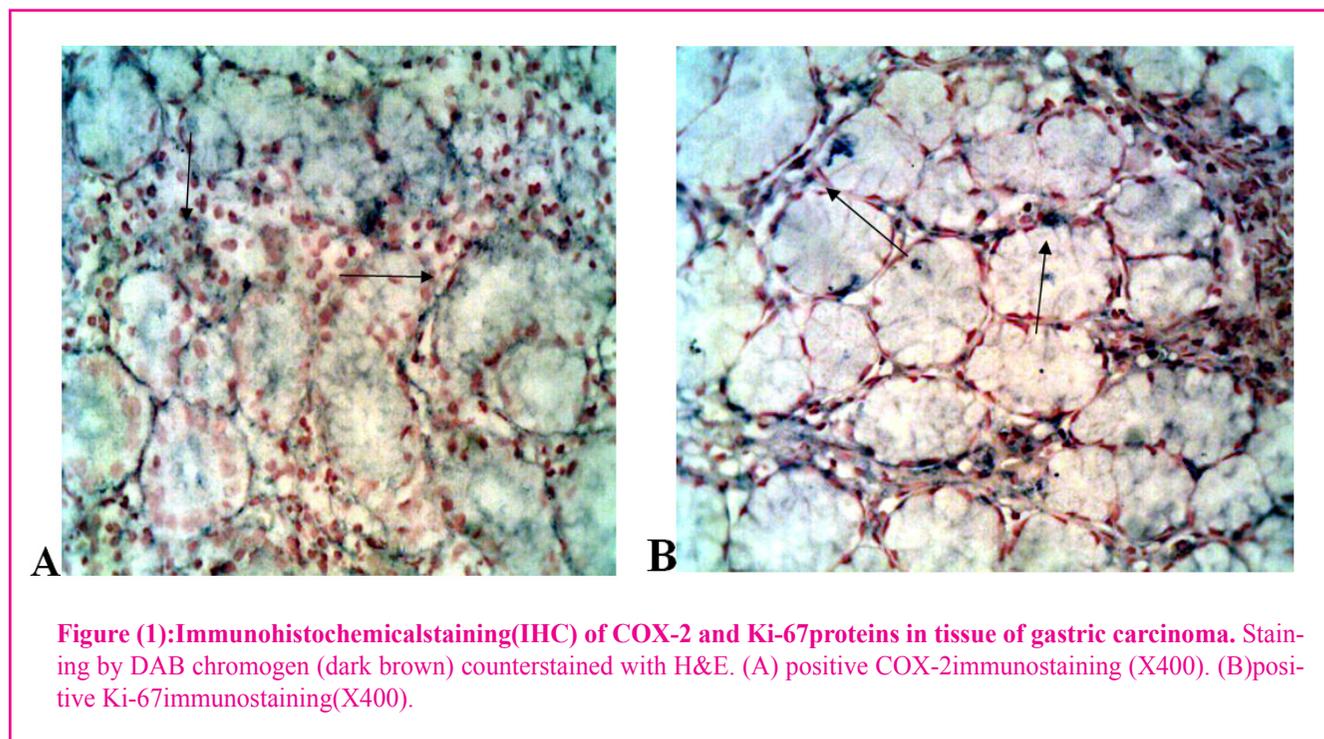
Marker	Marker expression in patients with gastric cancer	
	Negative (No %)	Positive (No %)
COX - 2	14	31
Ki - 67	17	23

The correlation of COX-2 and Ki –67 protein in gastric carcinoma is revealed in table- 4. There was a significant correlation between COX-2 and Ki –67 in gastric cancer patients (p < 0.05).

Table (4): Correlation (r) between COX-2 and Ki-67 in studied group.

Variable	Studied group	Correlation coefficient	P value
COX- 2 and Ki- 67	Control	0.260	<0.05
	Gastric cancer patients	0.163	< 0.05

Positive expression of COX-2 and Ki-67 protein was showed asdark brown staining in the tissue of gastric cancer shown in figure -1.Staining by DAB chromagen (dark brown) counterstain with H &E.

Figure (1):Immunohistochemicalstaining(IHC) of COX-2 and Ki-67proteins in tissue of gastric carcinoma. Staining by DAB chromogen (dark brown) counterstained with H&E. (A) positive COX-2immunostaining (X400). (B)positive Ki-67immunostaining(X400).

Discussion:

In this study, we investigated the relationship between gastric cancer and COX-2 which is considered as a potential target in cancer prevention. Studies carried out suggest that COX-2 is important in term of carcinogenesis in gastrointes-tinal cancers (14). According to our results, the mean COX-2value was significantly higher (P < 0.01) in gastric cancer patients than control group. This finding concordant with the results obtained by Lazar, et. al. 2008.

Most of antineoplastic effects of aspirin and other non-steroidal anti-inflammatory drugs have been linked to their

ability to suppress prostaglandin synthesis through inhibition of the activity of the inducible isoform COX-2 (15, 16).

Over production of COX-2 and prostaglandin has been found to accompany the development and progression of various human cancers including gastric cancer (17).

The mechanism by which COX-2is up regulated in cancer is not clear. It has been suggested that cancer cell may become intrinsically more in expressing COX-2 than normal cells (18).

Ki-67 is recognized as a nuclear antigen presented in proliferated cells but absent in resting quiescent cell. The Ki-67 LI determined by immunohistochemistry, is well known proliferation marker and has been extensively used to estimate the growth fraction of tumor (19, 20).

In addition,demonstrated that COX-2expression in gastric cancer tissues was positively correlated with cell proliferative activity analyzed by Ki-67.This result was agreement with the result obtained by Yamagishiet al. (2004) and also, Sawaoket al. (1994) reported that both selective an non- selective COX-2 inhibitor exerted minimal effects on cell proliferation of human gastric cancer cell line, which expressed lower levels of COX-2, but suppressed cell proliferation of human gastric cancer cell lines that overexpressed COX-2 (17).The result suggests that COX-2 expression associated with modulation of cellular proliferation and transformation during the evaluation of H. pylori associated gastritis to gastric cancer (23, 24) and imply that COX-2 play a critical role in tumor cell proliferation of gastric cancer.

In conclusion, with this study we showed that COX-2 and Ki-67 protein was increased in cancerous tissue. COX-2 and Ki-67 immunoreaction have turned positive more frequently in gastric carcinoma. A significant correlation in the immunohistochemical expression of COX-2 and Ki-67 in gastric carcinoma (P< 0.05).

References:

1. Moa, X.; Wang, X,; LV, X.; Xy, I. and BOham, C. (2007): COX-2 expression in gastric cancer and its relationship with angiogenesisused tissue microarray , World journal Gastroenterology. 13 (25):3466-3471.

2. Abbas, N. F.; EL-sharkawy, S. L.; Fadel, M. T.; AL- Shaer, M. T.; Abd El- Megid, M. B.; and Abd El-Aal, w.s.(2010): Combinedexpression ofand P53 in human gastric carcinoma.Reporte and Opinion. 2(11).

3. Dubois, R. N,;Abromson, S. B.; lipsky, B. E.; (1998): Cyclooygenase in biology and Disease. FASEB J 12: 1037 – 1063.

4. Uefuji, K.;IchiKura, T and Mochizuki, H. (2005): Increased expression of IL-1 alpha and COX-2 in human gastric cancer. Apossible role in tumor progression. Anticancer research 25:3225- 3230.

5. Honjo, S,;Kase, S.; Osaki, M.; Ardyanto, D.; Kiabara, N.andIlo, H. (2004): Cox-2 expression in human gastric tubular adenomas and carcinomas, correlation with Intratumoralmicrovessel density and apoptotic index, Anticancer research; 24: 1439- 1444.

6. Demeke, W.; Grothery, A. and SchMoll, H. (2001): Cyclooygenese – 2: A noval target for cancer chemotherapy J cancer Res. Clin. Oncol. 127:411-417.

7. Rodrigues, L.; Cogliati, B.; Guerra, J.; Dagli, M. and Lucas, S. (2011): An immunohistochemical study of Cyclooxygenese – 2 expressions in canine multicentral lymphoma. AV.VET (Murcia); 27:43-49.

8. Simon, L. (1999):Role and regulation of Cyclooxygenese – 2 during inflammation, Am. J. Mol.; 106: 537-542.

9. Samak, M.; Asmaa, A.; Abd-el- Wawed, M.; Kandil, A. and El-Kady.N. (2006): Cyclooxygenese – 2 expressions in chronic gastritis and gastric carcinoma, correlation with prognostic parameters. J. Egy.Nat, cancer Inst.; 18 (4): 363-374.

10. Sinicrop, F. and Sharelene, G. (2004): role of Cyclooxygenese – 2 in colorectal cancer. Cancer And Meta.; 23: 63-75.

11. Lzar, D,;Taban, S.; Ardele, C.; Simio, C.; Sporea, I.; Corntanu, M, and Vernic, T. (2008): immunohistochemical expression of the Cyclooxygenese – 2 in gastric cancer. The correlations of the tumor angiogenesis and patients survival. 4913: 371-79.

12. Joo, Y.; Chung, J.; Park, Y.; Koh, S.; Lee, N.; Kim, S.; Choi, K.; Rew, S. and Kim, J. (2006): expression of the Cyclooxygenese – 2, P53 and Ki67 in gastric carcinoma. J. Korean Med. Sci.; 21: 871-876.

13. Weidner, N.; Moore, D. and Vartanian, R. (1994): Correlation of Ki-67 antigen expression with mitotic figure and tumor grade in breast carcinomas using the noval paraffin – reactive M1B1 antibody. Hum. Pathol.; 25(4): 337 – 342.

14. Beekdemir, H.; Tekin, S.; Bilic, M.; CayIR, K. and Gundogdu, C. Cyclooxygenese– 2expression in gastric cancer. Int. J. Hemat.; 1(20): 34-41.

15. Danneberg, A.; Altorki, N.; Boyle, J.; Dang, C.; Howe, L. Weksler, B. andSubbaramaiah, K. (2001): Cyclooxygenese – 2: a pathological target for prevention of cancer. Lancet Oncol.; 2: 544-551.

16. Thun, M. (2000): NSAID use and decreased risk of gastrointestinal cancer. Gastrointest. Clin. North Am.; 25:333-348.

17. Joo, Y.; Kapp, Y.; Mino, S.; Park, C.; Park, S.; Choi, S.; Rew, J. and Kim, S (2006): expression of Cyclooxygenese – 2 protein in colorectal carcinoma. J. Gastrointest. Cancer. 31: 147-154.

18. Hazar, B.; Ergin, M.; Seyrek, E.; Erdogan, S.; Tuner, I. and Hakverdi, S. (2004): Cyclooxygenese – 2 expressions in lymphomas. Leuk. Lymphoma. 35: 1395- 1399.

19. William, C. and Dubois, R. (1996): Prostaglandin endoperoxidasesynthtase: Why two isoform. Am. J. Physiol.; 270: 393-400.

20. Tsujii, M.; Kawano, S. and Dubois, R. (1997): Cyclooxy-genese – 2 expression in human colon cancer cells increases metastatic potential. Proc. Nat. Acad. Sci.; 94: 3336-3340.

21. Cianchi, F.; Cortesini, C.; Bedi, P.; Fantappie, O.; Messerini, L.; Vannacci, A.; Sardi, I.; Baroni, G. and Boddi, V. (2001): Up regulation of Cyclooxygenese – 2 gene expression correlates with tumor angiogenesis in human colorectal cancer. Gastroeterology. 121: 1339-1347.

22. Yamagishi, M.; Noda, M.; Tatsumi, Y.; Mukaisho, K.; Sugihara, H.; Unanous, T. and Itattori, T. (2004): Correlation between Cyclooxygenese – 2, proliferative activity and mucin phenotype in human advanced gastric cancer, J. Gastroenterol.; 39:1143-1149.

23. Sawaoka, H.; Kawano, S.; Tsuji, S.; Murata, H. Hori, M. (1994): Effects of NSAIDs on proliferation of gastric cancer cells in vitro: Apossible implication of Cyclooxygenese – 2 in cancer development. J.Clin. Gastroenterol.; 27 (suppl 1): 47-52.

التقييم المناعي الكيميائي النسيجي لتعبير Ki-67 و Cox–2 في سرطان المعدة

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الخلاصه:

خصائص تكاثر الخلايا ربما تعكس خطورة اورام المعدة . الهدف من هذه الدراسة هو تقييم فيما اذا كانت الانشطة التكاثرية التي تحدد بواسطة Ki- 67 ربما تستخدم كمؤشر في المرضى الذين يعانون من سرطان المعدة وتحديد العلاقة بين Ki-67 و Cox–2 في هؤلاء المرضى. تم الكشف عن تعبير Ki-67 و Cox–2 في 40 مريض يعانون من سرطان المعدة و 35 شخص سليم بواسطة تقنية التحليل المناعي الكيميائي. ثبتت الخزعة النسيجية في 10 % من محلول الفورمالين وطمرت بالبرافين وصبغت بواسطة التحليل المناعي الكيميائي مع مضادات وحيدة النسيلة من Ki-67 و Cox–2. ظهر تعبير Ki-67 و Cox–2 اعلى في الورم مقارنة بمجموعة السيطرة ووجد هناك ارتباط بينهما. الاستنتاج: تعبير بروتين Ki-67, Cox–2 يرتبط بصورة قوية بسرطان المعدة.