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 and COX-2 was 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 isof orm 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 inhibits apoptosis 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.

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 charged slides.

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

Universal DakoCytomation streptavidin- biotin system was purchased from DakoCytomation (USA) Immunohistochemistry detection kit. Mucosal biopsies were immunostained with polyclonal antibodies to COX-2 (polyclonal, J1602, 1:50 dilution; 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 (X100 magnification). 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 immunoreactivity was 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 was 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.

<table>
<thead>
<tr>
<th>Studied group</th>
<th>No.</th>
<th>Mean ± SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>35</td>
<td>9.4 ± 1.4</td>
<td>P&lt; 0.001*</td>
</tr>
<tr>
<td>Gastric Cancer patients</td>
<td>40</td>
<td>70.3 ± 3.2</td>
<td></td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>Studied group</th>
<th>No.</th>
<th>Mean ± SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>35</td>
<td>25.2 ± 1.8</td>
<td>P&lt; 0.001*</td>
</tr>
<tr>
<td>Gastric Cancer patients</td>
<td>40</td>
<td>63.3 ± 7.5</td>
<td></td>
</tr>
</tbody>
</table>

*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>
<thead>
<tr>
<th>Marker</th>
<th>Marker expression in patients with gastric cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative (No %)</td>
</tr>
<tr>
<td>COX-2</td>
<td>14</td>
</tr>
<tr>
<td>Ki-67</td>
<td>17</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Studied group</th>
<th>Correlation coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX-2 and Ki-67</td>
<td>Control</td>
<td>0.260</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Gastric cancer patients</td>
<td>0.163</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

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

Figure (1): Immunohistochemical staining (IHC) of COX-2 and Ki-67 proteins in tissue of gastric carcinoma. Staining by DAB chromagen (dark brown) counterstained with H&E. (A) positive COX-2 immunostaining (X400). (B) positive Ki-67 immunostaining (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 gastrointestinal cancers (14). According to our results, the mean COX-2 value 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-2 is 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-2 expression 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:


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

الخلاصة:

خصصات تكاثر الخلايا ربما تعكس خطورة أورام المعدة. الهدف من هذه الدراسة هو تقييم فيما إذا كانت الإشارة التكاثرية التي تحدد بواسطة Cox-2 و Ki-67 في هؤلاء المرضى. تم التكشف عن تعبير Cox-2 و Ki-67 في سرطان المعدة وتحديد العلاقة بين Cox-2 و Ki-67 و Ki-67 على الورم. وشملت البيانات بتحليل المناعي الكيميائي مع مضادات وحيدة النسيلة من Cox-2 و Ki-67. مقارنة بمجموعة السيطرة وجدت هناك علاقة بينهما. الاستنتاج: تعبير Cox-2 و Ki-67 يرتبط بصورة قوية بسرطان المعدة.