

An investigation of the rate of cyclooxygenase-2 expression on the surface of adenomatous and colorectal adenocarcinoma polyps

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Abstract

Background: Colorectal cancer (CRC) (adenomatous, adenocarcinoma) is one of the major causes of mortality and morbidity in human societies. Considering the importance of cyclooxygenase-2 (COX-2) expression in the incidence of CRC, in this study, the rate of COX-2 gene expression on polyps and CRCs were addressed.

Materials and Methods: This is a cross-sectional descriptive analytic study carried out on the blocks of sampled tissue of adenomatous and colorectal adenocarcinoma polyps on 68 patients referred to Digestive Clinic in Isfahan Shariati Hospital in 2013. Patients were divided into two groups of polyps ($n = 52$) and cancer ($n = 16$). Given the presence of CRC or polyps by colonoscopy, samples were sent to the laboratory to measure the rate of COX-2 gene expression using immunohistochemistry.

Results: In polyp group, 41 individuals (78.8%) had two or <2 polyps, 24 cases (46.2%) had a tubular polyp, and about a third of all patients had a big polyp. The most frequency of the polyp site was related to sigmoid with 19 cases (36.54%), in cancer group, it was related to the rectum with 9 cases (56.25%) that there was no significant difference between two groups ($P < 0.05$). The overall prevalence of COX-2 expression was positive in 51 cases (75%) and negative in 17 cases (25%). COX-2 gene expression was separately observed in 38 individuals (73.10%) in the polyp group and in 13 cases (81.25%) in the cancer group, and no significant difference was found ($P > 0.05$).

Conclusion: There is no relationship between COX-2 gene expression and the surface of adenomatous and colorectal adenocarcinoma polyps.

Key Words: Adenomatous colorectal cancer, colorectal adenocarcinoma polyps, cyclooxygenase-2 expression

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Received: 05.03.2014, **Accepted:** 06.06.2015

INTRODUCTION

Colorectal cancer (CRC) is the fourth prevalent cancer in the world with approximately 783,000 new cases

per year.^[1] It is considered the third fatal cancerous disease in the world^[2] and constitutes 9.4% and 10.1% of all cancers in men and women respectively throughout the world.^[1,2]

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| Quick Response Code: | Website: www.advbiores.net |
|  | DOI: 10.4103/2277-9175.166136 |

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How to cite this article: Baghaei R, Beiraghdar M, Sobhani A, Rafei R, Kolahi L, Lotfolah F. An investigation of the rate of cyclooxygenase-2 expression on the surface of adenomatous and colorectal adenocarcinoma polyps. Adv Biomed Res 2015;4:200.

During the last 60 years, there have been a lot of controversies over numerous variables related to patients' survival. The extent of the tumor invasion into the bowl wall, metastasis to the adjacent lymph nodes, and metastasis of the tumor into other organs are variables known as factors influencing patients' survival.^[3]

During the last decades, the rate of mortality of this disease has been decreased or at least has not been increased in western countries. However, there has been a relative increase in the disease mortality and affliction in Iran. According to the report of the records of cancerous cases in 2006, CRC with 7.5% of recorded cancerous cases is the fourth prevalent cancer in Iran. The rate of affliction is equal among men and women up to 50 years old, but its outbreak increases afterward in men. Basically, it is a disease of elders.^[4]

Different studies show that cyclooxygenases (COX) increase different human cancers such as prostate, colon, and breast cancers.^[5]

In general, COX have an important role in all the stages of malignant tumor genesis including the increase of cell proliferation, apoptosis decrease, angiogenesis, and cancerous cells' mobility.^[6] These enzymes catalyze the formation of preglanidins, provestacyclenes, and thromboxanes in three isomeric forms of COX-1, COX-2, and COX-3.

COX-2 is an induced enzyme and increases dramatically in situations such as inflammation, stress, cancer, metastasis, and in case of cytokines. That is, why COX-2 gene expression is called instant response gene.^[7,8] This gene emerges in 40% of colon adenomas and 90% of colorectal adenocarcinomas.

COX-2 is always expressed in brain, epithelial cells of the trachea, kidney and Macula Densa. According to recent studies, COX enzyme has a short half-life; therefore, it mainly adjusts at the surface and transcription. Unlike COX-1, COX-2 expression is reduced by pro-inflammatory cytokines and growth factors.^[9]

The overexpression of COX-2 cause to increase the production of antigenic growth factors.

It also causes to produce the antigenic growth factors in colon cancer and may increase the production of vascular growth factors and the migration of endothelial cells through collagen matrix and formation of capillary-like networks in the laboratory.^[10-13]

According to some reports, the overexpression of COX-2 has been observed in 70–90% of patients with CRC. In a large study on 232 patients with CRC, the

rate of COX-2 expression was increased in 72% of the patients.^[14]

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin and ibuprofen are attached to the hydrophobic channel of COX enzyme and cause to inhibit it. Aspirin is the only COX inhibitor by which the covalent bond (acetylation) is established. Acetylation of the amino acid serine 530 inhibits binding arachidonic acid to the active site and, as a result, the irreversible inhibition of the enzyme.

Aspirin, indomethacin and ibuprofen act as nonselective inhibitors of COX. Population studies show that individuals regularly taking aspirin or other NSAID suffer from CRC 40% to 50% less than others. Primary attempts for understanding the molecular basis of these observations indicated that the rate of COX-2 in colorectal tumors is high, whereas its rate in the normal mucosa is minimal.^[15]

Given the importance of COX-2 in the expression of Adenomatous and colorectal adenocarcinoma polyps which causes to increase the growth of this tumor in the colon and given that studies on this issue are negligible all over the world and especially in Iran, and so far, no study has been conducted on the relationship between COX-2, cancers and colorectal adenomatous polyps, it is necessary to conduct a study in this field. Another significance of our study is to show that if COX-2 expression on adenomatous and colorectal adenocarcinoma polyps is high, perhaps by more prospective studies on taking aspirin for reducing these tumors, it can be proved that according to the high rate of the gene expression in adenomatous and colorectal adenocarcinoma polyps in patients, without measuring COX-2, taking aspirin can be started.

MATERIALS AND METHODS

This is a cross-sectional descriptive-analytic study done on the blocks of sampled tissue of adenomatous and colorectal adenocarcinoma polyps on 68 patients suffered from this disease referred to Digestive Clinic in Isfahan Shariati hospital in 2013.

Through colonoscopy and sampling and then staining in the pathology laboratory, for 68 (39 female and 29 male) patients, adenomatous and colorectal adenocarcinoma polyps were diagnosed in 2013. These cases were randomly selected among from 1000 patients who had the indication of colonoscopy and had referred to the clinic in 2013. Then, they were called to the clinic and made satisfied about using the tissue blocks of their colorectal mass that have previously been put under the diagnosis of adenomatous and colorectal adenocarcinoma.

In this study, sampling method was as a convenient sampling in which available samples successively referring to the hospital and their race were Iranian and refugees living in Iran have been excluded and also having the inclusion criteria were selected and they entered into the study. Similarly, selecting samples was successively done to reach the required sample size.

Using the formula of estimating the maximum sample size was calculated to compare proportions and considering the significance level of 95% and the power test of 80%.

Written consent was obtained from the patients who had a tendency to participate in the study. For obtaining desired information, the prepared questionnaire was completed via interview and based on the patient's statements.

People whose colonoscopy results were adenoma or adenocarcinoma and consented to participate in the study were included and provided that they did not want to participate in the study, were excluded.

Given the presence of CRC or polyps (adenomatous type), samples were sent to the laboratory to measure the rate of COX-2 gene expression using immunohistochemistry (IHC) (chemically staining tissue).

In this method, tissue taken from adenomatous polyps and colorectal adenocarcinoma tumor was first entered in 10% formalin to fix and alcohol interred into tissue cells by Autotechnicon. Next, xylenol was replaced by alcohol and then, paraffin was finally replaced by xylenol. This process took 14 h. At the next stage, by microtome, sections of 3–5 micron were prepared, and these sections inserted into fore and in 60°C, paraffin into cells melts. Now, the prepared slides have been specially stained for the diagnosis of adenomatous and adenocarcinoma polyps. After confirming the diagnosis of adenoma or adenocarcinoma, the separated slides were again provided and at this stage, by antibodies against the COX-2 enzyme, the presence or absence of the enzyme was detected. Staining on tissue was performed through IHC method by marker COX-2 as follows:

First, the slides were put in tumor at 74°C for 50 min to embed paraffin, and then they were put in two containers with xylenol (5 min), absolute alcohol (5 min), and 96% alcohol (2 min), respectively. For antigen retrieval, the slides were put on citrate buffer with pH = 6 in boiling water bath for 1 h. Then, the slides were put in phosphate buffer and

then in 3% hydrogen peroxide for 10 min and were re-washed with PBS (Phosphate Buffered Saline). Primary antibodies were poured on slides for 60 min and the slides were placed in PBS and then put in Envision Dual Link System Peroxidase for 1 h. The slides were washed again in phosphate buffer and put in DAB (3, 3'- Diaminobenzidine) slides for 3–5 min (Corrosion) and then, washed by distilled water. Finally, slides were put into hematoxylin and dewatering process (taking on alcohol) was performed. By putting coverslip and pasting slides by special IHC, staining was performed.

After editing and eliminating the defects, the collected data were analyzed by SPSS for Windows, version 20.0 (SPSS, Chicago, IL). The quantitative data have been reported as a mean \pm standard deviation and the qualitative data as number (percent). In order to analyze data, Fisher's exact test, Chi-square and *t*-tests were used. In all comparisons, the significant level was considered <0.05 .

RESULTS

The present study was performed on two groups of polyps ($n = 52$) and a group of cancer ($n = 16$) that among from them, 39 patients (57.4%) were female and 29 cases (42.6%) were male. Furthermore, the mean age of individuals is generally 56.5 ± 13.4 , 56.7 ± 13.6 for the polyp group and 55.75 ± 12.7 for cancer group that using independent sample *t*-test, no significant difference was found between two groups in terms of age and thus, both groups are similar in terms of age ($P > 0.05$) [Table 1].

Table 1: Frequency distribution and descriptive statistics of features in two studied groups

| Variable | Groups (%) | | P |
|----------------------|-----------------|------------------|-------|
| | Polyp (n=52) | Cancer (n=16) | |
| Age | 56.7 \pm 13.6 | 55.75 \pm 12.7 | 0.796 |
| Number of the polyps | | | |
| ≤ 2 | 41 (78.8) | - | - |
| > 2 | 11 (21.2) | - | - |
| Size of the polyps | | | |
| Small (< 1 cm) | 34 (65.4) | - | - |
| Great (≥ 1 cm) | 18 (34.6) | - | - |
| Type of the polyps | | | |
| Tubular | 24 (2/43) | - | - |
| Tubulovillous | 16 (30.8) | - | - |
| Villous | 12 (23.1) | - | - |
| Polyp or cancer site | | | |
| Sigmoid | 19 (36.54) | 6 (37.5) | 0.033 |
| Rectum | 11 (21.15) | 9 (56.25) | |
| Transverse colon | 8 (15.39) | 1 (6.25) | |
| Cecum | 8 (15.39) | 0 (0) | |
| Ascending colon | 6 (11.53) | 0 (0) | |
| Decreasing colon | 0 (0) | 0 (0) | |

In polyp group, 41 individuals (78.8%) had 2 or <2 polyps, 24 cases (46.2%) had a tubular polyp and about a third of all patients had a big polyp. The most frequency of the polyp site was related to sigmoid with the frequency of 19 cases (36.54%) and in contrast, in cancer group, the most frequency of the cancer site was related to the rectum with 9 cases (56.25%) that according to Chi-square test, cancer and polyp sites had no significant difference between two groups ($P < 0.05$) [Table 1].

In all the samples, the overall prevalence of COX-2 expression was positive in 51 cases (75%) and negative in 17 cases (25%). Also, COX-2 gene expression was separately observed in 38 individuals (73.10%) in the polyp group and in 13 cases (81.25%) in cancer group that are using Fisher's exact test, no significant difference was found between two groups ($P > 0.05$); on the other hand, COX-2 gene expression had no relationship with the level of adenomatous and colorectal adenocarcinoma polyps [Table 2 and Figure 1].

Finally, investigating the relationship between COX-2 gene expression and factors such as age, sex and mass site in all sample presented in the study, revealed that none of these factors affected COX-2 expression and, therefore, no significant correlation was found between them ($P > 0.05$) [Table 3].

DISCUSSION

It seems that COX enzyme-2 plays an important role in the induction of CRCs in a way that the correlation between COX-2 expression and tumor growth has become a controversial issue. Also, it has been expressed that inhibiting this enzyme causes to decrease the malignancy risk the gastrointestinal tract. On the other, there is evidence that in patients with metastatic CRC, in addition to improving the

quality of life, the inhibitor of COX enzyme increases the rate of survival too.^[16]

Given that CRC (adenomatous, adenocarcinoma) is one of the major causes of mortality and morbidity in human societies and is the cause of death in the United States of America and in Iran and considering the importance of COX-2 expression in the incidence of CRC and that studies conducted in Iran is negligible in this field, in the present study, the rate of COX-2 gene expression on polyps and CRC were addressed.

The study results indicated that among from 68 studied individuals, COX-2 gene was observed in 51 patients (75%) that 38 cases (73.10%) were in the polyp group and 13 cases (81.25%) were in cancer group that no statistically significant difference was found between two groups ($P > 0.05$). Also, no significant correlation was observed between COX-2 expression and factors of age, sex and tumor site.

The study results were consistent with the study conducted by Yamac *et al.* in 2005; in investigating the importance of prognostic COX-2 expression (using IHC method) on 83 patients with CRC; they found that there was no relationship between gene expression and size, histopathologic differentiation, site and the tumor vascular invasion.^[17]

Also, in the study by Lim *et al.* in 2008, no significant association was observed between COX-2 expression in patient's survival and CRC prognosis.^[18]

Table 2: Comparing the frequency distribution of COX-2 gene expression in two studied groups

| Groups | Positive (%) | Negative (%) | P |
|--------|--------------|--------------|-------|
| Polyp | 38 (73.10) | 14 (26.90) | 0.509 |
| Cancer | 13 (81.25) | 3 (18.75) | |

COX-2: Cyclooxygenase-2

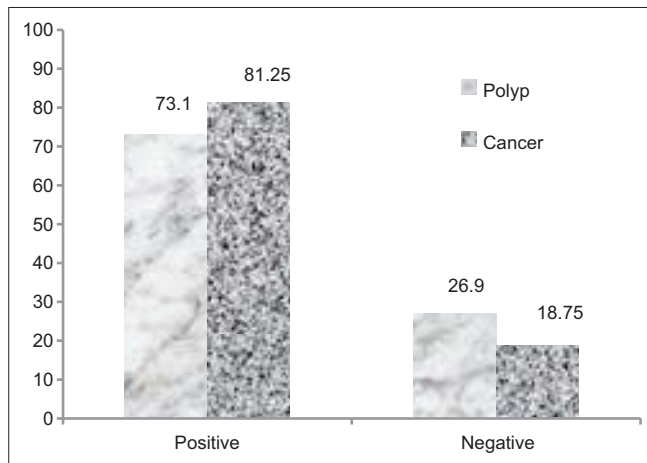


Figure 1: Bar chart of the frequency percentage of cyclooxygenase-2 gene expression in two studied groups

Table 3: The comparative study of factors affecting COX-2 gene expression in general

| Factors | COX-2 (%) | | P |
|----------------------|-----------------|-----------------|-------|
| | Positive (n=51) | Negative (n=17) | |
| Age (year) | 56.47±13.4 | 56.6±13.7 | 0.960 |
| Sex | | | |
| Male | 22 (43.1) | 7 (41.2) | 0.887 |
| Female | 29 (56.9) | 10 (58.8) | |
| Polyp or cancer site | | | |
| Sigmoid | 22 (41.2) | 4 (23.5) | 0.069 |
| Rectum | 17 (33.3) | 3 (17.6) | |
| Transverse colon | 6 (11.8) | 3 (17.6) | |
| Cecum | 3 (5.9) | 5 (29.4) | |
| Ascending colon | 4 (7.8) | 2 (11.8) | |
| Decreasing colon | 0 (0) | 0 (0) | |

COX-2: Cyclooxygenase-2

Results from the study by Zhan *et al.* in 2004 revealed that although COX-2 protein cannot be considered as a marker for early detection of developed CRC, it can be considered as a prognostic independent risk factor for the patient in postoperative advanced CRC.^[19]

In their study in 2011, Yoshinaga *et al.* reported that both PPAR and COX-2 genes expression in tissues may lead to liver metastasis and as a result, poor prognosis in patients with CRC.^[20]

In 2012, in spite of the results from the present study, in examining both COX-2 and E-cadherin proteins in the primary colorectal adenocarcinoma and relationship with clinicopathological features, Miladi-Abdennadher *et al.*, concluded that there is a statistical significant relationship between this enzymes expression and age at the time of vascular diagnosis and invasion.^[21]

The different results emerged from various studies indicate that the present studies are not sufficient in order to confirm the effect of COX-2 expression on the CRC and also, it seems that more prospective studies are required to assess fully this gene.

CONCLUSION

As the general conclusion of this study, it can be said that there is no relationship between COX-2 gene expression and the surface of adenomatous and colorectal adenocarcinoma polyps.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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