Influence of age and gender on association between -765G > C COX-2 genetic polymorphism and gastric adenocarcinoma risk: a case-control study in Iran

Masumeh Rostami¹, Mehdi Kadivar², Mozaffar Aznab³, Mina Abachi³
¹ Science and Research Branch, Islamic Azad University, Tehran, Iran
² Biochemistry Department, Pasteur Institute of Iran, Tehran, Iran
³ Emam Reza Hospital, Kermanshah, Iran

ABSTRACT

Aim: The purpose of this study was to investigate the possible influence of age and gender on association between -765G > C COX-2 genetic polymorphism and gastric adenocarcinoma risk in Iranian patients.

Background: The promoter polymorphism of COX-2 gene -765G>C has been described to play an important role in many cancers such as gastric cancer.

Patients and methods: We carried out single-nucleotide polymorphism analysis in Iranian samples including 91 patients and 91 control normal using PCR-RFLP technique.

Results: Statistical analysis revealed no significant association between GG, GC and CC genotypes and risk of gastric adenocarcinoma. However differences were considered significant (P=0.043) for female subjects with C carrier genotypes (GC and CC) and gastric adenocarcinoma when compared with male patients (P=0.645) and control groups (P=0.653). Also, there was a statistically significant difference between increasing of age and susceptibility for gastric adenocarcinoma (Odd Ratio = 1.125, 95% CI=1.089-1.162).

Conclusion: These results suggested that Iranian C carrier females can be more susceptible for gastric adenocarcinoma in comparison with control group. Also increasing of age should be considered as a risk factor for this disease.

Keywords: Gastric adenocarcinoma, COX-2 Polymorphism, PCR-RFLP.

Introduction

Gastric cancer (GC) is identified to be the second widespread neoplasm around the world (1). In spite of advances in surgical treatment and chemotherapy, gastric cancer remains a main overall health burden. A variety of etiologic factors have been related with this disease (2). Environmental factors such as infection and diet have been considered to play critical roles in GC. Although the genetic supposed to encompass an impressive capacity in GC development, this aspect has not been proved comprehensively (3). Moreover, there is an association between infection of H pylori and the possibility of GC occurrence (4, 5) In addition; several studies have demonstrated smoking can be one of the causes of GC (6, 7). Cyclooxygenase (COX) known as prostaglandin endoperoxide H
synthase (PGHS) and is a rate-limiting enzyme in prostaglandin synthesis. It converts free arachidonic acid into important prostanoids (PGs) and eicosanoids such as prostaglandin H2 (8). Three different isoforms of human COX, COX-1, COX-2 and COX-3 with independent genes and different expression pattern have been reported (9). Normally, COX-2 is undetectable in most of tissues but it can be activated by several inducers like cytokines, growth factor, tumor promoters and lipopolysacharides (LPS). Some studies reported its stimulation by cigarette smoking leading to carcinogenesis (10, 11). It is also accounted for inflammatory responses and tumor progression (12). Over expression of COX-2 is considered to be an indispensable part of tumor development, angiogenesis, metastasis and inhibition of apoptosis (13). Additionally, the expression of COX-2 is controlled by convoluted signaling pathways such as β-catenin signal transduction (14). The involvement of important nuclear proteins like NF-kB, CRE, PEA3 and NF-IL-6 in interacting with the COX-2 promoter region is essential for regulating the expression of the gene (15). Elevated level of COX-2 has been related to development of many human cancers such as colorectal and breast (16-18) and in particular gastric cancer (19, 20). The vulnerability to the progression of human cancers like prostate and breast can be as a consequence of genetics variations such as single nucleotide polymorphisms (SNP) (21, 22). Regarding the COX-2 gene, only a few polymorphisms observed in its promoter region appeared to have an efficient impact on the gene transcription (23). Nevertheless, the difference in mRNA expression level of the gene under the existence of carcinogens among individuals is due to different patterns of SNPs in the operative regions of the gene (24, 25). An acknowledged polymorphism in the promoter region of COX-2 gene, featured by a guanine (G) to cytosine (C) transition at position 765 (-765G > C) performs disruptions in a positive transcription activator, stimulatory protein 1 (24). Unfortunately, limited studies have been performed to date to scrutinize COX-2 polymorphisms in different forms of cancer and various diseases (26-29). It has been demonstrated that in Iranian population, gastric cancer incidence is high and the rate of this disease have increased about two fold when compared with the data of 40 years ago (30). This study was performed to investigate the influence of age and gender on association between -765G > C COX-2 genetic polymorphism and the development of gastric adenocarcinoma among Iranian samples.

**Patients and Methods**

A simple random sampling was used for used for collecting samples. This study was executed between two groups of healthy people with no clinical presentation of cancer including 91 subjects and individuals with evident gastric adenocarcinomas (n=91).

Five ml of peripheral blood was collected after obtaining written informed consent from patients. Blood samples were stored in a -20º C freezer. Using a standard salting-out protocol genomic DNA was isolated from white blood cells (26). Optical density (OD) and concentration was measured using nanophotometer at 260 and 280 nm.

The promoter region of COX-2 was amplified by polymerase chain reaction (PCR). A 157 bp DNA segment was amplified using the following primers: forward 5`-ATTCTGGCCTGCAGCGCTTC-3` and reverse 5`-CTCCCTTGTTCTTGGAAGAGAGC-3`. PCR reactions were carried out using 10 µl of Prime Taq Premix (2X) (GENET BIO, Korea), 0.7 µl of each forward and reverse primers, 0.5 µl genomic DNA and 8.1 µl dH2O in a total volume of 20 µl under following thermal conditions: 94º C for 5 min intended for the initial denaturation, 94º C (1 min) for denaturation, 59º C (1 min) for annealing and 72º C (1 min) for extension repeating 35 cycles (Master cycler gradient Eppendorf, USA). 9 µl PCR reaction products were subjected to 0.7 µl Bsh1236I restriction endonuclease (Fermentas, Vilnius,
Table 1. Mean ± standard deviation age of case and control groups

|                    | Gastric Adenocarcinoma (n=91) | Control (n=91) | Odd Ratio (95% CI) | P-value |
|--------------------|-------------------------------|----------------|-------------------|---------|
|                    | Female           | Male           | Total             | Female | Male | Total |                     |                     |
| Age                | 58.5±14.5        | 61.9±15.5      | 60.5±15.1         | 35.8±12.8 | 36.4±12.3 | 36.1±12.5 | 1.25(1.089-1.162) | <0.001 |

Table 2. Frequency of 765G>C polymorphism genotypes among different countries

| Population       | case/control | GG (%)  | GC (%)  | CC (%)  | C-carrier |
|------------------|--------------|---------|---------|---------|-----------|
| Portugal         | 73/210       | 49/62   | 44/32   | 7/6     | 51/38     |
| Netherland       | 96/100       | 76/59   | 20/32   | 4/9     | 24/41     |
| India            | 62/241       | 22.6/77 | 46.8/25.7 | 30.6/3.3 | 77.44/29 |
| Iran (present study) | 91/91 | 27.5/34 | 61.5/68.1 | 4.4/4.4 | 65.9/72.5 |

In this study, Logistic regression and $\chi^2$-test analysis was applied to analysis of data. Overall, 91 gastric adenocarcinoma patients (44 female and 47 male, mean age: 60.5±15.1) and 91 control normal (47 female and 44 male, mean age: 36.1±12.5) were enrolled. (Table1).

Results

After PCR-RFLP analysis of -765G > C COX-2 polymorphism, the wild-type homozygous -765GG was cut into fragments of 134 bp and 23 bp, however the uncut homozygous -765 CC was 157 bp. The presence of all the three fragments (157, 134 and 23 bp) showed a heterozygous -765 GC. The 23 bp fragment was not identified in the agarose gel due to primer-dimer band. A sample of PCR-RFLP gel electrophoresis has been presented in figure 1.

There was a statistically significant difference between increasing of age and gastric adenocarcinoma risk (Odds ratio=1.12, 95% CI=1.08-1.16; P<0.001). The genotype prevalence and allele frequencies are shown in (Table 2). The frequency of the -765>CC,GC and GG genotypes were 4.4%, 61.5% and 34% in controls and 4.4%, 68.1% and 27.5% in gastric adenocarcinoma
patients respectively. Statistical analysis revealed no significant association between the genotypes and risk of gastric adenocarcinoma. However, differences were considered significant (p=0.04) for female patients with C carrier genotypes (sum: GC and CC) and gastric adenocarcinoma when compared with male patients (p=0.65) and control groups (p=0.65).

Discussion

Based on the present results, we found that age and gender are important factors which are involved on association between -765G > C COX-2 genetic polymorphism and gastric adenocarcinoma risk. Although COX-1 is constitutively expressed, COX-2 expression is associated with various pathophysiological conditions, including inflammatory diseases and different cancers such as adenocarcinoma tissues and gastric precancerous (31, 32). Molecular mechanisms and other pathways that are responsible for over expression of COX-2 protein has not elucidate completely. The current consensus is that both transcriptional and post-transcriptional events are important (33). Many studies suggest an important role of COX-2 in the carcinogenesis pathway including in the inhibition of apoptosis, tumor growth, angiogenesis, invasion and metastasis (34). One of the most important polymorphism in COX-2 gene consisted on G/C transversion 765 nucleotides upstream from the transcription start (35). For these reasons, COX-2 is an important target for molecular intervention. Previously Pereira et al have investigated the influence of the COX2 -765G/C polymorphism on gastric cancers (36, 37). They have reported not only a nearly 3-fold increased risk of progression from gastric lesions into gastric cancer, but also described an associated between C-allele and gastric cancers. Sitarz et al. demonstrated that Cox2- 765G allele promoter polymorphism is associated with most kinds of gastric cancers (34).

In the present study, we investigated the presence of this nucleotide variant and recognized allelic frequencies of G-765C in the Iranians for the first time, similar to those formerly reported in other studies (5, 23, 32). We found no statistically significant between the presence of CC, GC and CC genotypes –765G>C polymorphisms and risk of gastric cancer. However, we observed a borderline statistically significant (P=0.043) for female subjects with C- carrier genotypes when compared with male subjects (P=0.645) and control groups (P=0.653). These results appear that gender may be a risk factor for the development of gastric adenocarcinoma related to COX-2 polymorphism in Iranian samples. Although the frequency of GC genotype is much higher than other similar published studies (5, 26, 34), the frequency of GG (27.5%) and C-carrier (72.5%) genotypes are similar to Indian population (5). Thus, it can be concluded that COX2 -765G/C polymorphism perhaps play a similar role in susceptibility to gastric cancer between Asian populations. We found that, age factor can be excessively marker for GC because we observe a statistically significant difference between increasing of age and GC risk (OR=1.125, 95CI=1.089-1.162). In general, COX2 -765G/C polymorphisms possibly play a role in cancer development under specific situation or in specific populations. Finally, we conclude that age and gender are important factors which can be involved on association between -765G > C COX-2 genetic polymorphism and gastric adenocarcinoma risk in Iranian samples.

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