Gastroenterology and Hepatology From Bed to Bench

ORIGINAL ARTICLE

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 Rostami[1], Mehdi Kadivar[2], Mozaffar Aznab[3], Mina Abachi[3]

[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.

(Please site as: **Rostami M, Kadivar M, Aznab M, Abachi M. Influence of age and gender on association between -765G > C COX-2 genetic polymorphism and gastric adenocarcinoma risk: a case-control study in Iran. Gastroenterol Hepatol Bed Bench 2012;5(1):29-34).**

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 endoproxide H

Received: 12 August 2011 *Accepted*: 18 November 2011
Reprint or Correspondence: Mehdi Kadivar, PhD. Biochemistry department, Pasteur Institute of Iran, Tehran, Iran
E-mail: kadivar@pasteur.ac.ir

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-κB, 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´-ATTCTGGCCTCGCCGCTTC-3´ and reverse 5´-CTCCTTGTTTCTTGGAAAGAGACG-3´. PCR reactions were carried out using 10 µ*l* of Prime Taq Premix (2X) (GENET BIO, Korea), 0.7 µ*l* of each forward and reverse primers, 0.5 µ*l* genomic DNA and 8.1 µ*l* ddH_2O in a total volume of 20 µ*l* 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 µ*l* PCR reaction products were subjected to 0.7 µ*l* B*sh*1236I 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

Lithuania), 2 μ*l* of R buffer and 2.3 μ*l* of ddH_2O for 11 h at 37º C. 3% agarose gel was utilized for electrophoresis and then DNA fragments were separated based on their specific length. In all samples, experiments were performed three times to confirm the genotypes.

Statistical analyses were performed applying SPSS version 15 (SPSS Inc., Chicago, IL). Chi-square test was applied for comparing the nominal and ordinal variables. A P-value less than 0.05 was considered statistically significant. An interview including a planned questionnaire was performed among subjects to gather information. Variables were age, gender and COX-2 (G, C) alleles.

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.

In this study, Logistic regression and χ^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).

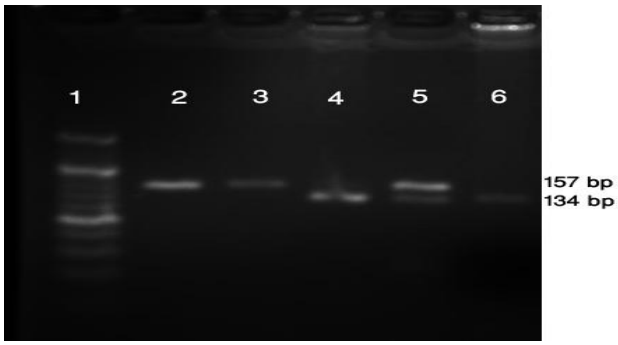

Figure 1. PCR-RFLP analysis of -765 G > C *COX-2* polymorphism; Lane 1: 20 bp DNA ladder; Lane 2: PCR product (undigested); Lane 3: Homozygous -765 CC genotype; Lanes 4, 6: Homozygous -765 GG genotype; Lane5: Heterozygous-765 GC genotype.

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 border line 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.

Acknowledgements

This project was supported by Pasteur Institute of Iran. The authors would like to thank Mrs. Nastaran Ghahhari for preparing manuscript and Dr. Ehsan Mostafavi for analysis of data.

References

1. Bornschein J, Rokkas T, Selgrad M, Malfertheiner P. Gastric cancer: clinical aspects, epidemiology and molecular background. Helicobacter 2011; 16:45-52.

2. Correa P, Piazuelo MB, Camargo MC. The future of gastric cancer prevention. Gastric Cancer 2004; 7:9-16.

3. Palli D, Galli M, Caporaso NE, Cipriani F, Decarli A, Saieva C, et al. Family history and risk of stomach cancer in Italy. J Cancer Epidemiol Biomarkers Prev 1994; 3:15-18.

4. Prinz C, Schwendy S, Voland *P. H pylori* and gastric cancer: shifting the global burden. World J Gastroenterol 2006; 12: 5458-64.

5. Saxena A, Prasad KN, Ghoshal UC, Roshan M,Krishnani N, Husain N. Polymorphism of -765G > C COX-2 is a risk factor for gastric adenocarcinoma and peptic ulcer disease in addition to H pylori infection: a study from northern India. World J Gastroenterol 2008; 14:1498-503.

6. Agudo A, Sala N, Pera G, Capella G, Berenguer A, Garcı N, et al. Polymorphisms in metabolic genes related to tobacco smoke and the risk of gastric cancer in the European prospective investigation into cancer and nutrition. J Cancer Epidemiol Biomarkers Prev 2006; 15:2427-34.

7. Vogel U, Segel S, Dethlefsen C, Tjønneland A, Saber AT, Wallin H, et al. Associations between COX-2 polymorphisms, blood cholesterol and risk of acute coronary syndrome. J Atherosclerosis 2009; 24:1-8.

8. Sadjadi A, Nouraie M, Mohagheghi MA, Mousavi-Jarrahi A, Malekezadeh R, Parkin DM, et al. Cancer occurrence in Iran in 2002, an international perspective. Asian Pac J Cancer Prev 2005; 6:359-63.

9. Wang B, Glatzle J, Mueller MH, Kreis M, Enck P, Grundy D. Lipopolysaccharide-induced changes in mesenteric afferent sensitivity of rat jejunum in vitro: role of prostaglandins. J Physiol Gastrointest Liver Physiol 2005; 289:254-60.

10. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, et al. Cyclooxygenase in biology and disease. FASEB J 1998; 12:1063-73.

11. Chandrasekharan NV, Dai H, Roos KL, Evanson NK, Tomsik J, Elton TS, et al. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. J Proc Natl Acad Sci U S A 2002; 99: 13926-31.

12. Wang D, Mann JR, DuBois RN. The role of prostaglandins and other eicosanoids in the gastrointestinal tract. J Gastroenterology 2005; 128:1445-61.

13. FitzGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. N Engl J Med 2001; 345:433-42.

14. Anto RJ, Mukhopadhyay A, Shishodia Sh, Gc Gairola, Aggarwal BB. Cigarette smoke condensate activates nuclear transcription factor-kappaB through phosphorylation and degradation of IkappaB (alpha): correlation with induction of cyclooxygenase-2. J Carcinogenesis 2002; 23:1511-18.

15. Hida T, Kozaki K, Ito H, Miyaishi M, Tatematsu Y, Suzuki T, et al. Significant growth inhibition of human lung cancer cells both in vitro and in vivo by the combined use of a selective cyclooxygenase 2 inhibitor. JTE-522 and conventional anticancer agents. Clin Cancer Res 2002; 8:2443-47.

16. Fujimura T, Ohta T, Oyama K, Miyashita T, Miwa K. Role of cyclooxygenase-2 in the carcinogenesis of gastrointestinal tract cancers: a review and report of personal experience. World J Gastroenterol 2006; 12:1336-45.

17. Jaime L, Masferrer K M, Alane T, Ben S Z, Settle SL, Woerner BM, et al. Antiangiogenic and Antitumor Activities of Cyclooxygenase - 2 Inhibitors. J Cancer Research 2000; 60:1306-11.

18. Cao Y, Prescott SM. Many actions of cyclooxygenase-2 in cellular dynamics and in cancer. J Cell Physiol 2002; 190:279-86.

19. Dixon DA. Regulation of COX-2 expression in human cancers.J Prog Exp Tumor Res 2003; 37: 52-71.

20. Castellone MD, Teramoto H, Williams BO, Druey KM, Gutkind JS. Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. J Science 2005; 310: 1504-10.

21. Dannenberg AJ, Altorki NK, Boyle JO, Dang C, Howe LR, Weksler BB , et al. Cyclo-oxygenase 2: a pharmacological target for the prevention of cancer. J Lancet Oncol 2001; 2: 544-51.

22. Dannenberg AJ, Altorki NK, Boyle JO, Lin DT, Subbaramaiah K. Inhibition of cyclooxygenase-2: an approach to preventing cancer of the upper aerodigestive tract. J Ann N Y Acad Sci 2001; 952:109-15.

23. Uefuji K, Ichikura T, Mochizuki H. Cyclooxygenase-2 expression is related to prostaglandin biosynthesis and angiogenesis in human gastric cancer. J Clin Cancer Res 2000; 6:135-38.

24. Ribeiro R, Vasconcelos A, Costa S, Pinto D, Morais A, Oliveira J, et al. Overexpressing leptin genetic polymorphism (-2548 G/A) is associated with susceptibility to prostate cancer and risk of advanced disease. J Prostate 2004; 59: 268-74.

25. Pinto D, Vasconcelos A, Costa S, Pereira D, Rodrigues H, Lopes C, et al. HER2 polymorphism and breast cancer risk in Portugal. Eur J Cancer Prev 2004; 13:177-81.

26. Pereira C, Sousa H , Ferreira P, Fragoso M, Moreira-Dias L , Lopes L, et al. -765G > C COX-2 polymorphism may be a susceptibility marker for gastric adenocarcinoma in patients with atrophy or intestinal metaplasia. World J Gastroenterol 2006; 12: 5473-78.

27. Papafili A, Michael R. H, David JB, Robin JM, Marshall RP, Humphries SE , et al. Common promoter variant in cyclooxygenase-2 represses gene expression: evidence of role in acute-phase inflammatory response. J Arterioscler Thromb Vasc Biol 2002; 22:1631-36.

28. Cok SJ, Morrison AR. The 3'-untranslated region of murine cyclooxygenase-2 contains multiple regulatory elements that alter message stability and translational efficiency. J Biol Chem 2001; 276: 23179-85.

29. Hu Z, Miao X, Ma H, Wang X,Tan W, Wei Q, et al. A common polymorphism in the 3'UTR of cyclooxygenase 2/prostaglandin synthase 2 gene and risk of lung cancer in a Chinese population. J Lung Cancer 2005; 48: 11-17.

30. Szczeklik W, Sanak M, Szczeklik A. Functional effects and gender association of COX-2 gene polymorphism G-765C in bronchial asthma. J Allergy Clin Immunol 2004; 114: 248-53.

31. Kang S, Kim YB, Kim MH, Yoon KS, Kim JW, Park NH, et al. Polymorphism in the nuclear factor kappa-B binding promoter region of cyclooxygenase-2 is associated with an increased risk of bladder cancer. J Cancer Lett 2005; 217:11-16.

32. Cipollone F,Toniato E, Martinotti S, Fazia M, , Iezzi A, Cuccurullo C, et al. A polymorphism in the cyclooxygenase 2 gene as an inherited protective factor against myocardial infarction and stroke. JAMA 2004; 291: 2221-28.

33. Harper KA, Tyson-Capper AJ. Complexity of COX-2 gene regulation. J Biochem Soc Trans 2008; 36: 543-45.

34. Sitarz R, Leguit RJ , Leng WW, Polak M, Morsink FM, Bakker O, et al. The COX-2 promoter polymorphism -765 G>C is associated with early-onset, conventional and stump gastric cancers. J Mod Pathol 2008; 21: 685-90.

35. Upadhyay R, Jain M, Kumar SH, Ghoshal UC, Mittal B. Functional polymorphisms of cyclooxygenase-2 (COX-2) gene and risk for esophageal squmaous cell carcinoma. J Mutat Res 2009; 663:52-59.

36. Li HX, Chang XM, Song ZJ, He SX. Correlation between expression of cyclooxygenase-2 and angiogenesis in human gastric adenocarcinoma. World J Gastroenterol 2003; 9: 674-77.

37. Crew KD, Neugut AI. Epidemiology of gastric cancer. World J Gastroenterol 2006; 12: 354-62.