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Original Research

The COX2 genetic variants in oral squamous cell carcinoma in Turkish population

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Abstract: Oral squamous cell carcinoma (OSCC) is a common type of cancer that genetic and environmental factors also lifestyle habits, infections play important roles in the pathogenesis of disease. Cyclooxygenase 2 (COX2) is the inducible isoform of enzyme which convert arachidonic acid to prostaglandins. It was known that alterations in COX2 gene functions contribute to the inflammation process thus induce cancer progression, including cell proliferation, apoptosis, adhesion, invasion and metastasis. A total of 114 cases 165 healthy individuals were included in present study. We aimed to evaluate possible association between the COX2; -765, -1195 polymorphisms and the risk of OSCC. The genotypes were determined by using polymerase chain reaction restriction fragment length polymorphism techniques. In our study group the carriers of COX2 -765 C allele were statistically higher in patients compared with controls and individuals who had CC genotype had a 3,4 fold high risk for OSCC ($p < 0,05$). We also observed the COX2 -1195 AA genotype frequency was higher in cases that of healthy group and individuals who had AA genotype showed a 1,7 fold increased risk for OSCC ($p < 0,05$). Haplotype analysis confirmed our result and revealed that the frequencies of COX2 -765C, -1195A haplotype frequencies were significantly higher in patients as compared with those of controls. In conclusion we suggest that COX2, -765, -1195 polymorphisms appear to be an important predictive factor and may be a prognostic biomarker for risk of OSCC. Further investigations with larger study groups are needed to fully elucidate the role of COX2 -765, -1195 variations in the development of OSCC.

Key words: Oral squamous cell carcinoma; Cyclooxygenase; Gene variation.

Introduction

Oral cancer is one of the most frequently diagnosed cancer with high morbidity and mortality and important health problem in various regions of the world (1,2). The etiology of the disease is multifactorial but multiple genetic and molecular variable stimuli that include distinction in the genome products as a result differentiated expression of proteins, chemical mediators have significant effects in the development of OSCC (3).

Studies revealed that inflammation is associated with the expression of oncogenes and tumor suppressor genes and also very effective in promoting neoplastic transformation (4). According to studies the chemical mediators such as transforming growth factor beta (TGF-β), tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, (COX2), and matrix metalloproteinase-7 (MMP-7) are found to be frequently expressed by upregulation in inflammation process (5,6). COX, also known as prostaglandin endoperoxide synthetase (PTGS) is a key enzyme which catalyze convertion of arachidonic acid to prostaglandins (PGs) and observed in the inflammatory progression (7). The two isoforms of COX which were identified as COX1 and COX2 are encoded by different genes even so catalyse the same molecular reaction (8).

It was known that cell proliferation may have effects in carcinogenesis and cyclooxygenases (COXs) play important roles in this process by inducing tumorogic pathways including apoptosis, cell adhesion, invasion, metastasis and angiogenesis (9,10-12). To our knowledge increased level of COX2 expression is observed in stromal cells and cancerous cells at the invasive front in OSCC (13,14) thus COX2 influence the local invasion and metastasis process (15) and it has been suggested that increased levels of COX2 is associated with high rate of recurrence after treatment and also poor prognosis (16).

The gene encoding COX2 is located on the chromosome 1q25.2-q25.3, approximately less than 8 kb pairs in size and consists of 10 exons (17). Certain single nucleotide COX2 polymorphisms (SNPs) have been investigated in different types of cancers and the polymorphic variants of -765G/C (rs20417) and -1195 A/G (rs689466) has been detected to have a functional effect such as altering the enzyme function of COX2 by differential regulation of COX2 expression and transcription (18,19). According to the studies high levels of COX2 mRNA and protein were detected in some types of epithelial tumors that include breast, lung (20-21).

Although the effects of COX2 polymorphism have been tested in different types of malignancy there are limited number of studies that subjected directly to research oral squamous cell carcinoma also variable results even in the studies which were conducted in the same regions and populations. The present study was designed to define the role of COX2 gene polymorphism in OSCC development and risk using a case-control design.

Materials and Methods

Patient selection and clinical investigation

A total of 114 oscc patients and 165 healthy controls were included in the current study. All of the cases were treated at Istanbul University Faculty of Dentistry Department of Oral Surgery and Medicine in 6 months time. The protocol followed was consistent with the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects). Local Ethical Committee approval was obtained for the study. After obtaining informed consent, the blood specimens were collected from the patients before any treatment had been started (chemotherapy or radiotherapy). 165 healthy partipiciants were selected from the volunteers without any symptoms of oscc and any kind of cancer history in their families.

DNA isolation

Blood specimens were collected in tubes containing EDTA and DNA samples were extracted from whole blood by a salting out procedure (22). Genotyping was performed by polymerase chain reaction (PCR) and restriction fragment length polymorphism, We used 5′ AGG CAG GAA ACT TTA TAT TGG 3′ (forward) and 5′ ATG TTT TAG TGA CGA CGC TTA 3′ (reverse) primers for detection of the COX2 -765G/C. 50-100 ng genomic DNA were amplified with 1x PCR buffer, 0.2 mM of each dNTP, 3 mM MgCl2, 0.2 mM of each primer and 0.5 U of Taq polymerase (MBI Fermentas) in a 25 µl reaction volume. The initial denaturation step of 94°C for 5 min followed by 35 cycles of 94°C for 45 s, 56°C for 45 s, and 72°C for 45 min and a further 72 for 5 min. The PCR product was digested with AciI restriction enzyme (MBI Fermentas) at 37°C for 16 hours and was electrophoresed in 2% agarose gels and stained with ethidium bromide (23).

COX2 -1195A/G PCR amplification product was generated by using the primers 5′ CCCTGAGCACTACCCATGAT 3′ (forward) and 5′ GCCTTCATAGGAGATACTGG 3′ (reverse). The reaction mix contained 50-100 ng genomic DNA amplified with 1x PCR buffer, 0.2 mM of each dNTP, 3 mM MgCl2, 0.2 mM of each primer and 0.5 U of Taq polymerase (MBI Fermentas, Lithuania) in a 25 µl reaction volume. After denaturating the DNA for 5 min at 95°C, the reaction mixture was subject to 35 cycles of denaturating for 45 s at 94°C, 45 s annealing at 58°C and 72°C for 45 s. The final step was at 72°C for 5 min. The PCR products were digested with Pvu II restriction enzyme (MBI Fermentas) at 37°C for 2 h followed by electrophoresis in 2% agarose gel containing ethidium bromide (23).

Table 1. Characteristics of patients with oral squamous cell carcinoma and control group.

Variables	Controls n:165	Cases n:114	p value
Age	55,13±9,66	57,79±12,8	0,062
Gender			
Female	63,0%	31,6%	0,000
Male	37,0%	68,4%	
Smoking Status			
Yes	30,9%	65,8%	0,000
No	69,1%	34,2%	
Drinking Status			
Yes	7,9%	26,3%	0,000
No	92,1%	73,7%	

Statistical analysis

Statistical analyses were performed using the SPSS software package (revision 11.5 SPSS Inc., Chicago, IL, USA). Data are expressed as means±SD. Differences in the distribution of COX2 genotypes or alleles between cases and controls were tested using X^2 the test. Relative risk at 95% confidence intervals (CI) was calculated as the odds ratio (OR). Linkage disequilibrium between COX2 -765, -1195 polymorphisms was assessed using $D^0$and r^2values obtained through the Haploview program (http://www.broad.mit.edu/mpg/haploview/documentation.php). A multivariate logistic regression model was performed to investigate possible effects genotypes and alleles after adjustment for age. Values for P\0.05 were considered as statistically significant.

Results

The characteristics of patients with OSCC and healthy controls which included this study are summarized in Table 1. The mean ages of patients were 57,79±12,8 and control group were 55,13±9,66 years, respectively. There was no statistically significant difference in mean age of our study groups but we observed high significant results for demographic variables such as smoking and drinking status between cases and controls. The clinical and pathological characteristics of our patients with OSCC were shown in Table 2.

Table 2. The clinical and pathological parameters of our patients with OSCC.

Patient characteristics	Cases (%)
Location of the Tumor	
Oral tongue	49,1
Lip	8,8
Floor of Mouth	14,9
Gingiva alveolar crest	14,0
Retromolar trigon	4,4
Hard palate	4,4
Buccal	4,4
Mechanical trauma	
(Positive %)	33,3
(Negative%)	66,7
Keratinization	
(Positive %)	64,0
(Negative%)	36,0
Tumor size (%)	
<4cm	69,8
≥4 cm	30,2
Lymph node metastasis	
Negative	64
≤3cm	22,8
3-6 cm	13,2
Stage (%)	
Early	42,1
Advanced	57,9
Differentiation	
Good	86,8
Poor	13,2

Table 3. Genotype and allele frequencies of study group and controls.

Genotypes/Alleles	Controls *N*: 165 *n* (%)	Patients *N*: 114 *n* (%)	*p* value
COX2 -765(rs20417)			
GG	35(21,2)	11(9,6)	
GC	85(51,5)	39(34,2)	
CC	45(27,3)	64(56,1)	**0,000**
G allele	155 (46,9)	61(26,7)	
C allele	175 (53,03)	167(73,2)	**0,000**
COX2-1195(rs689466)			
AA	98(59,4)	82(71,9)	
AG	67(40,6)	31(27,2)	
GG	0(0)	1(0,9)	**0,038**
A allele	263(79,6)	195(85,5)	
G allele	67 (20,4)	33 (14,5)	**0,07**

p-Value obtained by chi-square test, p<0.05.

The distributions of genotype and allelic frequency of COX2; -765, -1195 polymorphisms in our patients and controls were demonstrated in Table 3. The frequencies of COX2; -765 polymorphisms GG and GC, CC genotypes in controls and cases were 21,2%, 51,5%, 27,3% and 9,6%, 34,2%, 56,1% respectively. We observed individuals with COX2 -765 CC genotype had 3,4 fold increased risk for development of the OSCC (p:0,000) also the carrying of the COX2 -765 C allele frequency was higher in patients (73,2%) compared with controls (53,03%) and this difference was statistically significant (p:0,011) In consistent with our result we detected the G allele seem to be protected from OSCC (p:0,000).

When we analysed COX2 -1195 genotypes for controls and cases we found the distribution of AA 59,4%, AG 40,6%, GG 0%, and AA 71.9%, AG27.2%, GG0.9%, respectively. There were statistically significant differences in the genotype frequencies between patients and controls (p:0,038). Individuals with the AA genotype had 1,7 fold increased risk for OSCC (p:0,031). In addition we observed the frequency of the COX2 -1195; G allele higher in controls compared to cases (p:0,031)

When we performed stratification analyses by regarding tumor characteristics, such as lymph node-negative status, tumor stage, differentiation and other demographic variables; we did not detect any significant correlation between the genotypes distribution and these prognostic parameters (data not shown).

In addition to SNP analyses, haplotypes were evaluated for possible relation with OSCC There was significant association of COX2; -1195,-765 gene variants (Table 4). According to haplotype analysis the 765C: 1195A haplotype frequencies were significantly higher and COX2 -765G: -1195G haplotype frequencies were significantly lower in cases when compared with those of controls.

There was a linkage disequilibrium between COX2 -765G/C and -1195A/G gene variations (D': 0,295, r2: 0,012).

While gender, smoking, alcohol status, COX2 -765 CC and COX2 -1195 AA genotypes and haplotype -765C: -1195A were associated with OSCC in univariate analysis, gender, smoking COX2 -765 CC, COX2 -1195 AA were associated with this disease in multivariate logistic regression analysis.

Discussion

COX2 products play important roles in different physiological and pathophysiological mechanisms such as the control of blood pressure and renal hemodynamics, endothelial thromboresistance, pain and inflammation, and some type of malignancy (24, 25). Researchers demonstrated that polymorphisms induce the expression levels and enzymatic activity of COX2, therefore, they are substantially related with inflammatory response and individual variations in the susceptibility to oral cancers (26,27). To our best knowledge the present study was the first that evaluate the role of the COX2; -765,-1195 gene variations in oral squamous cell carcinoma in Turkish patients.

Different nucleotide polymorphisms are defined in the COX2 regions. Several molecular epidemiological studies have examined the influence of COX2; -765, -1195 polymorphisms. It was shown that the -765G/C polymorphism which lies in the promoter region of the COX2 gene results substitution of guanine (G) to cytosine (C) variation at position -765 and this variation (rs20417) located at the transcription start site prevents stimulatory protein (Sp1) binding but occurs a new E2 promoter factor (E2F) binding site, leading to increased transcription activity (26,27), also researchers were reported the COX2 -1195 created a c-MYB-binding site, resulting in higher transcriptional activity of the COX2 gene (28) and overexpression of c myb and COX2 induce cell division processes such as angiogenesis, proli-

Table 4. The haplotype analyses of COX-2 gene in patients and controls.

Haplotype Associations 765:1195	Frequencies	Case,Control Ratios	Chi-Square	P value
CA	0,482	0,601, 0,400	21,928	$2{,}8.10^{-6}$
GA	0,338	0,254, 0,396	12,165	$5{,}10^{-4}$
CG	0,131	0,131, 0,131	0,0	0,9943
GG	0,049	0,014, 0,073	10,301	0,0013

feration and apoptosis (29).

In our study group the COX2 -765 C allele frequency was statistically higher in patients that of controls and individuals who had -765 CC genotype showed a 3,4 fold increased risk for OSCC. We also observed that individuals with the COX2 -1195 AA genotype had 1,7 fold increased risk for OSCC while COX2 -765G and -1195G alleles seem to be protected against to OSCC. Similarly, haplotype analysis revealed that the -765C: -1195A haplotype frequencies were significantly higher and COX2 -765G: -1195G haplotype frequencies were significantly lower in cases when compared with those of controls.

There were various results about the effects of COX2; -765,-1195 polymorphism in the literatures. For instance Chiang et al. did not find any significant differences for the COX2 -765GC variant in Taiwan population but they demonstrated statistical differences for COX2 -1195 polymorphism. When they compared the COX2 -1195 GG homozygote, those with the AA homozygote they observed that the carrying of the AA genotype had a 1,55 fold increased risk of OSCC but those with the AG heterozygote displayed no statistically significant risk (30).

In another study which was performed by Mittal et al. in Asian Indians population, they did not report any significant differences in the frequency of COX2 -765 and -1195 variations between patients and controls (31).

There were also different studies have explored the possible association between SNPs of COX2 gene and several types of human cancer. For instance, COX2; -765, -1195 and 8473 variations were examined by Moraes at al. in lung cancer cases and they reported there was no association between these polymorphisms and this tumor type in Brazilian population (32). In another study which was conducted by Shin et al. the frequencies of COX2 -765 genotypes were not found different among their Korean study groups while they suggested that the COX2 -1195AA genotype may make the subjects more susceptible to diffuse type gastric cancer in Korea (33). As the same results Liu et al. reported an association between the COX2 -1195AA genotype and gastric cancer in China population (18). Meanwhile Zhang et al. documented an increased risk for the 1195AA genotype and esophageal cancer (28).

Current studies reveal that the genetic backgrounds and geographical regions may be responsible from genetic variations and different allelic frequencies. In one comprehensive meta analysis which includes 65 articles with 29,487 cancer cases and 39,212 non-cancer controls Wang et al. (34). reported that COX2 -765 C allele carriers had an increased risk of cancer especially gastric cancer. In the same study they also interestingly revealed an association between the COX2 -765 C allele and decreased cancer risk in Caucasian population compared with Asian population (34). When we evaluated our finding considering this parameter we observed consistent results with statistically high significant risk for carriers of the COX2 -765 C allele in our patient group.

There were also meta analysis for COX2 -1195 in the literature according to study researchers reported significant association between COX2 -1195 polymorphism and increased risk of gastric cancer, pancreatic cancer, hepatocellular carcinoma and other cancers. In addition they had stratified a sub-group analysis by ethnicity, increased cancer risk was observed among Asians instead of Caucasians, Africans and mixed populations (35). We also found statistically significant association between COX2 -1195 polymorphism and cancer risk.

The present study has some limitations for the number of cases and controls. However, we have chosen our group of patients according to strict criteria. If these findings confirmed in larger patient groups may have clinical value in assessment of the genetic risk and tumor progression thus opening new perspectives for the study of molecular factors underlying the mechanisms of OSCC.

In conclusion, present study indicates that functional genetic variants of COX2 -765 CC and -1195 AA genotypes may have potential risk in the development of the oral squamous cell carcinoma in Turkish patients

Conflicts of Interest Statement

No conflicts of interest were declared.

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References

1. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 2009; 45 (4-5): 309 - 16.

2. Fuller C, Camilon R, Nguyen S, Jennings J, Day T, Gillespie M. B. Adjunctive diagnostic techniques for oral lesions of unknown malignant potential: systematic review with meta-analysis. Head Neck. 2015; 37(5):755-62.

3. Pérez-Sayáns M, Somoza-Martín JM, Barros-Angueira F, Reboiras-López MD, Gándara Rey JM, García-García A. Genetic and molecular alterations associated with oral squamous cell cancer (Review). Oncol Rep. 2009; 22(6):1277-82.

4. Schetter AJ, Heegaard NH, Harris CC. Inflammation and cancer: interweaving microRNA, free radical, cytokine and p53 pathways. Carcinogenesis. 2010;31(1):37–49.

5. Mignogna MD, Fedele S, Lo Russo L, Lo Muzio L, Bucci E. Immune activation and chronic inflammation as the cause of malignancy in oral lichen planus: is there any evidence? Oral Oncol. 2004; 40(2): 120–130.

6. Li TJ, Cui J. COX-2, MMP-7 expression in oral lichen planus and oral squamous cell carcinoma. Asian Pac J Trop Biomed. 2013; 6(8): 640–643.

7. Noguchi K, Ishikawa I. The roles of cyclooxygenase-2 and prostaglandin E2 in periodontal disease. Periodontol 2000. 2007;43:85-101.

8. Miyauchi M, Hiraoka M, Oka H, Sato S, Kudo Y, Ogawa I, et al. Immuno-localization of COX-1 and COX-2 in the rat molar periodontal tissue after topical application of lipopolysaccharide. Arch Oral Biol. 2004;49:739-46.

9. Chandrasekharan NV, Simmons DL. The cyclooxygenases. Genome Biol 2004; 5(9):241.

10. Tsujii M, DuBois RN: Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell. 1995; 83(3):493-501.

11. Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell. 1998; 93(5):705-16.

12. Nozoe T, Ezaki T, Kabashima A, Baba H, Maehara Y.Significance

of immunohistochemical expression of cyclooxygenase-2 in squamous cell carcinoma of the esophagus. Am J Surg 2005; 189(1):110-115.
13. Pontes HA, Pontes FS, Fonseca FP, de Carvalho PL, Pereira EM, de Abreu MC, et al. Nuclear factor kappa B and cyclooxygenase-2 immunoexpression in oral dysplasia and oral squamous cell carcinoma. Ann Diagn Pathol. 2013;17(1):45–50.
14. Mauro A, Lipari L, Leone A, Tortorici S, Burruano F, Provenzano S, Gerbino A, Buscemi M. et al. Expression of cyclooxygenase-1 and cyclooxygenase-2 in normal and pathological human oral mucosa. Folia Histochem Cytobiol. 2010;48(4):555–563.
15. Salimi M, Esfahani M, Habibzadeh N, Aslani HR, Amanzadeh A, Esfandiary M, Sedaghati B, Bidgoli SA, Ghahremani MH. Change in nicotine-induced VEGF, PGE2 AND COX-2 expression following COX inhibition in human oral squamous cancer. J Environ Pathol Toxicol Oncol. 2012;31(4):349–356.
16. Goulart Filho JA, Nonaka CF, da Costa Miguel MC, de Almeida Freitas R, Galvão HC. Immunoexpression of cyclooxygenase-2 and p53 in oral squamous cell carcinoma. Am J Otolaryngol. 2009;30(2):89–94.
17. Kosaka T, Miyata A, Ihara H, Hara S, Sugimoto T, Takeda O, Takahashi E, and Tanabe T. Characterization of the human gene (PTGS2) encoding prostaglandin- endoperoxide synthase 2. Eur J Biochem. 1994; 221:889–897.
18. Liu F, Pan K, Zhang X, Zhang Y, Zhang L, Ma J, Dong C, Shen L, Li J, Deng D, Lin D, You W. Genetic variants in cyclooxygenase-2: expression and risk of gastric cancer and its precursors in a Chinese population. Gastroenterology 2006;130(7):1975-84.
19**.** Kristinsson JO, van Westerveld P, te Morsche RH, Roelofs HM, Wobbes T, Witteman BJ, Tan AC, van Oijen MG, Jansen JB, Peters WH. Cyclooxygenase-2 polymorphisms and the risk of esophageal adeno- or squamous cell carcinoma. World J Gastroenterol. 2009;15(28):3493-3497.
20. Hwang D, Scollard D, Byrne J, Levine E. Expression of cyclooxygenase-1 and cyclooxygenase-2 in human breast cancer. J Natl Cancer Inst. 1998;90(6):455-60.
21. Wolff H, Saukkonen K, Anttila S, Karjalainen A, Vainio H, Ristimaki A. Expression of cyclooxygenase-2 in human lung carcinoma. Cancer Res. 1998 ;58(22):4997-5001.
22. Miller SA, Dykes DD, Polesky HF. A simple saltingout procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16(3):1215.
23. Mozaffari E, Doosti A, Arshi A, Faghani M. Association of COX-2 Promoter Polymorphisms -765G/C and -1195A/G with Migraine. Iran J Public Health. 2016 ;45(12):1625-1635.
24. Smyth EM, Grosser T, FitzGerald GA. Lipid-derived autacoids: eicosanoids and platelet-activating factor In: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12th edn, eds Brunton L, editor; Chabner B, editor; , Knollman B, editor. McGraw-Hill; New York, 2011; 937–57.
25. Dovizio M, Alberti S, Sacco A, Guillem-Llobat P, Schiavone S, Maier TJ, et al. Novel insights into the regulation of cyclooxygenase-2 expression by platelet-cancer cell cross-talk. Biochem Soc Trans. 2015; 43: 707–14.
26.Li D, Hao SH, Sun Y, Hu CM, Ma ZH, Wang ZM, Liu J, Liu HB, Ye M, Zhang YF, Yang DS, Shi G. Functional Polymorphisms in COX-2 Gene Are Correlated with the Risk of Oral Cancer. Biomed Res Int. 2015;2015:580652. doi: 10.1155/2015/580652. Epub 2015 Apr 21.
27. Zhu W, Wei BB, Shan X, Liu P. -765G>C and 8473T>C polymorphisms of COX-2 and cancer risk: a meta-analysis based on 33 case-control studies. Mol Biol Rep. 2010; 37(1):277-88.
28. Zhang X, Miao X, Tan W, Ning B, Liu Z, Hong Y, et al. Identification of functional genetic variants in cyclooxygenase-2 and their association with risk of esophageal cancer. Gastroenterology 2005;129:565–76.
29. Ramsay RG, Barton AL, Gonda TJ. Targeting c-Myb expression in human disease. Expert Opin Ther Targets. 2003;7(2):235-48.
30. Chiang SL, Chen PH, Lee CH, Ko AM, Lee KW, Lin YC, Ho PS, Tu HP, Wu DC, Shieh TY, Ko YC. Up-regulation of Inflammatory Signalings by Areca Nut Extract and Role of Cyclooxygenase-2 −1195G>A Polymorphism Reveal Risk of Oral Cancer. Cancer Res. 2008; 68(20):8489-98.
31. Mittal M, Kapoor V, Mohanti BK, Das SN. Functional variants of COX-2 and risk of tobacco-related oral squamous cell carcinoma in high-risk Asian Indians. Oral Oncol. 2010;46(8):622-6.
32. Moraes JL, Moraes AB, Aran V, Alves MR, Schluckbier L, Duarte M, Toscano E. et al. Functional analysis of polymorphisms in the COX-2 gene and risk of lung cancer. Mol Clin Oncol. 2017 ; 6(4): 494-502.
33. Shin WG, Kim HJ, Cho SJ, Kim HS, Kim KH, Jang MK, Lee JH, Kim HY. The COX-2-1195AA Genotype Is Associated with Diffuse-Type Gastric Cancer in Korea. Gut Liver. 2012;6(3):321-7.
34. Wang XF, Huang MZ, Zhang XW, Hua RX, Guo WJ. COX-2-765G>C Polymorphism Increases the Risk of Cancer: A Meta-Analysis PLoS One. 2013;8(9):e73213. doi: 10.1371/journal.pone.0073213. eCollection 2013.
35. Wang Y, Jiang H, Liu T, Tang W, Ma Z. Cyclooxygenase-2 -1195G>A (rs689466) polymorphism and cancer susceptibility: an updated meta-analysis involving 50,672 subjects. Int J Clin Exp Med. 2015; 8(8): 12448–12462.