

## Original Article

**Running Title:** COX-2 Polymorphisms and Bladder Cancer

Received: September 20, 2020; Accepted: May 30, 2021

### Prostaglandin Synthase 2/Cyclooxygenase 2 Gene Polymorphisms and Susceptibility to Urothelial Bladder Cancer in an Iranian Population

Kamyar Mansori<sup>\*</sup>, PhD, Mohammad Reza Nowroozi<sup>\*\*</sup>, MD, Amirreza Farzin<sup>\*\*</sup>, MD, Solmaz Ohadian Moghadam<sup>\*\*♦</sup>, PhD

<sup>\*</sup>*Department of Epidemiology and Biostatistics, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran*

<sup>\*\*</sup>*Uro-Oncology Research Center, Tehran University of Medical Sciences, Tehran, Iran*

#### ♦Corresponding Author

Solmaz Ohadian Moghadam, PhD

Uro-Oncology Research Center,

Tehran University of Medical Sciences, Tehran, Iran

Tel/Fax: +98 21 66 43 79 69

Email: [s-ohadian@sina.tums.ac.ir](mailto:s-ohadian@sina.tums.ac.ir)

#### Abstract

**Background:** Prostaglandin-endoperoxide synthase 2, recognized as cyclooxygenase 2 (COX-2), is an important enzyme contributing to the generation of proinflammatory prostaglandins. It can play a role in increased tumor angiogenesis, apoptosis inhibition, metastasis, and invasion of tumors. Single nucleotide polymorphisms (SNPs) of the COX-2 promoter may associate with the cancer predisposition. In the present work, we aimed to explore whether SNPs of COX-2 gene affect both the risk of development and grade of bladder cancer.

**Method:** This case-control study was performed and the genetic polymorphisms of six COX-2 SNPs including, intron 1 (rs2745557), intron 5 (rs16825748), intron 6 (rs2066826), T+8473C (rs5275), G-765 (rs20417), and A-1195G (rs68946) were genotyped in 80 healthy controls and 80 bladder cancer patients using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). To select independent prognostic factors, the univariate and multivariate analyses were implemented.

**Results:** Univariate logistic regression model indicated a significant association between COX-2 765G>C heterozygous GC genotype and greater risk of bladder cancer (OR: 2.07; 95% CI: 1.03 - 4.15;  $P= 0.04$ ). However, the multivariate logistic regression analysis showed no associations between COX-2 variants and bladder cancer development.

**Conclusion:** We concluded that COX-2 polymorphisms do not contribute to the genetic susceptibility to urothelial bladder cancer in an Iranian population. However, the only genotype in which the frequency of alleles significantly differed between the two groups of high-grade tumors and low-grade tumors was COX -2 8473T> C (rs5275). Moreover, our findings showed that both smoking and family history of cancer play a role in susceptibility to bladder cancer.

**Keywords:** Urinary bladder neoplasms, Polymorphism, Single nucleotide, Cyclooxygenase 2, Case-control studies, Restriction fragment length polymorphisms

## Introduction

Bladder cancer is among the major causes of cancer-related death all over the world with an estimated 400,000 new cases and 165,000 deaths annually.<sup>1,2</sup> It is the seventh most prevalent cancer among men worldwide and the second in Iran as a Middle Eastern country.<sup>3</sup> The prevalence of bladder cancer considerably varies in different regions, ranging from 5.9 in Asia to 23.9 in Europe.<sup>4</sup> Despite the improvements in cancer treatment, survival rate after radical cystectomy still remains unchanged.<sup>5</sup> There are several accepted patients and tumor-related prognostic factors for bladder cancer. Further studies are also under assessment. However, still there is a lack of knowledge regarding these factors. Therefore, developing novel biomarkers is urgently required to choose an appropriate decision for the treatment and prediction of survival and recurrence.<sup>6</sup> Smoking and family history of cancer are also considered to be important environmental risk factors for cancer.<sup>5</sup> There are a few studies on the association between these factors and the risk of bladder cancer, whose results are inconsistent.<sup>5</sup>

Genetic polymorphisms have a potential role in the susceptibility to bladder cancer.<sup>6</sup> Cyclooxygenases (COXs) are important enzymes for the synthesis of prostaglandins (PGs) and other eicosanoids from arachidonic acid. The gene for PG-endoperoxide synthase 2 is recognized as *COX-2* gene and harbors various functional single-nucleotide polymorphisms (SNPs). There are several gene polymorphisms described previously for the *COX-2* gene, which might affect its expression. However, only some of them are significant and associated with diseases. Among these, the most well-known and identified polymorphisms are 8473T>C in the 3'-untranslated region (UTR) and -765G>C, 1195G>A in the promoter region, which have been reported to have a role in cancer development. Moreover, polymorphism 1290 A>G, intron 1 (rs2745557), intron 5 (rs16825748), and intron 6 (rs2066826) appear to have functional implications.<sup>7</sup> The COX has two isoenzymes, namely COX-1 and COX-2. The first one is constitutively expressed while the latter one is an extremely inducible protein and is correlated with inflammation and other pathologies, such as cancer proliferation.<sup>8</sup>

Overexpression of *COX-2* gene can result in both proliferation increase and apoptosis inhibition, which have been shown in several types of human cancers.<sup>9</sup> However, the association of *COX-2* genotypes with bladder cancer has not been properly explored. There may be differences in mRNA and protein levels of *COX-2* among individuals and this variability may depend on *COX-2* single nucleotide polymorphisms (SNPs).<sup>10</sup> Accordingly, this study aimed to explore the relationship between the genetic polymorphisms of six *COX-2* SNPs including, intron 1 (rs2745557), intron 5 (rs16825748), intron 6 (rs2066826), T+8473C (rs5275), G-765 (rs20417), and A-1195G (rs689466) with bladder cancer development risk. Moreover, the association between both family history of cancer and smoking with bladder cancer risk was investigated.

## Materials and Methods

### *Study subjects*

From 2018 until 2019, a total of 160 samples comprising 80 histologically confirmed bladder cancer patients (as cases) and 80 sex and age-matched healthy controls were enrolled in the current case/control study. Both groups completed a questionnaire for demographic and clinical characteristics and provided blood sample. Staging and grading were performed according to the American Joint Committee on Cancer's 2002 TNM staging system and World Health Organization (WHO), respectively. The exclusion criteria for the controls were as follow: the presence of malignancies or chronic diseases at the same time, history of any other malignancies and/or genetic diseases, cancer history, and cancer metastasized to bladder from another origin. All the participants signed informed consent. The present work was approved by our institutional review board (IR.TUMS.VCR.REC.1397.342).

### *Genotyping assay*

Whole-blood samples of both cases and controls were collected in tubes with ethylenediaminetetraacetic acid (EDTA) for further DNA extraction and genotyping. The genomic DNA of peripheral blood leukocytes was extracted from (QIAamp DNA Blood Mini Kit, Qiagen) based on the manufacturer's guideline.

To examine the SNP polymorphism of *COX-2* gene [intron 1 (rs2745557), intron 5 (rs16825748), intron 6 (rs2066826), T+8473C (rs5275), G-765 (rs20417), and A-1195G (rs689466)] in both controls and cases, polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay was accomplished as described previously.<sup>8</sup> Genomic DNA was amplified using PCR method and subsequently PCR products were digested with suitable restriction enzymes; the products were then visualized on 2% agarose gel that was stained with GelRed. The utilized primers, amplification conditions, and appropriate restriction enzymes were previously described in detail<sup>8</sup>. To confirm the accuracy of genotyping, 10% of the cases and control's samples were randomly re-genotyped by a different researcher and all the results indicated 100% concordant. The people performed the assays were blinded to the case and control status.

#### **Statistical analysis**

All the data were analyzed via stata14. For quantitative variables in descriptive analyses, mean and standard deviation (SD) were employed and for qualitative variables, number and relative frequencies were used. Moreover, to determine the association between demographic variables and allele frequencies of the *COX-2* gene polymorphism with bladder cancer risk, univariate and multiple logistic regression analysis were used. The crude and adjusted OR with 95% confidence interval (CI) were computed as well. Additionally, the area under a receiver operator characteristic (ROC) curve was used to assess the discriminative ability of the multivariable logistic regression analysis.  $P \leq 0.05$  was considered to be statistically significant.

#### **Results**

A total of 80 controls (non-cancer patients) and 80 cases (cancer patients) were recruited for the present study. Table 1 depicts the demographic characteristics of the controls and cases. Moreover, this table shows the association between the demographic characteristics with the bladder cancer risk using univariate logistic regression model. As could be seen, there were statistically significant associations between smoking and family history of cancer with

bladder cancer risk. The odds of bladder cancer for smoking and positive family history of cancer were respectively 2.25 (95% CI: 1.14 - 4.45) and 4.21 (95% CI: 2.15-8.23) times more than those who did not have this history.

Table 2 demonstrates the frequencies of different *COX-2* gene genotypes in the cases and controls. *COX-2* 765G>C heterozygous GC variant was associated with the higher bladder cancer risk (OR: 2.07; 95% CI: 1.03 - 4.15;  $P = 0.04$ ).

Subsequent to univariate logistic regression model, to exclude possible confounding variables, all the variables that seemed to be significantly associated in univariate logistic regression model ( $P \leq 0.20$ ) were included in a multivariate logistic regression analysis. The findings of multivariate logistic regression analysis, after adjusting for the confounding variables, showed a significant statistical association between smoking (adjusted OR: 2.36; 95% CI: 1.11 – 5.01;  $P = 0.025$ ) and cancer family history (adjusted OR: 4.04; 95% CI: 1.99 – 8.19;  $P < 0.001$ ) and bladder cancer risk. However, alleles of the *COX-2* gene polymorphism did not show a statistically significant relationship with bladder cancer risk ( $P$ -Value>0.05) (Table 3).

In our study, the area under an ROC curve was utilized to assess the discriminative ability of the multivariable logistic regression analysis (Figure 1).

Table 4 represents the associations between the type of bladder tumor with genotypes and allele frequencies of the *COX-2* gene in the case group. An intragroup analysis (in the bladder cancer group=cases) was performed to compare the frequency of *Cox-2* polymorphisms alleles in people with low-grade tumors and those with high-grade tumors. Chi-square test indicated that the frequency of TT and TC alleles in the patients with low grade tumor was significantly higher than that in those with high grade tumor. The only genotype in which the frequency of alleles significantly differed between the two groups of high-grade tumors and low-grade tumors was *COX-2* 8473T> C (rs5275) ( $P$ -Value=0.034).

## Discussion

Our findings revealed that COX-2 polymorphisms do not contribute to the genetic susceptibility to urothelial bladder cancer in an Iranian population. However, the only genotype in which the frequency of alleles significantly differed between the two groups of high-grade tumors and low-grade tumors was COX -2 8473T> C (rs5275).

In the current case-control study, we assessed the relationship between various COX-2 polymorphisms and the susceptibility to bladder cancer in an Iranian population. Among the seven polymorphic sites studied, univariate logistic regression results indicated a statistically significant relationship between COX-2 765G>C heterozygous GC genotype and increased risk of bladder cancer ( $P$ -Value = 0.04). However, the results of multivariate logistic regression model after adjusting for the confounding variables revealed no statistically significant associations between alleles of the COX-2 gene polymorphism and bladder cancer risk ( $P$ -Value>0.05)( Table 3). It should be noted that for COX-2 765G>C heterozygous GC, the obtained  $P$ -value in multivariate logistic regression model was very close to the significance level (0.072). Therefore, it might become significant with a larger sample size. Thus, carriers for 765C genotype are likely to be at higher risk for bladder cancer. Our findings also indicated no associations between 1290G>A, 8473T>C, and 1195G>A polymorphisms of COX-2 and bladder cancer risk. Inducible cyclooxygenase (COX-2) is a significant enzyme associated with inflammation, which is considered as a significant factor in the pathogenesis of several diseases, including malignant tumors. Similar to numerous gene variants, SNPs are also inherited. The SNP of COX-2 gene were observed to be associated with the susceptibility to a number of cancers, such as non-small cell lung cancer (NSCLC), esophageal squamous cell carcinoma, and prostate cancer.<sup>11-13</sup> The COX-2 765G>C region was recognized to have a role in modulation of the COX-2 gene expression and was established to be associated with numerous human diseases.<sup>14</sup> The rs20417 polymorphism is located at the promoter region of COX-2 gene (position -765 nucleotides) and contributes to

the reduction of COX-2 expression.<sup>10</sup> The nucleotide alteration of 765G>C polymorphism in a stimulatory protein1 (SP1) binding motif can form a binding site for E2F transcription factor which is recognized as adenovirus-induced and regulates the expression of numerous genes. Therefore, increased transcription activity as well as elevated expression of COX-2 occurs, which may cause the development of different cancers.<sup>10</sup> There are limited number of case-control studies on the COX-2 polymorphism and susceptibility to the cancers and the existing data are controversial. The association of this polymorphism with increased risk of other cancers, such as gastric cancer,<sup>15</sup> esophageal cancer,<sup>16</sup> colorectal cancer,<sup>17</sup> NSCLC<sup>18</sup> and nasopharyngeal carcinoma (NPC)<sup>19</sup>, has been previously indicated. The association between rs20417 variant and enhanced risk of bladder cancer has been indicated in Taiwanese, Indians, and Chinese populations.<sup>7,20</sup> On the contrary, a decreased susceptibility to bladder cancer has been reported for rs20417 polymorphism in American population.<sup>4</sup> Furthermore, enhanced risk of colon cancer among Singapore Chinese has been reported to be associated with carrying -765-C allele.<sup>21</sup> However, carrying -765-C allele have been associated with reduced colon cancer risk in American Caucasians.<sup>19</sup> These dissimilar results could be due to the variations in the frequency of COX-2 -765 alleles amongst various populations. Otherwise, it could be concluded that the contribution of the COX-2 -765 G/C polymorphism in susceptibility to cancers differ significantly according to the tumor type or ethnicity.

In the present study, the only genotype in which the frequency of alleles differed between the two groups of high-grade tumors and low-grade tumors was COX -2 8473T> C (rs5275) ( $P$ -Value=0.034). The results of Chi-square test demonstrated that the frequency of TT and TC alleles of in the patients with low grade tumor was significantly higher than that in those with high grade tumor. The human COX-2 gene comprises 10 exons and various polymorphism sites. The 8473 T > C polymorphism is located in the 3'UTR of this gene. Previous studies have revealed the association between this polymorphism and the mRNA level of COX-2

gene.<sup>22</sup> Our result is consistent with the findings of a previous meta-analysis indicating the association of COX -2 8473T>C polymorphism with a reduced risk of bladder cancer as well as nasopharyngeal cancer. Their stratified analysis also indicated the significant association of this polymorphism with greater risk of skin cancer and esophageal cancer. Moreover, they claimed that there are no significant associations between COX -2 1195G>A and COX-2 765G>C variants and bladder cancer risk.<sup>4</sup> Several previous studies have also shown the association between 8473C>T genotype and higher risk of non-small cell lung cancer,<sup>23</sup> breast cancer,<sup>24,25</sup> and colorectal cancer<sup>26</sup>. However, some other researchers have explained a protective role of this polymorphism in lung cancer.<sup>27</sup> These different results could be due to ethnic dissimilarities, various etiologies of different types of tumors, and gene-environment interactions, including smoking, which could stimulate COX-2 expression and be considered as the major risk factor for lung cancer.<sup>25</sup>

In the current study, the findings of multivariate logistic regression analysis adjusted for the confounding variables revealed a significant correlation between smoking and family history of cancer with bladder cancer risk. These findings are consistent with those of earlier studies, in which a positive family history of cancer has been associated with increased risk of bladder cancer.<sup>5,28</sup>

Similar to other studies, our work also encountered certain limitations, namely cross-sectional design and low number of participants.

### Conclusion

We concluded that COX-2 polymorphisms do not contribute to the genetic susceptibility to urothelial bladder cancer in an Iranian population. The findings of the current study could be compared to experimental evidences from different ethnic populations. Therefore, further investigation is required to explore whether COX-2 polymorphisms could be considered as a potential diagnostic biomarker for early diagnosis of bladder cancer or a prognostic factor for prediction of disease outcome. Moreover, our findings showed that both smoking and family history of cancer have

potential roles in susceptibility to bladder cancer risk.

### Acknowledgements

We would like to thank all the members of Uro-Oncology Research Center for their helpful discussions and friendly support. This study was supported by Tehran University of Medical Sciences Tehran, Iran (Grant no: 97-01-114-37205).

### Conflict of Interest

None declared.

### References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin.* 2018;68(1):7-30. doi: 10.3322/caac.21442.
2. Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder cancer incidence and mortality: A global overview and recent trends. *Eur Urol.* 2017;71(1):96-108. doi: 10.1016/j.eururo.2016.06.010.
3. Rafiemanesh H, Lotfi Z, Bakhtazad S, Ghoncheh M, Salehiniya H. The epidemiological and histological trend of bladder cancer in Iran. *J Cancer Res Ther.* 2018;14(3):532-6. doi: 10.4103/0973-1482.172134.
4. Wan GX, Chen P, Yu XJ, Di QS, Yu YD, Lei JH, et al. Cyclooxygenase-2 polymorphisms and bladder cancer risk: a meta-analysis based on case-control studies. *Int J Clin Exp Med.* 2015;8(3):3935-45.
5. Lin J, Spitz MR, Dinney CP, Etzel CJ, Grossman HB, Wu X. Bladder cancer risk as modified by family history and smoking. *Cancer.* 2006;107(4):705-11.
6. Wu X, Gu J, Grossman HB, Amos CI, Etzel C, Huang M, et al. Bladder cancer predisposition: a multigenic approach to DNA-repair and cell-cycle-control genes. *Am J Hum Genet.*

- 2006;78(3):464-79. doi: 10.1086/500848.
7. Gangwar R, Mandhani A, Mittal RD. Functional polymorphisms of cyclooxygenase-2 (COX-2) gene and risk for urinary bladder cancer in North India. *Surgery*. 2011;149(1):126-34.
  8. Chang WS, Yang MD, Tsai CW, Cheng LH, Jeng LB, Lo WC, et al. Association of cyclooxygenase 2 single-nucleotide polymorphisms and hepatocellular carcinoma in Taiwan. *Chin J Physiol*. 2012;55(1):1-7. doi: 10.4077/CJP.2012.AMM056.
  9. Pereira C, Sousa H, Ferreira P, Frago M, Moreira-Dias L, Lopes C, 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(34):5473-8. doi: 10.3748/wjg.v12.i34.5473.
  10. Papafili A, Hill MR, Brull DJ, McAnulty RJ, Marshall RP, Humphries SE, et al. Common promoter variant in cyclooxygenase-2 represses gene expression: evidence of role in acute-phase inflammatory response. *Arterioscler Thromb Vasc Biol*. 2002;22(10):1631-6. doi: 10.1161/01.atv.0000030340.80207.c5.
  11. Cheng I, Liu X, Plummer SJ, Krumroy LM, Casey G, Witte JS. COX2 genetic variation, NSAIDs, and advanced prostate cancer risk. *Br J Cancer*. 2007;97(4):557-61. doi: 10.1038/sj.bjc.6603874.
  12. 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. doi: 10.1016/j.oraloncology.2010.06.002.
  13. Wang W, Fan X, Zhang Y, Yang Y, Yang S, Li G. Association between COX-2 polymorphisms and lung cancer risk. *Med Sci Monit*. 2015;21:3740-7. doi: 10.12659/msm.894839.
  14. Tomitão MTP, Nahas SC, Kubrusly MS, Furuya TK, Diniz MA, Marie SKN, et al. Cyclooxygenase-2 gene polymorphisms and susceptibility to colorectal cancer in a Brazilian population. *J Gastrointest Oncol*. 2017;8(4):629-635. doi: 10.21037/jgo.2017.03.05.
  15. Luo MX, Long BB, Li F, Zhang C, Pan MT, Huang YQ, et al. Roles of Cyclooxygenase-2 gene -765G > C (rs20417) and -1195G > A (rs689466) polymorphisms in gastric cancer: A systematic review and meta-analysis. *Gene*. 2019;685:125-35. doi: 10.1016/j.gene.2018.10.077.
  16. 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(2):565-76. doi: 10.1016/j.gastro.2005.05.003.
  17. Tan W, Wu J, Zhang X, Guo Y, Liu J, Sun T, et al. Associations of functional polymorphisms in cyclooxygenase-2 and platelet 12-lipoxygenase with risk of occurrence and advanced disease status of colorectal cancer. *Carcinogenesis*. 2007;28(6):1197-201. doi: 10.1093/carcin/bgl242.
  18. Liu L, Zhou F, Ren S, Chen X, Li X, Li W, et al. Prognostic value of cyclooxygenase-2 gene polymorphisms in advanced non-small cell lung cancer patients treated with first-line platinum-based chemotherapy. *Asia Pac J Clin Oncol*. 2016;12(2):e339-46. doi: 10.1111/ajco.12258.
  19. Ben Nasr H, Chahed K, Bouaouina N, Chouchane L. PTGS2 (COX-2) -765 G > C functional promoter polymorphism and its association with risk and lymph

- node metastasis in nasopharyngeal carcinoma. *Mol Biol Rep.* 2009;36(1):193-200. doi: 10.1007/s11033-007-9166-3.
20. Chang WS, Tsai CW, Ji HX, Wu HC, Chang YT, Lien CS, et al. Associations of cyclooxygenase 2 polymorphic genotypes with bladder cancer risk in Taiwan. *Anticancer Res.* 2013;33(12):5401-5.
  21. Koh WP, Yuan JM, van den Berg D, Lee HP, Yu MC. Interaction between cyclooxygenase-2 gene polymorphism and dietary n-6 polyunsaturated fatty acids on colon cancer risk: the Singapore Chinese Health Study. *Br J Cancer.* 2004;90(9):1760-4. doi: 10.1038/sj.bjc.6601797.
  22. Li Q, Ma C, Zhang Z, Chen S, Zhi W, Zhang L, et al. Association between cyclooxygenase-2 (COX-2) 8473 T>C polymorphism and cancer risk: a meta-analysis and trial sequential analysis. *BMC Cancer.* 2018;18(1):847. doi: 10.1186/s12885-018-4753-3.
  23. Campa D, Zienolddiny S, Maggini V, Skaug V, Haugen A, Canzian F. Association of a common polymorphism in the cyclooxygenase 2 gene with risk of non-small cell lung cancer. *Carcinogenesis.* 2004;25(2):229-35.
  24. Gao J, Ke Q, Ma HX, Wang Y, Zhou Y, Hu ZB, et al. Functional polymorphisms in the cyclooxygenase 2 (COX-2) gene and risk of breast cancer in a Chinese population. *J Toxicol Environ Health A.* 2007;70(11):908-15. doi: 10.1080/15287390701289966.
  25. Langsenlehner U, Yazdani-Biuki B, Eder T, Renner W, Wascher TC, Paulweber B, et al. The cyclooxygenase-2 (PTGS2) 8473T>C polymorphism is associated with breast cancer risk. *Clin Cancer Res.* 2006;12(4):1392-4. doi: 10.1158/1078-0432.CCR-05-2055.
  26. Cox DG, Pontes C, Guino E, Navarro M, Osorio A, Canzian F, et al. Polymorphisms in prostaglandin synthase 2/cyclooxygenase 2 (PTGS2/COX2) and risk of colorectal cancer. *Br J Cancer.* 2004;91(2):339-43. doi: 10.1038/sj.bjc.6601906.
  27. Sørensen M, Autrup H, Tjønneland A, Overvad K, Raaschou-Nielsen O. A genetic polymorphism in prostaglandin synthase 2 (8473, T-->C) and the risk of lung cancer. *Cancer Lett.* 2005;226(1):49-54. doi: 10.1016/j.canlet.2005.03.037.
  28. Murta-Nascimento C, Silverman DT, Kogevinas M, García-Closas M, Rothman N, Tardón A, et al. Risk of bladder cancer associated with family history of cancer: do low-penetrance polymorphisms account for the increase in risk? *Cancer Epidemiol Biomarkers Prev.* 2007;16(8):1595-600. doi: 10.1158/1055-9965.EPI-06-0743.

Table 1. Demographic characteristics of the cases and controls and Univariate Logistic Regression model

<b>Variable</b>		<b>Case (%)</b>	<b>Control (%)</b>	<b>Crude OR (95% CI*)</b>	<b>P-Value</b>
<b>Age (year)</b>	≤55	16 (20)	20 (25)	Reference	0.450
	>55	64 (80)	60 (75)	1.33 (0.63 -2.81)	
<b>Sex</b>	Female	16 (20)	18 (22.5)	Reference	0.699
	Male	64 (80)	62 (77.5)	1.16 (0.54 -2.48)	
<b>Smoking</b>	No	19 (23.8)	33 (41.3)	Reference	<b>0.019</b>
	Yes	61 (76.2)	47 (58.7)	2.25 (1.14 – 4.45)	
<b>Family History of Cancer</b>	No	21 (26.3)	48 (60)	Reference	<b>&lt;0.001</b>
	Yes	59 (73.7)	32 (40)	4.21 (2.15 – 8.23)	
<b>Type of Bladder Tumor</b>	HG TCC	36 (45)			
	LG TCC	44 (55)			
<b>Type of Treatment</b>	RC	13 (16.2)			
	TURBT	67 (83.8)			
<b>Muscle Invasion</b>	No	34 (42.5)			
	Yes	46 (57.5)			
<b>Tumor Recurrence</b>	No	26 (32.5)			
	Yes	54 (67.5)			

\*CI: Confidence Interval, **RC**: Radical cystectomy; **TURBT**: trans urethral resection of bladder tumor; **HG TCC**: High grade transitional cell carcinoma; **LG TCC**: Low grade transitional cell carcinoma



Table 2. Genotype and allele frequencies of the *COX-2* gene polymorphism among the cases and controls (Univariate Logistic Regression model)

Variable		Case (%)	Control (%)	Crude OR (95% CI*)	P-Value
<b>COX-2 765G&gt;C (rs20417)</b>	GG	49 (61.3)	61 (76.2)	Reference	-
	GC	30 (37.5)	18 (22.5)	2.07 (1.03 – 4.15)	<b>0.04</b>
	CC	1 (1.3)	1 (1.3)	1.24 (0.07 – 20.41)	0.878
<b>COX -2 8473T&gt;C (rs5275)</b>	TT	33 (41.3)	27 (33.8)	Reference	
	TC	34 (42.5)	42 (52.5)	0.66 (0.33 – 1.31)	0.235
	CC	13 (16.3)	10 (12.5)	1.06 (0.40 – 2.80)	0.901
<b>COX -2 1195G&gt;A (rs689466)</b>	GG	64 (80)	57 (71.2)	Reference	
	GA	14 (17.5)	22 (27.5)	0.56 (0.26 – 1.21)	0.143
	AA	2 (2.5)	1 (1.3)	1.78 (0.16 – 20.17)	0.641
<b>COX-2 1290A&gt;G</b>	AA	62 (77.5)	55 (68.8)	Reference	
	AG	15 (18.8)	21 (26.3)	0.63 (0.30 – 1.35)	0.237
	GG	3 (3.8)	3 (3.8)	0.88 (0.17 – 4.58)	0.886
<b>Intron 1 (rs2745557)</b>	GG	56 (70)	51 (63.8)	Reference	
	GA	23 (28.7)	26 (32.5)	0.80 (0.41 – 1.58)	0.532
	AA	1 (1.3)	3 (3.8)	0.30 (0.03 – 3.01)	0.309
<b>Intron 5 (rs16825748)</b>	TT	66 (82.5)	67 (83.2)	Reference	
	AT	12 (15)	8 (10)	1.52 (0.58 – 3.96)	0.389
	AA	2 (2.5)	5 (6.3)	0.41 (0.07 – 2.16)	0.292
<b>Intron 6 (rs2066826)</b>	GG	70 (87.5)	74 (92.4)		
	GA	7 (8.8)	5 (6.3)	1.48 (0.45 – 4.88)	0.520
	AA	3 (3.7)	1 (1.3)	3.17 (0.32 – 31.21)	0.323

\*CI : Confidence Interval

Table 3. Odds ratio and 95% confidence interval derived from Multivariate Logistic Regression model

Variable		Case (%)	Control (%)	Crude OR (95% CI*)	P-Value
<b>Smoking</b>	No	19 (23.8)	33 (41.3)	Reference	<b>0.025</b>
	Yes	61 (76.2)	47 (58.7)	2.36 (1.11 – 5.01)	
<b>Family History of Cancer</b>	No	21 (26.3)	48 (60)	Reference	<b>&lt;0.001</b>
	Yes	59 (73.7)	32 (40)	4.04 (1.99 – 8.19)	
<b>COX-2 765G&gt; (rs20417)</b>	GG	49 (61.3)	61 (76.2)	Reference	
	GC	30 (37.5)	18 (22.5)	2.02 (0.94 – 4.37)	0.072
	CC	1 (1.3)	1 (1.3)	0.42 (0.02 – 7.29)	0.556
<b>COX -2 1195G&gt;A (rs689466)</b>	GG	64 (80)	57 (71.2)	Reference	
	GA	14 (17.5)	22 (27.5)	0.47 (0.20 – 1.09)	0.079
	AA	2 (2.5)	1 (1.3)	0.68 (0.05 – 8.34)	0.769

\*CI : Confidence Interval

Table 4. The association between the type of bladder tumor with genotype and allele frequencies of the *COX-2* gene polymorphism in the case group (Chi-squared Test)

		<b>COX-2 765G&gt; (rs20417)</b>			<i>P</i> -Value
		<b>GG (%)</b>	<b>GC (%)</b>	<b>CC (%)</b>	
<b>Bladder Tumor</b>	<b>HG TCC</b>	20 (55.6)	15 (41.7)	1 (2.8)	0.392
	<b>LG TCC</b>	29 (65.9)	15 (34.1)	0 (0.0)	
		<b>COX -2 8473T&gt;C (rs5275)</b>			<i>P</i> -Value
		<b>TT (%)</b>	<b>TC (%)</b>	<b>CC (%)</b>	
<b>Bladder Tumor</b>	<b>HG TCC</b>	14 (38.9)	12 (33.3)	10 (27.8)	<b>0.034</b>
	<b>LG TCC</b>	19 (43.2)	22 (50.0)	3(6.8)	
		<b>COX -2 1195G&gt;A (rs689466)</b>			<i>P</i> -Value
		<b>GG (%)</b>	<b>GA (%)</b>	<b>AA (%)</b>	
<b>Bladder Tumor</b>	<b>HG TCC</b>	26 (72.2)	9 (25.0)	1 (2.8)	0.270
	<b>LG TCC</b>	38 (86.4)	5 (11.4)	1 (2.3)	
		<b>COX-2 1290A&gt;G</b>			<i>P</i> -Value
		<b>AA (%)</b>	<b>AG (%)</b>	<b>GG (%)</b>	
<b>Bladder Tumor</b>	<b>HG TCC</b>	25 (69.4)	10 (27.8)	1 (2.8)	0.169
	<b>LG TCC</b>	37 (84.1)	5 (11.4)	2 (4.5)	
		<b>Intron 1 (rs2745557)</b>			<i>P</i> -Value
		<b>GG (%)</b>	<b>GA (%)</b>	<b>AA (%)</b>	
<b>Bladder Tumor</b>	<b>HG TCC</b>	27 (75.0)	9 (25.0)	0 (0.0)	0.504
	<b>LG TCC</b>	29 (65.9)	14 (31.8)	1 (2.3)	
		<b>Intron 5 (rs16825748)</b>			<i>P</i> -Value
		<b>TT (%)</b>	<b>AT (%)</b>	<b>AA (%)</b>	
<b>Bladder Tumor</b>	<b>HG TCC</b>	30 (83.3)	5 (13.9)	1 (2.8)	0.961
	<b>LG TCC</b>	36 (81.8)	7 (15.9)	1 (2.3)	
		<b>Intron 6 (rs2066826)</b>			<i>P</i> -Value
		<b>GG (%)</b>	<b>GA (%)</b>	<b>AA (%)</b>	
<b>Bladder Tumor</b>	<b>HG TCC</b>	31 (86.1)	3 (8.3)	2 (5.6)	0.742
	<b>LG TCC</b>	39 (88.6)	4 (9.1)	1 (2.3)	

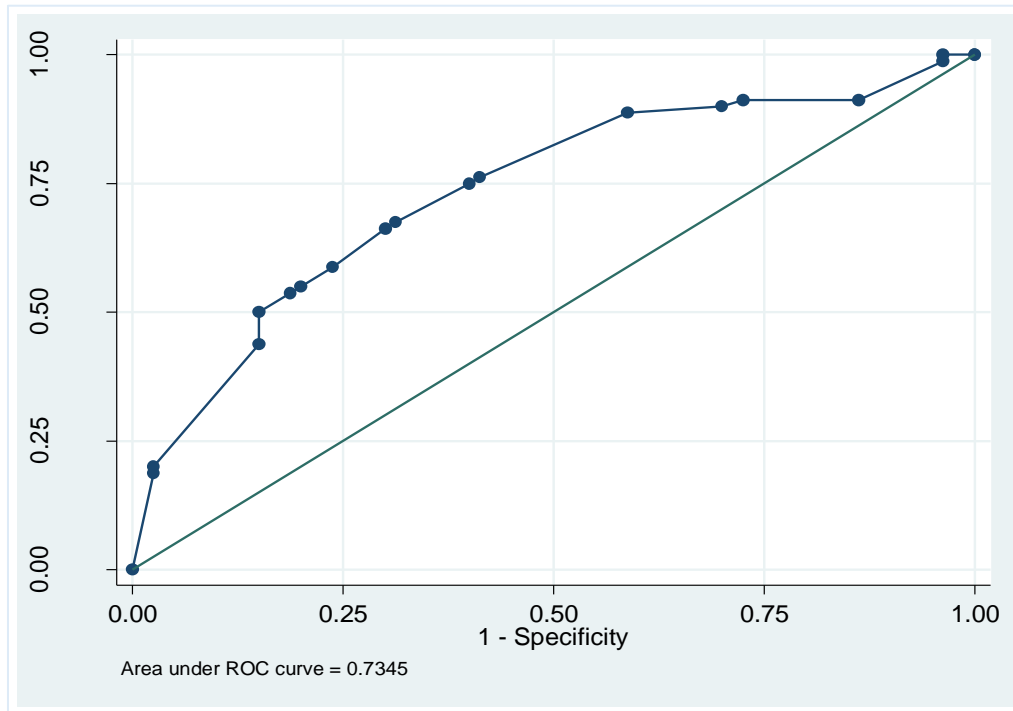


Figure 1. The discriminative ability of the multivariable logistic regression analysis was assessed by the area under a ROC curve.  
ROC: Receiver operator characteristic