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

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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.

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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.[1,2] It is the seventh most prevalent cancer among men worldwide and the second in Iran as a Middle Eastern country.[3] The prevalence of bladder cancer considerably varies in different regions, ranging from 5.9 in Asia to 23.9 in Europe.[4] Despite the improvements in cancer treatment, survival rate after radical cystectomy still remains unchanged.[5] 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.[6] Smoking and family history of cancer are also considered to be important environmental risk factors for cancer.[5] There are a few studies on the association between these factors and the risk of bladder cancer, whose results are inconsistent.[5]

Genetic polymorphisms have a potential role in the susceptibility to bladder cancer.[6] 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 3í-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.[7] 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.[8] Overexpression of *COX-2* gene can result in both proliferation increase and apoptosis inhibition, which have been shown in several types of human cancers.[9] 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 SNPs.[10] 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.[8] 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.[8] 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.

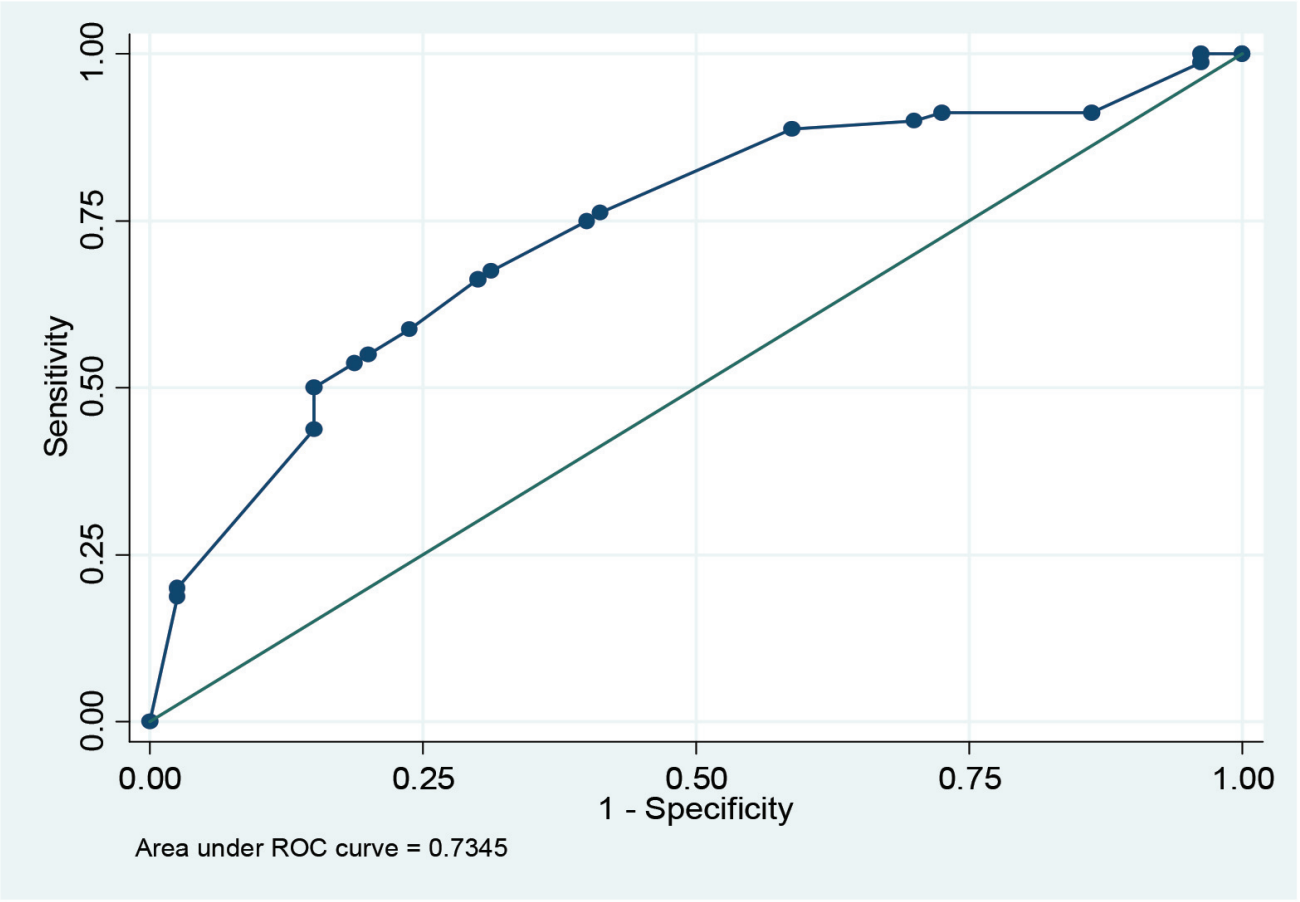

Figure 1. The discriminative ability of the multivariable logistic regression analysis was assessed by the area under a ROC curve.
ROC: Receiver operator characteristic

Table 1. Demographic characteristics of the cases and controls and univariate logistic regression model

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

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 a 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

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
COX-2 765G>C (rs20417)	GG	49 (61.3)	61 (76.2)	Reference	-
	GC	30 (37.5)	18 (22.5)	2.07 (1.03 - 4.15)	0.04
	CC	1 (1.3)	1 (1.3)	1.24 (0.07 - 20.41)	0.878
COX -2 8473T>C (rs5275)	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
COX -2 1195G>A (rs689466)	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
COX-2 1290A>G	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
Intron 1 (rs2745557)	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
Intron 5 (rs16825748)	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
Intron 6 (rs2066826)	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; OR: Odds ratio; COX-2: Cyclooxygenase-2

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

Table 3. OR and 95% CI derived from multivariate logistic regression model

Variable		Case (%)	Control (%)	Crude OR (95% CI)	*P*-Value
Smoking	No	19 (23.8)	33 (41.3)	Reference	0.025
	Yes	61 (76.2)	47 (58.7)	2.36 (1.11 - 5.01)	
Family History of Cancer	No	21 (26.3)	48 (60)	Reference	<0.001
	Yes	59 (73.7)	32 (40)	4.04 (1.99 - 8.19)	
COX-2 765G> (rs20417)	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
COX -2 1195G>A (rs689466)	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; OR: Odds ratio; COX-2: Cyclooxygenase-2

of cancers, such as non-small cell lung cancer (NSCLC), esophageal squamous cell carcinoma, and prostate cancer.[11-13] 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.[14] 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.[10] 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.[10] 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,[15] esophageal cancer,[16] colorectal cancer,[17] NSCLC[18] and nasopharyngeal carcinoma (NPC)[19], has been previously indicated. The association between rs20417 variant and enhanced risk of bladder cancer has been indicated in Taiwanese, Indians, and Chinese populations.[7,20] On the contrary, a decreased susceptibility to bladder cancer has been reported for rs20417 polymorphism in American population.[4] Furthermore, enhanced risk of colon cancer among Singapore Chinese has been reported to be associated with carrying -765-C allele.[21] However, carrying -765-C allele have been associated with reduced colon cancer risk in American Caucasians.[19] 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.[22] 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.[4] Several previous studies

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)

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

COX-2: Cyclooxygenase-2; HGTCC: High grade transitional cell carcinoma; LG TCC: Low grade transitional cell carcinoma

have also shown the association between 8473C>C genotype and higher risk of non-small cell lung cancer,[23] breast cancer,[24,25] and colorectal cancer.[26] However, some other researchers have explained a protective role of this polymorphism in lung cancer.[27] 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.[25]

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.[5,28]

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.

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Conflict of Interest

None declared.

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