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Original Article

Detection of CYP1A1 and GSTP1 gene polymorphisms in bladder cancer patients in a Turkish population using a polymerase chain reaction-restriction fragment length polymorphism method

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ABSTRACT

Objective: Understanding genetic polymorphisms might facilitate the analysis of differences between individuals in their susceptibility to developing cancers as a result of environmental carcinogens. Skin, lung, colon and bladder cancers emerge from biological defects in GSTM1, GSTT1 and GSTP1 gene expressions. In this study, we aimed to investigate whether there was an association between CYP1A1 and GSTP1 gene polymorphisms and bladder cancer in a Turkish population.

Material and methods: Blood samples were collected from 120 individuals (60 patients with bladder cancer and 60 healthy individuals), and their DNAs were isolated. A polymerase chain reaction-restriction fragment length polymorphism (PCR - RFLP) method was used to detect the frequencies of CYP1A1 NM_000499.3: c.*1189T > C and GSTP1 NM_000852.3: c.313A > G polymorphisms in bladder cancer patients.

Results: The frequency of the CYP1A1: c.*1189 TC genotype and C allele were significantly different between bladder cancer patients and healthy individuals (p=0.001 and p=0.005, respectively). However, there was no significant difference for the GSTP1: c.313 AG genotype or G allele between both study groups (p=0.699 and p=0.360, respectively).

Conclusion: A polymorphic site of the CYP1A1 gene might be involved in the development of bladder cancer. However, the investigated GSTP1 polymorphic site did not represent an important risk factor for the development of bladder cancer in a Turkish population.

Keywords: Bladder cancer; cytochrome; gene; glutathione; polymorphism.

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Introduction

Bladder cancer is the 11th most commonly diagnosed cancer in the world, and the age-standardized incidence rate (per 100,000 person-years) is 8.9 for men and 2.2 for women.[1] The mutual interaction of various genetic and carcinogenic factors is thought to be an important determinant. Genetic polymorphisms causing changes in enzyme activities leading to biotransformation were reported to have a major role in the development and progression of cancer.[2] Endogenous and exogenous xenobiotics are activated and deactivated in two metabolic steps by phase I and phase II enzymes. Human P450 enzymes are oxidative enzymes with a role in the activation and deactivation of anticancer agents and the activation of various precarcinogen-metabolic conversions.

Smoking is the most important factor assumed to cause bladder cancer. The relationship between bladder cancer and smoking has been revealed by many epidemiological studies.[3,4] Smoking is the main risk factor for bladder cancer for both men and women and it is responsible for 1/3 of bladder cancers in men and for 1/4 in women. Cigarettes have over

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60 carcinogen compounds, including polycyclic aromatic hydrocarbons (PAH), such as benzopyrene, and aromatic amines, such as 2 - naphthylamine and 4 - aminodiphenyl.[5] The emergence of hazardous effects of these metabolic compounds depends on metabolic activation.[6] In particular, the carcinogenic effects of PAHs emerge from the stimulation of the metabolic activation that occurs as a result of the epoxidation and hydrolysis of these agents.[5] The metabolic activation of PAHs and aromatic amines is followed by the binding of their metabolites to the DNA structure, which elicits the main tumorigenic effects. Enzymes, generally within the cytochrome P450 (CYP450) family, initiate the metabolic effects of PAHs. In this first step, xenobiotics are transformed into hydrophilic and disposable derivations. In particular, CYP450 enzymes work as catalysts in the oxidation of many endogenous and exogenous compounds. Although most of these metabolites transform into detoxified forms, some gain electrophilic characteristics and interact with DNA.[7] The human *CYP1A1* gene participates in the activation of tobacco and pro-carcinogens, including PAH and aromatic hydrocarbons, and it was reported as a potential genetic biomarker for some cancer types.[8] More than 11 alleles (gene pairs) that code amino acid changes have been identified for the *CYP1A1* gene.[9] The effects of *CYP1A1* gene polymorphisms on the risk of bladder cancer remain controversial in different populations. A meta-analysis revealed that the c.*1189C > T polymorphism is not associated with bladder cancer risk in Chinese, Turkish, and French populations.[10] However, several studies have indicated that the *CYP1A1* gene might be strongly correlated with an increased risk of bladder cancer in populations of China and North India.[11,12]

Phase II enzymes (epoxide hydrolase, glutathione S-transferase, sulfotransferase, glucuronosyltransferase) conjugate with glutathione and glucuronides to produce excretable hydrophilic products, which results in the detoxification of primary metabolites. In total, the balance between these activation and deactivation systems determines the biologically active toxic dose and reveals the disease risk.[13] Deletions occurring in either the *GSTM1* or *GSTT1* genes cause the total loss of enzyme activity. Any amino acid change in the *GSTP1* gene causes a change in the activation of this enzyme.[14] Skin, lung, colon and bladder cancers emerge from the biological outcomes of the defects occurring in *GSTM1*, *GSTT1* and *GSTP1* gene expressions.[15,16]

Some individuals have increased cancer risk depending upon DNA damage. Understanding genetic polymorphisms might facilitate the analysis of differences between individuals in terms of susceptibility to developing cancers as a result of environmental carcinogens. Obtaining information about gene pair variants in the metabolism of xenobiotics, DNA damage and events causing mutations may help in the diagnosis and treatment of bladder cancer. Many studies have shown a relationship between biometabolism and bladder cancer.[14,17] This case-control study examined genetic polymorphisms in the *CYP1A1* and *GSTP1* genes and their relationship with bladder cancer.

Material and methods

Patients

The study included 60 patients with bladder cancer and 60 healthy individuals. Patients with bladder cancer were diagnosed as having transitional cell carcinoma by histopathological examination. Blood samples were collected from 60 patients after bladder cancer diagnosis. Also blood samples from 60 healthy humans were taken and analyzed as the control group. All patients in the study group were informed about the study after taking their detailed medical history. All patients gave written informed consent before being recruited. The study was approved by the ethics committee of the Medical Faculty of Harran University (Şanlıurfa, Turkey). Exclusion criterion was receival of any form of radiotherapy or chemotherapy for the treatment of cancer.

DNA extraction

In this study, blood samples were collected in both groups and DNA was extracted according to the salting out technique from the bloods with EDTA. In the PCR, 30 ng DNA was used for each patient.[18]

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) Technique

The *CYP1A1* (NM_000499.3; GI: 1543):c.*1189T > C (rs4646903, in 3'-untranslated region (3'-UTR))and *GSTP1* (NM_000852.3; GI: 2950):c.313A > G (rs1695, in exon 5) polymorphic sites were investigated by the PCR - RFLP technique. Four primers (forward and reverse) were used to determine the genotype and allele status of both genes (Table 1). With the selected primers, the DNA was amplified in a 10 - μl reaction volume containing 1xPCR buffer, 2 mM $MgCl_2$, 0.2 μM primers (BioBasic Inc, Ontario, Canada), 200 μM each deoxynucleotide triphosphate (dNTPs, Fermentas, St. Leon-Rot, Germany), 30 ng of genomic DNA, and 0.5 U of Taq DNA polymerase (Fermentas). For the*CYP1A1*: c.*1189T > C polymorphic site, the PCR program was applied at 94°C for 3 min for the initial denaturation, then 35 cycles with 94°C for 45 sec, 59°C for 45 sec, and 72°C for 45 sec, and finally 72°C for 5 min for the final extension. For the *GSTP1*: c.313A > G polymorphism, the PCR program was the same but with an annealing temperature of 60°C.

Ten microliters of PCR product in a 30-μL volume for *CYP1A1*: c.*1189T > C and *GSTP1*: c.313A > G were separately digested with 1.5 Units of *Msp*I (*Hpa*II) and *Alw26*I (*Bsm*AI) (Fermentas, St. Leon-Rot, Germany), respectively, at 37°C for 2 hours.

Table 1. Primer sequences selected on CYP1A1 ve GSTP1 genes

Primers	Primer sequences	PCR products (bp)
CYP1A1: c.*1189T > C	5'-CAGTGAAGAGGTGTAGCCGC-3'	342
	5'-TAGGAGTCTTGTCTCATGCC-3'	
GSTP1: c.313A > G	5'-ACCCCAGGGCTCTATGGGAA-3'	176
	5'-TGAGGGCACAAGAAGCCCCT-3'	

Table 2. Distribution of clinical and demographical parameters in patients with bladder cancer and healthy controls

	Patients with bladder cancer	Healthy control group	p
Individuals (n)	60	60	
Gender (M/F)	52/8	51/9	1.000
Age (years)	64.3±12.7	63.6±12.7	0.775
BMI (kg/m^2)	25.8±3.9	26.7±4.6	0.260
Diabetics	6	16	0.32
Smoking	53	39	0.035
Hypertension	21	27	0.352

Average ± standard deviation, M: male; F: female

The digested PCR products were separated on a 2% agarose gel prepared with 10 mM lithium borate and analyzed using the Alpha Imager System (AlphaInnotech, San Leandro, California, USA). The digested *CYP1A1*: c.*1189 T allele yielded two fragments of 209 and 133 bp, and the C allele yielded a 342 bp fragment (Figure 1).

The *GSTP1*: c.313 A allele yielded a fragment of 176 bp, and the G allele yielded three fragments of 176, 93, and 83 bp (Figure 2).

Statistical analysis

Student's *t* - test and chi-square tests were used to determine differences in the means of the demographic and clinical profiles. The genotype and allele frequencies of *CYP1A1*: c.*1189T > C and *GSTP1*: c.313A > G were tested for Hardy-Weinberg Equilibrium using the chi-square test. The genotype and allele frequencies of these polymorphisms were analyzed with Fisher's exact test using the Statistical Package for the Social Sciences 11.0 (SPSS In.; Chicago, IL, USA). Statistical significance was defined as $p< 0.05$.

Results

With regard to the patient characteristics, the number of patients; patient gender, age, and body mass index; and the number of patients with diabetes and hypertension were similar between the two groups. Only the number of smoking patients was significantly different ($p<0.05$). The baseline characteristics of the patients and controls are presented in Table 2.

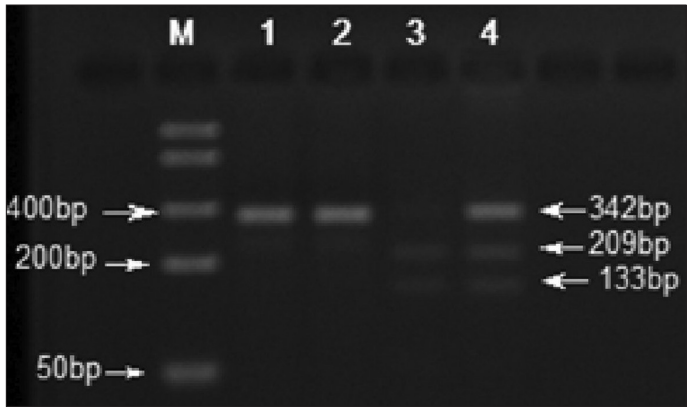

Figure 1. The restriction profile of CYP1A1: c.*1189T > C (MspI, HpaIIC > T). DNA Ladder (50-1500bp, Bio Basic Inc., Canada); lane 1 : undigested PCR product; lane 2: CC genotype (homozygous, polymorphic) ; lane 3 : TT genotype (homozygous, wild type); and lane 4 : TC genotype (heterozygous)

The distribution of the genotypes for the *CYP1A1*: c.*1189T > C and *GSTP1*: c.313A > G polymorphic sites were consistent with Hardy-Weinberg equilibrium in the bladder cancer and control groups ($p>0.05$). For *CYP1A1*: c.*1189, the TC heterozygous genotype and C allele frequencies in bladder cancer individuals were higher than those in healthy individuals (48.3% and 25.8% vs. 20.0% and 11.4% respectively, $p<0.05$). This difference was found to be significant. Furthermore, there was no significant association between bladder cancer patients and healthy controls for the *GSTP1*: c.313 GG genotype (5.0% vs. 8.3%) or G allele (20.8% vs. 25.8%) frequencies (Table 3).

Discussion

Genetic differences between detoxification systems in humans affect the extent to which environmental factors are involved in bladder cancer. Our study primarily examined the frequency of three genotypes of the *CYP1A1* and *GSTP1* genes in bladder cancer patient and control groups.

The relationship between occupational exposure to smoking and aryl amines has been well defined in terms of bladder cancer etiology.[19] Individual differences in the occurrence of cancer were defined by polymorphic variables in the bioactivation and

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Table 3. SNP polymorphisms in patients with bladder cancer and control group

SNPgenotype/allele	Bladder cancer patients (n=60)	Healthy control (n=60)	X^2	OR (95% CI)	p
CYP1A1: c.*1189T > C					
TT	30 (50.0%)	47 (78.3%)	10.474	Reference	Reference
TC	29 (48.3%)	12 (20.0%)	10.707	3.742 (1.664 - 8.414	0.001
CC	1 (1.7%)	1 (1.7%)	0.000	1.000 (0.061 -16.366)	1.000
T	89 (74.2%)	106 (88.3%)	7.904	Reference	Reference
C	31 (25.8%)	14 (11.4%)	7.904	2.637 (1.321 - 5.264)	0.005
GSTP1: c.313A > G					
AA	38 (63.3%)	34 (56.7%)	0.556	Reference	Reference
AG	19 (31.7%)	21 (35.0%)	0.150	0.861 (0.403 - 1.840)	0.699
GG	3 (5.0%)	5 (8.3%)	0.536	0.579 (0.132 - 2.540)	0.464
A	95 (79.2%)	89 (74.2%)	0.839	Reference	Reference
G	25 (20.8%)	31 (25.8%)	0.839	0.756 (0.414 - 1.378)	0.360

X^2: Chi-square; OR: odds ratio; CI: confidence interval; SNP: single nucleotide polymorphism

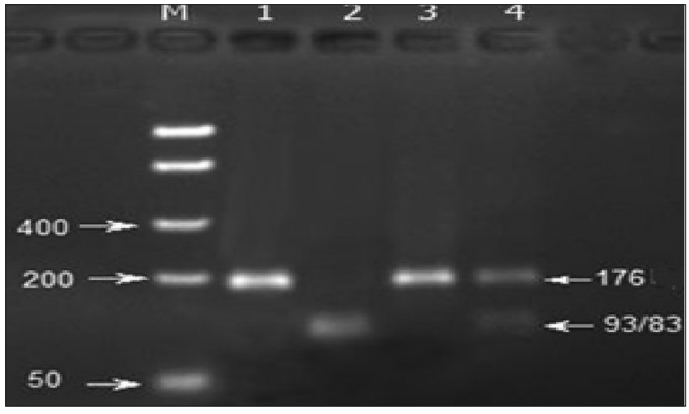

Figure 2. The restriction profile of the GSTP1: c.313A > G (Alw26I, BsmAI A>G). DNA Ladder (50-1500bp, Bio Basic Inc., Canada); lane 1: undigested PCR product; lane 2: AG genotype (heterozygous); lane 3: AA genotype (homozygous, wild type); and lane 4: GG genotype (homozygous, polymorphic) (the 93-and 83-bp fragments could not be separated from each other on the gel)

detoxification of carcinogens.[20] For example, cigarette smoke contains many carcinogenic compounds, including PAH and aryl amines, and these are metabolized by the CYP450 and GST enzyme families. With regards to PAH, it is not known whether the changes in the catalytic activities enabling the oxidation of xenobiotics are caused by amino acid changes in the CYP450 enzymes.[9] Some studies have shown that *CYP1A1* gene polymorphisms convey bladder cancer risk in some Asian populations.[11,12] Our study have demonstrated that the c.*1189 TC (heterozygote) genotype of the *CYP1A1* gene was significantly higher in patients (48.3%) than in the controls (20%). Additionally, the c.*1189 C allele rates of the *CYP1A1* gene were found to be significantly higher in patients (25.8%) than in the controls (11.4%). However, a meta-analysis has indicated that the *CYP1A1* gene c.*1189C > T polymorphism is not associated with bladder cancer risk in Chinese, Turkish, or French populations.[10] The effects of *CYP1A1* gene polymorphisms on the risk of bladder cancer remain controversial in different populations.

However, no relationship was observed between the genotype and allele rates of the c.313A > G polymorphism of the *GSTP1* gene and bladder cancer in the present study.

Although a relationship between the GSTP1 Ile Val genotype and bladder cancer was observed in a previous study performed in an Iranian population, this relationship was not observed in German or Japanese populations.[21] Epidemiological studies have revealed differences in the distribution frequency of alleles and polymorphic alleles of different ethnic groups.[22] There are few studies examining the relationship between bladder cancer and gene polymorphisms in Turkey. Altaylı et al.[23] investigated the relationship between bladder cancer and *CYP1A2*, *CYP2D6*, *GSTM1*, *GSTP1* and *GSTT1* gene polymorphisms. Although no relationship was found between *CYP1A2*, *CYP2D6*, *GSTM1*, or *GSTP1* gene polymorphisms and bladder cancer in this study, a statistical correlation was found with the *GSTT1* gene and bladder cancer (OR=3.94, 95% CI=1.70-9.38, $p<0.05$). In addition, a significant relationship was identified between smoking and bladder cancer. In our patient group, even though the relationship between the *CYP1A1* gene and bladder cancer was determined for the "TC" genotype, the "CC" genotype was not found to be significantly

different as it was found in only one case. No statistically significant relationship was found for any variation in the GSTP1 gene (OR=0.86; 95% CI=0.40-1.84). Although our findings are in agreement with those of study performed by Grando et al.[24] in Brazil, they differ from those reported by Altaylı et al.[23]

There is no consensus in the literature on the extent to which variant gene forms are effective in the activation and detoxification of carcinogens or on the sources of differences within populations. Although Agundez et al.[25] reported a relationship between some cytochrome P450 enzyme polymorphisms and various cancer types, they did not clearly state the relationship with bladder cancer. In a case-control study, Brockmöller et al.[26] investigated some relevant gene polymorphisms in terms of their effects on drug metabolism and did not find any relationship between *CYP1A1* gene polymorphisms and bladder cancer. Katoh et al.[27] found no relationship between *CYP1A1* gene polymorphisms and bladder cancer in a Japanese sample. Johns and Houlston[28] suggested that the *CYP1A1* polymorphism played a secondary role rather than creating a predisposition for cancer. Contrary to these findings, some studies have shown that the presence of a*CYP1A1* allele in combination with *GSTM1* null alleles is associated with an increase in cancer incidence.[28-30] In a study a relationship between a*GSTM1* polymorphism, which causes gene mutations over TP53, and bladder cancer was indicated.[31] However, we did not investigate this gene polymorphism in our study.

Single nucleotide polymorphisms are important as they stress that drug metabolism is not activated on its own, even though many studies have found no significant relationship between these polymorphisms and bladder cancer. This result indicates that carcinogenesis requires the interaction of more than one gene for genetic predisposition in bladder cancer. Naccarati et al.[32] reported that different relationships with genetic polymorphisms caused differing individual sensitivities to genotoxicity and carcinogens. The present study investigated a potential relationship between different genotype variants (*CYP1A1* and *GSTP1*) and bladder cancer.

Some recent studies have shown that *CYP1A1* protein levels increase in exfoliative urothelial cells in smokers.[33] It was reported that *CYP1A1*-mediated materials are deposited in bladder tissues in smokers. Our study found a positive relationship between bladder cancer and the *CYP1A1* C allele, and 28 patients (47%) in this group had a history of smoking. It is well known that bladder cancer is not only related to smoking but also it may be related to occupational exposure to carcinogens. In our study, no difference was found between the two gene groups according to gender in terms of the allele distribution ($p>0.05$).

Although some studies from other countries reported a relationship between *GSTP1* polymorphisms and bladder cancer, a Turkish study by Altaylı et al.[23] found no relationship. The results of our study support this finding. Contrary to our findings, Cao et al.[30] demonstrated a relationship between *GSTP1* II and IV gene variants and bladder cancer. Similar to the results of our study, some studies conducted in Turkey did not find any relationship between the *GSTP1* gene polymorphism and laryngeal squamous cancers, stomach cancer or colon cancer.[34,35] According to the comparable results of these studies, we can infer that, in Turkish society, there is no relationship between *GSTP1* gene polymorphisms and cancers.

Epidemiological studies show varying polymorphic allele distributions among different ethnic groups.[22] However, people in different origin commonly live in our city. Genetic variations associated with ethnicity may result in a non-homogenous distribution in the study and control groups and therefore different results may be obtained. A future study based on ethnic origins may address this issue.

One of the main deficiencies of our study is the small number of cases in our patient group. The "TC" (heterozygote) *CYP1A1* genotype was more frequently seen in patients (48.3%) than in healthy controls (20%). As the "CC" (homozygote) *CYP1A1* genotype was found only in one patient, it was not included in our study. A larger study cohort may help to reveal any potential relationship between the frequency of the "CC" homozygote *CYP1A1* genotype and bladder cancer.

In conclusion, the results of our study suggest that the bladder cancer risk is high in individuals with the "TC" allele of the *CYP1A1* gene, and predisposition to bladder cancer can be predetermined in these individuals. The*GSTP1* c.313A > G gene polymorphic site did not represent an important risk factor for the development of bladder cancer in a Turkish population. However, larger case series studies are required to generate further evidence for this assumption.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Harran University School of Medicine (Project No: 1044).

Informed Consent: Written informed consent was obtained from all patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.S., A.A.; Design - A.A., E.Y.; Supervision - E.Y., M.S.; Resources - K.G.; Materials - A.A., F.D.; Data Collection and/or Processing - F.D., M.M.U.; Analysis and/or Interpretation - A.A., F.D., M.S.; Literature Search - A.A., İ.K., D.A.; Writing Manuscript - A.A., M.S.; Critical Review - E.Y., M.S., A.A.; Other - K.G., İ.K.

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