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DNA Repair Gene Polymorphisms and Bladder Cancer Susceptibility in a Turkish Population

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Abstract. *Background: Occupational exposure and life style preferences, such as smoking are the main known environmental susceptibility factors for bladder cancer. A growing list of chemicals has been shown to induce oxidative DNA damage. Base excision repair (BER) genes (X-ray repair cross complementing 1, XRCC1 and human 8-oxoguanine DNA glycosylase 1, OGG1) may play a key role in maintaining genome integrity and preventing cancer development. Materials and Methods: We tested whether polymorphisms in XRCC1 and OGG1 are associated with bladder cancer risk by using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay. In addition, the possible modifying affect of cigarette smoking was evaluated. Results: No studies, to date, have examined the association between genetic polymorphisms in DNA repair genes and bladder cancer susceptibility, in the Turkish population. We found the OGG1 Cys326Cys genotype to be more frequent among bladder cancer patients (odds ratio (OR): 2.41 (95% CI, 1.36-4.25)). However, in the case of XRCC1, there was no significant difference in susceptibility to bladder cancer development between patients with the Arg399 and these with the Gln399 allele (OR: 0.72 (95% CI, 0.41-1.26)). Conclusion: Our data showed that OGG1 genetic polymorphisms might be useful as prognostic genetic markers for bladder cancer in the clinical setting.*

Bladder cancer is the sixth most common cancer in the United States, with 53200 incident cases estimated for the year 2000 (1). In the Turkish population, it is the third most common cancer in men and the eighth in women (2). Oxidative DNA damage induced by reactive oxygen species (ROS) is thought to be involved in the processes of carcinogenesis and aging (3). In the case of cancer, oxidative damage to DNA is associated with mutations that activate oncogenes or inactivate tumor suppressor genes (4). Cigarette smoking is the dominant risk factor for several epithelial cancers, including the lung, bladder, oral cavity, pharynx and larynx (5). Smoking constitutes the single most important cause of bladder cancer, with cigarette smokers having 2- to 4-fold higher incidences than nonsmokers, and causes direct and indirect damage to DNA (6, 7). The repair of DNA damage is under genetic control. DNA repair genes may play a key role in maintaining genome integrity and preventing cancer development. At least 135 single nucleotide polymorphism (SNPs) in 17 DNA repair and repair related genes have been identified, including genes for nucleotide excision repair (NER), base excision repair (BER), homologous recombination repair (HRR) for double-strand breaks, and cell-cycle check point. Amino acid substitution variants of DNA repair genes could alter either DNA repair capacity or fidelity, and may contribute to cancer susceptibility (8). Polymorphisms in DNA repair genes resulting in variation of DNA repair efficiency may therefore be associated with bladder cancer susceptibility (9). Among DNA damage induced by ROS, 8-hydroxyguanine (8-OHG) is highly mutagenic, yielding GC to TA transversions upon its replication by DNA polymerases. The primary pathway for the repair of 8-OHG is short patch BER (10). *X*-ray repair cross complementing 1 (XRCC1) protein probably participates in this pathway (11). Although the precise function of XRCC1 is not known, it may have an important role in DNA single-strand breaks (SSBR) and BER (12). XRCC1 is an abundant nuclear zinc finger protein and part of a DNA binding protein complex. Although the XRCC1 protein has no enzymatic activity itself, it interacts with key enzymes involved in BER including poly (*ADP*-ribose) polymerase, *DNA polymerase β*, *DNA ligase III*, and *AP* endonuclease (*APE1*), a rate-limiting enzyme in BER. Therefore, polymorphisms causing amino acid substitutions may impair the interaction of XRCC1 with other enzymatic proteins, and alter the BER activity (13). There are three polymorphisms in the XRCC1 gene, which result in amino acid changes at evolutionarily conserved regions. Another key component of BER pathway is human 8-oxoguanine DNA glycosylase 1 (*OGG1*) which catalyzes the

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removal of 8-OHdG. Studies on the genetic structure of *OGG1* revealed the presence of several polymorphisms within this locus (14). Among them, *OGG1 Ser326Cys* polymorphism is of particular interest, since it may play a major role in different kinds of cancers. The amino acid substitution from serine to cysteine in codon 326 is the result of a C/G substitution at position 1245 in the 1-specific exon 7 of *OGG1* (15). Several studies have suggested that the *Cys326* variant is associated with increased risk for lung, bladder, and stomach cancer (13, 16). In this study, we investigated the potential contribution of XRCC1 (*Arg399Gln*) and *OGG1* (*Ser326Cys*) polymorphisms to bladder cancer, in a case/control study in the Turkish population.

Materials and Methods

Five milliliters of peripheral blood was collected from 75 bladder cancer patients and 100 age-matched controls in EDTA containers *via* venipuncture. A detailed description of the patients has been published (17). The mean age of the bladder cancer patients was 59.87 years, with a standard deviation and range of 12.54 and 25-87 years, respectively; the mean age of the control group was 59.33 years, with a standard deviation and range of 13.58 and 23-79 years, respectively. The control subjects had no previous or present history of malignancies and were excluded from the study if there was any evidence of major organ disease, family history of cancer and or if taking any medication. The patients were diagnosed at the Hacettepe University Medical School and Ankara Numune Hospital, Turkey. Informed consent was obtained from all study participants. In addition to clinical information and tumor histopathology, subjects completed a questionnaire. Smoking status information was based on self report, and only 48 patients gave information on smoking preferences. Genomic DNA was extracted from whole blood using a sodium perchlorate / chloroform extraction method, as described elsewhere (18).

All patients and control subjects were screened for polymorphic sites both *OGG1* and *XRCC1* genes. A simple PCR-RFLP method was used to identify the *Ser326Cys* variant described by Karahalil and Kocabas (18), because the *C* to *G* transversion creates a new Fnu4HI restriction site. Briefly, a 207 bp fragment was amplified by PCR in a 30 μl reaction volume that contained 100 ng genomic DNA, 0.2 mM of dNTP, 15 mM $MgCl_2$, 18 pmol of the *OGG1* sense (5'-ACT GTCACTAGTCTCACCAG-3') and antisense (5'-TGAATTC GGAAGGTGCTTGGGGAAT-3') primers and 3 units of Taq DNA polymerase (Fermentas, Vilius, Lithuania). Cycling conditions were as follows: initial denaturation at 94˚C for 2 min, then amplification was done using 33 cycles of denaturation at 94˚C for 15 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 35 sec, followed by final extension at 72˚C for 3 min. Five μl of each PCR sample was digested with 6 units of Fnu4HI (Fermentas, Vilius, Lithuania) at 37˚C overnight and resolved on 6% polyacrylamide gels to detect 107 bp and 100 bp fragments for the *326Cys* allele in RFLP patterns. The digested products were photographed using a Genecam System (Spectronics GL-2000, MA, USA).

The *XRCC1* alleles were detected using PCR-RFLP which was a modification of the method described by Sturgis *et al.* (1999) (19). The sequences of the PCR primers were 5'-CAGTGGTGCTA ACCTAATC-3' (forward) and 5'-AGTAGTCTGCTGGCTCTGG-3' (reverse), to generate an 871 bp product. A 30 ml PCR reaction was performed using approx 0.2 to 0.5 μg genomic DNA, 0.25 mM each primer, 200 μM of each dNTP, 1.5 U Taq polymerase (Promega, Madison, WI, USA) in the 1xPCR buffer supplied by the manufacturer (50 mM KCl, 10 mM Tris-HCl, pH 9,0. 1% TritonX-100). After an initial melting step at 95˚C for 5 min, amplification was carried out for 35 cycles by denaturing at 95˚C for 30 min, annealing at 58˚C for 45 min, extending at 72˚C for 45 min, and a final extention at 72˚C for 10 min for 1 cycle. The PCR products were digested with Nci I restriction enzyme (New England Biolabs, UK) to distinguish the point mutation at 28152 of cDNA sequence at exon 10. The digestion of 6 μl of PCR products was carried out using 8U Nci I and the 1xNEB4 buffer at 37˚C overnight. The wild allele (*i.e.* 28152 *G*) has an Nci I restriction enzyme site and gives rise to two fragments of 461 and 182 bp. The mutant allele (i.e. 28152 A) loses the Nci I site resulting in a single 593 bp fragment. To analyze the restriction fragments, 3% 1:1 ratio of Agarose: Gamma microspore gel (Prone, Burgos, Spain) was used. The digested products were photographed using Genecam system (Spectronics GL-2000, MA, USA).

Statistical analysis. We used SPSS for statistical analysis including Fischer's exact test. We used standard methods for 2x2 contingency tables, including Fisher's exact test, as appropriate, to analyze categorical variables without adjustment for covariates. The strengths of associations between bladder cancer and the *OGG1* and *XRCC1* polymorphisms were measured as odds ratios (Ors). We classified *XRCC1* and *OGG1* genotypes as: homozygous wild-type (*Arg399Arg*), (*Ser326Ser*); heterozygous (*Arg399Gln*), (*Ser326Cys*); homozygous variant (*Gln399Gln*), (*Cys326Cys*). The *XRCC1* (*Arg399Arg*) and OGG1 (*Ser399Ser*) were designated as reference genotypes, since they are thought to have the highest enzymatic activity. We tested the genotype-genotype interaction, using Fisher's exact test.

Results

The frequency distributions of both genotypes and alleles for *XRCC1* and *OGG1* are shown in Tables I and II. In the case of *XRCC1 Arg399Gln* polymorphism, there was no significant difference in the frequency of the *399Gln* allele, between patients and controls [OR: 0.72 (95% CI, 0.41-1.26), $p=0.16$]. The role of the *Gln399Gln XRCC1* in bladder cancer susceptibility is unclear, because of inconsistent findings reported in the literature. Whereas some studies showed that the mutant allele had a protective effect in bladder cancer development, others found an increased risk (20). Neither a protective effect nor an increased risk due to the mutant allele was observed for bladder cancer in the present study (Table I). In the case of *OGG1 Ser326Cys* polymorphism, the mutant allele frequency among patients and controls was 0.36 and 0.28, respectively (Table II). We found that carriers of the *326Cys* allele were more frequent among bladder cancer patients with an OR of 2.41 (95% CI, 1.36-4.25, $p=0.002$). Stratification of the data according to smoking status did not change the results for either *XRCC1 Arg399Gln* or *OGG1 Ser326Cys* polymorphisms (data not shown). Although an inverse association between XRCC1 Arg399Gly and bladder cancer was observed (OR=0.28; 95% CI=0.08-1.02, $p=0.045$)

Table I. *XRCC1 genetic polymorphism and bladder cancer susceptibility.*

	XRCC1 genotype frequency			*XRCC1* allele frequency		Compared groups for *XRCC1*	Odds ratio (95% CI)[a]	*p*-value
	Arg399Arg	*Arg399Gln*	*Gln399Gln*	*Arg399*	*Gln399*			
Patients (n=100)*	49	38	13	68	32			
Patients (n=48)#	43.7	41.6	14.6	64.5	35	Patients* *versus* Controls	0.72 (0.41-1.26)	0.16
S (n=35)	25	37.5	10.5	43.7	29.0	Patients# *versus* Controls	0.89 (0.45-1.79)	0.44
NS (n=13)	18.7	4.1	4.1	20.8	6.0	Patients *versus* Control	1.52 (0.60-3.81)	0.26
						Patients *versus* Control	0.28 (0.08-1.02)	0.04[b]
Controls (n=100)	41	42	17	62	38			
S (n=43)	19	16	8	27	16			
NS (n=57)	22	26	9	35	22			

[a]Odds ratios with 95% confidence intervals (95% CI) and *p*-values were calculated for wild/wild genotype *versus* wild/mutant and mutant/mutant genotypes; [b]statistically significant. S, smokers; NS, non-smokers. *No information available about smoking habits; #information available about smoking habits.

Table II. *OGG1 Ser326Cys genetic polymorphism and bladder cancer susceptibility.*

	OGG1 genotype frequency			*OGG1* allele frequency		Compared groups for *OGG1*	Odds ratio (95% CI)[a]	*p*-value
	Ser326Ser	*Ser326Cys*	*Cys326Cys*	*Ser326*	*Cys326*			
Patients (n=99)*	40	47	12	64	36			
Patients (n=47)#	44.7	42.5	12.8	65.9	34	Patients* *versus* Controls	2.41 (1.36-4.25)	0.002[b]
S (n=34)	29.8	34.0	8.5	46.8	25.5	Patients# *versus* Controls	2.02 (1.00-4.08)	0.036[c]
NS (n=13)	14.9	8.5	4.3	19.1	8.5	Patients *versus* control	1.13 (0.45-2.81)	0.49
						Patients *versus* control	1.71 (0.51-5.82)	0.29
Controls (n=100)	62	20	18	72	28			
S (n=43)	27	9	7	32	11			
NS (n=57)	38	9	10	43	14			

[a]Odds ratios with 95% confidence intervals (95% CI) and *p*-values were calculated for wild/wild genotype *versus* wild/mutant and mutant/mutant genotypes; [b,c]statistically significant. S, smokers; NS, non-smokers. *No information available about smoking habits; #information available about smoking habits.

amongst those who never smoke (Table I), this finding needs to be tested with a larger study population.

Table III presents results of the analysis that evaluated the joint effects of polymorphism at *OGG1* codon 326 and *XRCC1* codon 399. We considered as the reference group for this analysis those carriers of the genotypes found to be at lowest risk of disease (*i.e.*, *Ser326Ser* for *OGG1* and *Arg399Arg* for *XRCC1*). As shown in Table III, there was no increased risk in individuals who carried only one of the two polymorphisms associated with bladder cancer (*i.e.*, *OGG1 Ser326Cys-Cys326Cys* or *XRCC1 Arg399Arg* (OR: 0.86; %95 CI, 0.35-2.13)).

Table III. *Gene-gene interaction and bladder cancer susceptibility.*

Gene-gene grouping		Patients	Controls	OR (% CI)
OGG1	*XRCC1*			
Ser326Ser	*Arg399Arg*	23	24	1.0
Ser326Cys+ Cys326Cys	*Arg399Arg*	14	17	0.86 (0.35-2.13)
Ser326Cys+ Cys326Cys	*Arg399Gln+ Gln399Gln*	33	21	1.64 (0.74-3.61)
Ser326Ser	*Arg399Gln+ Gln399Gln*	17	38	0.47 (0.21-1.04)

OR: Odds ratio determined using Fischer's exact test.

Discussion

Polymorphism in DNA repair genes resulting in variation of DNA repair efficiency may be associated with bladder cancer risk (9). The human genome encodes information to protect its own integrity (21). DNA repair enzymes continuously monitor chromosomes to correct damaged nucleotide residues, generated by exposure to carcinogens and cytotoxic

compounds (22). Several studies have screened DNA repair genes for the presence of polymorphic alleles (9, 23). It is possible that the inherited polymorphism of the recombinant repair pathway may affect the risk of bladder cancer. In our previous study, the allelic frequencies of the *XRCC1* gene in 166 of the healthy Turkish population were 0.60 for the *399Arg* polymorphism and 0.40 for the *399Gln* polymorphism and were similar to those for the Caucasian population (21).

Kim *et al.* carried out a study to determine whether polymorphism of tumor necrosis factor-alpha (*TNF*-alpha), vascular endothelial growth factor (*VEGF*), *OGG1*, glutathione S-transferase-μ (*GSTM1*), and glutathione S-transferase-φ (*GSTT1*) are risk factors for bladder cancer amongst Koreans (22). They showed that *GSTM1*-negative, *GSTT1*-positive, and *OGG1 Ser326Ser* and *Ser326Cys* genotypes are risk factors for bladder cancer. Our result for *OGG1* polymorphism in bladder cancer patients is similar to their findings. Stern *et al.* tested whether *XRCC1* polymorphism was associated with bladder cancer risk and also examined gene-environment interactions (20). They found no evidence of an association between the codon 280 variant and bladder cancer risk. On the other hand, they found evidence of a protective effect for patients that carried at least one copy of the codon *194* and *399* variant allele.

In conclusion, the role of *Gln399Gln XRCC1* is unclear, because of the contradictory data reported, regarding its protective effect and its association with an increased risk. Our data did not suggest a protective effect. On the other hand *OGG1 Cys326Cys* does appear to be a risk factor for bladder cancer.

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