RESEARCH ARTICLE

Possible Relation between the NOS3 Gene GLU298ASP Polymorphism and Bladder Cancer in Turkey

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Abstract

Endothelial nitric oxide synthase (eNOS), encoded by the NOS3 gene, has been suggested to play an important role in uncontrolled cell growth in several cancer types. The objective of this study was to evaluate the role of the NOS3 Glu298Asp polymorphism in bladder cancer susceptibility in a Turkish population. We determined the genotypes of 66 bladder cancer cases and 88 healthy controls. Genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism analysis. A significant association for NOS3 Glu298Asp heterozygotes genotypes and T allele were found between healthy controls and bladder cancer, respectively ($p<0.001$: $p=0.002$). There were no significant associations between any genotypes and the stage, grade, and histological type of bladder cancer. Our study suggested an increased risk role of NOS3 GT genotype in bladder cancer susceptibility in our Turkish population.

Keywords: DNA - cancer - nitric oxide - bladder cancer - polymorphism

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Introduction

Bladder cancer is the second most common malignancy of urinary tract cancer and fourth most frequent cancer diagnosed in men accounting for 7% of total cancers and the eight most common cancer in women in the United States (Demirel et al, 2008; Ozturk et al 2009). Lethality of the disease is in the tenth line for men and nineteenth line for women in Turkey while frequency among the women is three times higher than men (Health Ministry of Turkey, 2004). Bladder cancer is a multi-factorial disease that is mediated by both genetic and environmental factors such as occupational chemical exposure, tobacco use (Giovino et al., 2012) and genetic factors. The effects of genetic factors have been estimated to nearly thirty percent of the total risk on the bladder cancer (Ryk et al., 2011).

Nitric oxide (NO) is a short-lived and small molecule, which is implicated in several physiological functions in biological processes such as vasodilatation, neuronal transmission, smooth muscle relaxation and immunity and is released by endothelial cells (Shochina et al., 2001; Yang et al., 2007; Ryk et al., 2011). The general function of NO protects against the effects of free radicals but at excessive concentrations, NO or its derivatives may lead to DNA damage and impair the tumor suppressor function of p53, which may cause to cancer development (Yang et al., 2007).

Nitric oxide synthase (NOS) is the responsible enzyme in NO production from L-arginine. NOS enzymes are categorized in two functional classes. Constitutive class includes endothelial-NOS (eNOS-also known as NOS3) and neuronal-NOS (nNOS) while the other class contains inducible form of NOS (iNOS).

NOS isoforms are reported to be present in human solid tumors and tumor cell lines. eNOS is one of the isoform which was first defined in the vascular endothelial cells. Later, expression of this isoform has been found in other cell types including airway epithelia, neurons and certain types of cancers (Wang et al., 2005; Tecder et al., 2010).

NOS3 is known to be expressed in the bladder tumor endothelial cells and the endothelial-derived NO is catalyzed by NOS3. Experimental data suggested that NOS3 can contribute to bladder tumor development and progression. At the present time, several polymorphisms identified in human NOS3 gene but a few variations are assumed functional importance (Yang et al., 2007; Ryk et al., 2011). The NOS3 gene is located on chromosome 7q35. The G894T variation (rs1799983) is located in exon 7, which results in an amino acidic substitution of glutamic acid to aspartic acid position 298 of NOS3 gene (Venturelli et al., 2009; Ryk et al., 2011). The G894T variation may alter NOS3 activity or regulation.

This substitution was reported to alter the susceptibility to cleavage of NOS3 thus results decreased endothelial

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NO production (Tesauro et al., 2000; Ahsan et al., 2005; Choi et al., 2009).

NOS3 G894T polymorphism is associated with several diseases such as coronary artery disease, hypertension and various cancer types (Hong et al., 2007; Lee et al., 2009; Ryk et al., 2011). Up to date, there are some studies that point the involvement of this polymorphism in several cancer types however its role as a risk factor in cancer is controversial. The aim of this study was to evaluate the NOS3 G894T polymorphism as a risk factor for bladder cancer in a Turkish patient group.

Materials and Methods

Subjects

The patients and controls were selected from the urology clinic of a high volume tertiary center. Twenty-nine women and one hundred and twenty five men included in this study. We investigated the NOS3 G894T gene polymorphism in 66 bladder cancer patients and 88 healthy controls. Patients with primary bladder cancer were included in this study as the main case group. The control partipiciants were selected from the voluntiers without bladder cancer and individuals with any kind of cancer history were excluded from our study. Our study groups had similar distribution of age and gender. All participants signed an informed consent before enrollment and Institutional Ethical committee approval was obtained for the study.

Isolation of DNA

DNA was isolated and genotyping was performed as described previously. Genomic DNA was extracted from peripheral whole blood containing EDTA according to salting-out technique (Miller et al., 1988).

Polymorphism analysis

Extracted DNA was amplified with polymerase chain reaction (PCR). NOS3 Glu298Asp polymorphism was analyzed using primers (forward, 5' AAG GCA GGA GAC AGT GGA TGG A-3'; reverse, 5' CCC AGT CAA TCC CTT TGG TGC TCA 3'). PCR–restriction fragment–length polymorphism method was used genotyping (Miyamoto et al., 1998). PCR products were digested with suitable restriction enzymes than visualized and analyzed with agarose-gel electrophoresis.

Statistical analyses

Statistical analyses were performed with SPSS version 7.5 for windows (SPSS Inc, Chicago, USA) . Data are expressed as means±SD. Differences in the distribution of NOS3 G894T genotypes or alleles between cases and controls were tested using the Chi-square statistic, respectively. Relative risk at 95% confidence intervals (CI) was calculated as the odds ratio (OR). Values of $p<0.05$ were considered statistically significant.

Results

Table 1 shows the demographic characteristics of our patients groups. Genotypes and allele frequencies for NOS3 Glu298Asp in bladder cancer patients and controls are listed in Table 2. Genotype distributions for NOS3 Glu298Asp polymorphism in control group were in agreement with Hardy–Weinberg equilibrium (χ^2=0.168, p=0.682) but not in patient group (χ^2=15.7, p<0.0001). NOS3 Glu298Asp genotype and allele frequencies between bladder cancer patients and controls were statistically significant (p=0.0002, p=0.0029, respectively). The frequency of GT genotype were found 1.7 fold higher in bladder cancer patients than those with controls (p=0.0002). Among the patients with GT genotype, smokers who smoke 30 or more package of cigarettes per year (83,8 %) showed significantly increased risk of bladder cancer [p=0.04, OR=1,35(0.982-1.855)].

Moreover, patients having GG genotype showed significantly 3.3 fold lower risk for bladder cancer when compared with other genotypes [p<0.0001, OR=0.301(0.141-0.641)]. No significant association was found between clinical parameters such as age, gender, alcohol consumption or disease stage.

Table 1. Characteristics of Patients with Bladder Cancer

Parameters		Bladder Cancer Patients n=66	%
Sex	Male	58	87.9
	Female	8	12.10
Smoking status	Yes	60	90.9
	No	6	9.10
Alcohol consumption	Yes	0	0
	No	66	100
Family history of any kind of cancer	Yes	0	0
	No	66	100
Tumor grade	G1	35	56.5
	G2	15	24.2
	G3	12	19.40

*The pathological data of four patients are absent

Table 2. Genotypes and Allele Frequencies for NOS3 Glu298Asp in Bladder Cancer Patients and Controls

		Patients n (%)	Controls n (%)	OR	95%CI
Genotype	GG	7 (10.6)	31 (35.2)	0.30	0.14-0.64
	GT	49 (74.2)	44 (50)	1.48	1.15-1.91
	TT	10 (15.2)	13 (14.8)	1.02	0.48-2.19
	P Value	P=0.002			
Alleles	G	63 (47.7)	106 (39.8)		
	T	69 (52.3)	70 (39.8)		
	P Value	P=0.029			

Discussion

In this study, we represent the first demonstration of the NOS3 Glu298Asp gene polymorphism in Turkish patients with bladder cancer.

Endothelial nitric oxide synthase (eNOS) is an important biological messenger and plays an important role in the various physiological functions of diverse tissue. Almost 400 polymorphisms of NOS3 gene were determined so far and a number of them are known to related with numerous diseases, such as cardiovascular diseases and cancer. Specifically three of

these polymorphisms, including Glu298Asp seems to be functional thus under spot light as potential risk factors (Colomboet al., 2002; Lamblin et al., 2005; Yang et al., 2007; Tecder et al., 2010).

Several studies reported that some of the eNOS gene polymorphisms are significantly associated with development of several types of cancer (Hefler et al., 2002; Arıkan et al., 2012; Terrazzino et al., 2012). For instance, a recent study reported the A allele of eNOS4a/b polymorphism as a pottential risk factor for bladder cancer in a Turkish patient group (Amasyalı et al., 2012).

Importance of NOS gene polymorphisms may be relies on the contradictory role of NO in carcinogenesis. Some studies suggest the potential role of NO in carcinogenesis with its ability to promote tumor angiogenesis and metastasis (Jadeski et al., 2003) while it may inhibit DNA repair (Chien et al., 2004) and induce the DNA damage. On the other hand, there are also studies suggesting the protective role of NO such as reduction of tumor cell adhesion to endothelium (Kong et al., 1996) increasing the blood flow (Dhar et al., 2003) and modulation of apoptosis.(Choi et al., 2002; Fabbri et al., 2005; Gao et al., 2005; Lu et al., 2006).

One possible effect of eNOS polymorphisms is the alterations in the enzyme activity. Some of the eNOS gene polymorphisms may be related to alterations in the NOS enzyme activity (Serrano et al., 2004). Previous studies implicated the possible relationship between eNOS polymorphisms and abnormalities in transcription, mRNA stability, enzyme formation or enhanced degradation of the enzyme (Donesko et al., 2005). Some previous studies have reported that NOS3 894G>T alleles may causes low NO enzyme activity (Muller et al., 1985; Willich et al., 1992; Kario et al., 2001; Kario et al., 2003).

To our knowledge, a number of studies reported the lack of association between Glu298Asp substitution and cancer risk so far (Hefler et al., 2002; Lee et al., 2007; Ryk et al., 2011).

However there are some exceptions: Yang et al. (2007), reported that NOS3 genotypes are associated with the risk of post-menopausal breast cancer among smokers. In another study, it was reported the increased risk for breast cancer in the presence of Glu289Asp substitution in Caucasian women (Hefler et al., 2006). In our previous study, we observed significant difference in the distribution of NOS3 genotypes and allele frequencies between colorectal cancer patients and controls (Arıkan et al., 2012).

In a recent study, Ryk et al. (2011) reported that the NOS3 is expressed in the bladder tumor vessels, suggesting the possible relation between NOS3 and the bladder cancer. They found no increased risk of bladder cancer with Glu298Asp polymorphism however there was an association between the Glu298Asp and bladder tumor grade (Ryk et al., 2011).

We observed approximately 2 fold higher risks for bladder cancer for patients who have GG genetype with G3 tumor grade (16.7%) when compared to those with G1-G2 grade tumors (8%) however these results did not showed a significant difference.

Unlike Ryk et al. (2011) GT genotype was found to be a potential risk factor for bladder cancer with 1.7 fold increased frequency while GG genotype represents 3.3 fold lower risk for bladder cancer. A relation between NOS3 genotypes, smoking status and risk of breast cancer has been reported previously (Yang et al., 2007). Our results indicate such a similar relation for bladder cancer. For patients with GT genotype, smoking 30 or more package per year showed statistically significant risk when compared with other genotypes and smoking free control group.

A possible relation between Glu289Asp GT genotype, smoking status and the bladder cancer risk is represented in this study. One of the potential limitations of our study is the relatively small sample size. However, in such studies, creating a homogenized study group for age, gender and smoking status etc. is one of the most challenging issues. Here we represent the first results on Glu289Asp polymorphism and bladder cancer but further investigation is needed in order to clarify this relation.

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