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ORIGINAL ARTICLE

Detection of XRCC1 gene polymorphisms in Turkish head and neck squamous cell carcinoma patients: a comparative analysis with different populations

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Summary

Purpose: *X-ray repair cross-complementing (XRCC1) is one of the most important genes for the maintenance of genomic integrity and protection of cells from DNA damage. Although tobacco and alcohol consumption are the major risk factors for the development of head and neck squamous cell carcinoma (HNSCC), sequence variation in XRCC1 gene may alter HNSCC susceptibility. Reports on the relationship between HNSCC and polymorphisms in XRCC1 gene have been inconsistent so far. The aim of this study was to investigate the association of XRCC1 Arg194Trp and Arg399Gln single nucleotide polymorphisms (SNP), smoking and alcohol consumption with the risk of HNSCC in Turkish population and also to compare to these results with the ones from both Turkish and different populations in the literature. The frequencies of Arg194Trp and Arg399Gln SNPs were studied in 55 HNSCC and 69 healthy individuals.*

Methods: *Genomic DNA was isolated from peripheral blood and SNP was genotyped by PCR-RFLP method.*

Results: *The genotype and allele frequencies of both polymorphisms were not statistically different between the HNSCC and control groups. On the other hand, smoking and chronic alcohol consumption were associated with risk of HNSCC, but there was no association between Arg194Trp, Arg399Gln polymorphisms, smoking and alcohol consumption in HNSCC cases.*

Conclusion: *These results indicate that both Arg194Trp and Arg399Gln polymorphisms were not associated with the development of HNSCC in Turkish population. In addition, the allele frequencies of polymorphisms were in line with other Turkish population results that were studied previously. However, compared to different populations, there were marked differences in allele frequencies.*

Key words: *DNA repair,head and neck squamous cell carcinoma, single nucleotide polymorphism, XRCC1*

Introduction

HNSCC develops from the mucosal linings of the upper aerodigestive tract which comprises the nasal cavity, nasopharynx, hypopharynx, larynx, trachea, oral cavity and oropharynx. Squamous cell carcinoma (SCC) is the most common malignant tumor of the head and neck region, being the fifth most common type of cancer in the world [1]. Smoking and chronic alcohol consumption are the most important risk factors for developing HNSCC [2]. Some occupational exposures may be associ-

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ated with a higher risk. Significant associations for developing HNSCC were found with polycyclic hydrocarbons, metal dust, ionizing radiation, diesel exhaust etc [3]. On the other hand, it is known that genetic susceptibility plays an important role in the risk of this disease [4]. Identification of SNPs in candidate genes of various pathways is the focus of many researchers who are dealing with association studies on cancer susceptibility. Among those SNPs that are involved in DNA repair pathways are the most exciting ones due to their vital role in protecting the genome from the damaging effects of environmental carcinogens [5,6]. Alterations in the DNA repair genes can increase the risk of head and neck cancer since they can cause reduction of DNA repair capacity [7,8].

There are three important DNA repair pathways, including nucleotide excision repair (NER), base excision repair (BER), and double strand break (DSB). The XRCC1 gene is involved in the BER pathway and is implicated in single-strand break repair [9,10]. BER operates on small lesions such as oxidized or reduced bases, fragmented or nonbulky adducts, or those produced by methylating agents [11]. Cells that are carrying a mutant XRCC1 gene have increased sensitivity to ionizing radiation and alkylating agents [12]. The carcinogens present in tobacco products generate free radicals and may induce various types of DNA damage which cause uncontrolled cell growth in mutant cells [13].

Two common SNPs in the XRCC1 gene that lead to amino acid substitutions are Arg194Trp (rs#1799782) in exon 6, codon 194 results from C→T and Arg399Gln (rs#25487) in exon 10, codon 399 substitution of G→A, respectively [4,14]. These alterations of amino acids may affect the repair of DNA single-strand breaks by changing the protein-protein interactions between XRCC1 and other BER proteins [14].

The scope of this case-control study was to investigate the association between XRCC1 gene polymorphisms (Arg194Trp and Arg399Gln), smoking and chronic alcohol consumption with the risk of HNSCC in Turkish population and compare these results with the ones from both Turkish and different populations in the literature.

Methods

Study population

Fifthy five unrelated HNSCC patients, clinically diagnosed at Diskapi Yildirim Beyazit Training and Research Hospital, Department of Otorhinolaryngology and 69 unrelated healthy volunteers from different geographic regions of Turkey were included in this study. The exposure status of smoking and alcohol consumption and also demographic data (age and gender) were collected for all subjects. The individuals of the control group were selected to match the patients in terms of age and gender. Study approval of the local ethics committee was obtained from Diskapi Yildirim Beyazit Traning and Research Hospital (# 12/11) and all patients gave informed consent. The study was conducted in accordance with the guidelines of the Declaration of Helsinki.

DNA Isolation and Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (RFLP)

Genomic DNA was isolated from peripheral blood samples of both HNSCC patients and controls using NucleoSpin® DNA isolation kit (Macherey-Nagel, Germany) according to manufacturer's instructions. DNA amplification was carried out on a Techne TC-512 PCR system (N.Jersey, USA) in a 50 µl reaction mixture containing 10x PCR buffer (BioLabs Inc. New England, Hertfordshire, UK), 25 mM magnesium chloride (BioLabs Inc. New England, Hertfordshire, UK), 200 µM dNTP (BioLabs Inc. New England, Hertfordshire, UK), 10 pmol of forward (F) and reverse (R) primers (Iontek, Istanbul, Turkey), 0.5 µU Taq DNA polymerase (BioLabs Inc. New England, Hertfordshire, UK) and 50 ng genomic DNA. The PCR cycling conditions consisted of an initial denaturation step at 95°C for 5 min, followed by 40 cycles of 94°C for 1 min, 60oC for 1 min for Arg194Trp and 56°C for 1 min for Arg399Gln, 72°C for 1 min and final extension step at 72°C for 5 min. Then, 10 µl PCR products of Arg194Trp (304 bp) and Arg399Gln (616 bp) were digested with 10 U CutSmart,

Table 1. Primer sequences, restriction enzymes and RFLP fragment sizes of variant alleles of Arg194Trp and Arg399Gln polymorphisms

SNP	*Primer sequence*	*PCR product (bp)*	*Restriction enzyme*	*PCR fragment lenghts (bp) after digestion*
Arg194Trp	F : 5'- GGTAAGCTGTACCTGTCACTC-3' R : 5'-GACCCAGGAATCTGAGCC-3'	304	Msp I	Arg/Arg (wild type): 20+117+167 Trp/Trp (mutant): 137+167
Arg399Gln	F : 5'-TTGTGCTTTCTCTGTGTCCA -3' R : 5'-TCCTCCAGCCTTTACTGATA -3'	616	Msp I	Arg / Arg (wild type) : 376 + 240 Gln / Gln (mutant) : 616

Table 2. Genotype and allele frequencies of Arg194Trp polymorphism in HNSCC cases and controls

Genotype	*Control (N=69) N (%)*	*Cases (N=55) N (%)*	*p value*	*OR (95% CI)*
Arg/Arg	60 (87)	49 (89)		REF
Arg/Trp	9 (13)	4 (7)	0.174	0.523 (0.152-1.799) p=0.304
Trp/Trp	0 (0)	2 (4)		6.495 (0.306-138.153) p=0.2303
Allele				
Arg	129 (93)	102 (93)	0.831	REF
Trp	9 (7)	8 (7)		0.889 (0.332-2.387) p=0.816

OR: odds ratio, CI: confidence interval, REF: ORs with respect to wild type genotype/allele

Table 3. Genotype and allele frequencies of Arg399Gln polymorphism in HNSCC cases and controls

Genotype	*Control (N=69) N (%)*	*Cases (N=55) N (%)*	*p value*	*OR (95% CI)*
Arg/Arg	22 (32)	21 (38)		REF
Arg/Gln	35 (51)	27 (49)	0.670	1.319 (0.627-2.774) p=0.465
Gln/Gln	12 (17)	7 (13)		0.815 (0.412-1.613) p=0.558
Allele				
Arg	79 (57)	69 (63)	0.375	REF
Gln	59 (43)	41 (37)		1.257 (0.752-2.099) p=0.382

For abbrevations see footnote of Table 2

Msp I restriction endonuclease enzyme at 37°C for 15 min (New England Biolabs, Hertfordshire, UK). The digested and undigested PCR products were seperated on a 2.5% agarose gel electrophoresis at 120 V, stained with ethidium bromide (0.5 µ/ml) and visualized under a UV transilluminator (Vilber Lourmat, Marne-la-Vallée, France). Primer sequences, restriction enzymes and fragment lenghts are given in Table 1.

Statistics

The Statistical Package for Social Sciences (SPSS) version 16.0 software was used for statistical analysis. The genotypes and allele frequencies of Arg194Trp and Arg399Gln polymorphisms were obtained by direct count and departure from the Hardy-Weignberg equilibrium was evaluated by x^2 analysis. A p value <0.05 was considered as statistically significant. Odd ratios (OR) and 95% confidence intervals (CI) were also calculated.

Results

Figures 1 and 2 show the gel photographs which contain DNA fragments representing homozygote wild type, homozygote mutant type and heterozygote for Arg194Trp and Arg399Gln polymorphisms, respectively. Genotype distribution of Arg194Trp (x^2=3.380; p=0.064) and Arg399Gln (x^2=0.188; p=0.713) polymorphisms corresponds to Hardy-Weignberg expectation.

Table 2 shows the genotype and allele frequencies of Arg194Trp polymorphism in HNSCC patients and control cases. Among HNSCC patients 89% were found to be homozygote for wild type (Arg/Arg), 7% were heterozygote (Arg/Trp) and 4% were homozygote mutant type (Trp/Trp). The Arg allele frequency was 93% and Trp allele was 7%. Among the control cases, 87% were found to be homozygote for wild type and 13% were heterozygote. None of the control cases was found to be homozygote mutant type. The Arg allele frequency was 93%, whereas the Trp allele frequency was 7%.

Arg399Gln polymorphism in HNSCC patients and control group are listed in Table 3. Among patients 38% were found to be homozygote for wild type (Arg/Arg), 49% were heterozygote (Arg/Gln) and 13% were homozygote mutant type (Gln/Gln). The Arg allele frequency was 63% and the Gln al-

Table 4. Association between Arg194Trp polymorphism, smoking and alcohol consumption in HNSCC cases

Cases (N=55)	*Smoking (N=39)*	*Non-smoking (N=16)*	*p value*	*Alcohol consumption (N=14)*	*Not alcohol consumption (N=41)*	*p value*
Arg/Arg	35	14		14	35	
Arg/Trp	2	2	0.433	0	4	0.317
Trp/Trp	2	0		0	2	

Table 5. Association between Arg399Gln polymorphism, smoking and alcohol consumption in HNSCC cases

Cases (N=55)	*Smoking (N=39)*	*Non-smoking (N=16)*	*p value*	*Alcohol consumption (N=14)*	*Not alcohol consumption (N=41)*	*p value*
Arg/Arg	16	5		3	18	
Arg/Gln	17	10	0.396	9	18	0.317
Gln/Gln	6	1		2	5	

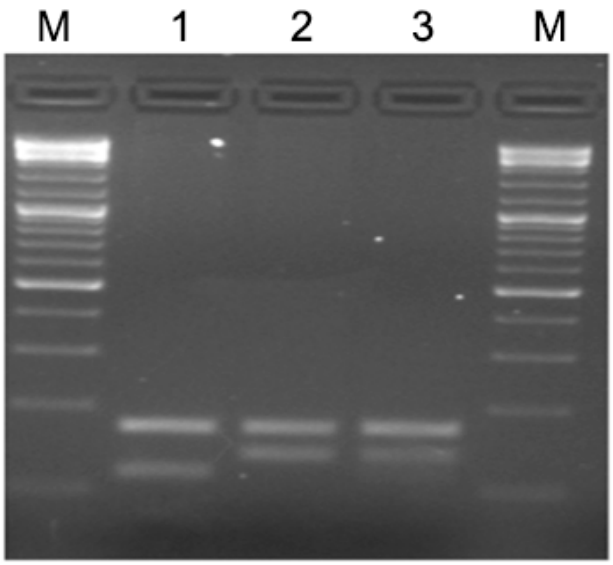

Figure 1. A representative agarose gel image of digested PCR products with MspI. M: 100 bp marker; lane 1: homozygote wild type (Arg/Arg); lane 2: homozygote mutant type (Trp/Trp); lane 3: heterozygote (Arg/Trp).

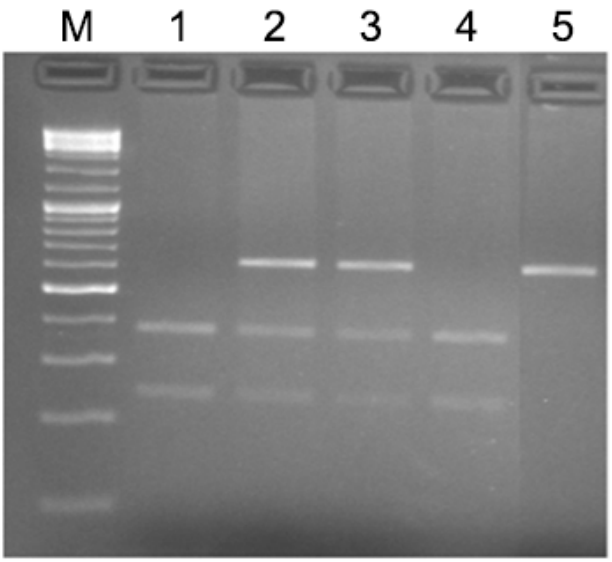

Figure 2. A representative agarose gel image of digested PCR products with Msp I. M: 100 bp marker; lane 1 and 4: homozygote wild type (Arg/Arg); lane 2 and 3: heterozygote (Arg/Gln); lane 5: homozygote mutant type (Gln/Gln).

lele was 37%. Among the control group, 32% were found to be homozygote for wild type, 51% were heterozygote and 17% were homozygote mutant type. The Arg allele frequency was 57%, whereas the Gln allele frequency was 43%.

In addition, we performed an analysis of the influence of smoking and chronic alcohol consumption on the risk of HNSCC and also association with Arg194Trp and Arg399Gln polymorphisms. Among 55 HNSCC patients, 71% were smokers and 25% were using alcohol, while for the control group these percents were only 23% and 3%, respectively ($p<0.05$). These results indicate that people who are smokers and chronic alcohol consumers have more risk in developing HNSCC compared to non-smokers and non-alcohol users. However, no association was noticed between both Arg194Trp and Arg399Gln polymorphisms, smoking and alcohol consumption among HNSCC cases (Tables 4 and 5).

Table 6. Population differences in the allele frequencies for Arg194Trp polymorphism

Reference	*Population*	*Arg allele frequency (%)*	*Trp allele frequency (%)*
42	American (Black)	95	5
42	American (White)	94	6
42	Taiwanese	83	17
45	Columbian	94	6
43	Chinese	68	32
44	Egyptian	70	30
41	Turkish	94	6
33	Turkish	94	6
This study	Turkish	93	7

Table 7. Population differences in the allele frequencies for Arg399Gln polymorphism

Reference	*Population*	*Arg allele frequency (%)*	*Gln allele frequency (%)*
42	American (Black)	83	17
42	American (White)	63	37
42	Taiwanese	75	25
45	Columbian	64	36
43	Chinese	74	26
30	Finland	70	30
46	Caucasians	68	32
47	Italian	66	34
39	Indian	67	33
35	Saudi Arabia	74	26
44	Egyptian	80	20
41	Turkish	65	35
33	Turkish	63	37
This study	Turkish	57	43

Discussion

The DNA repair pathways are one of the primary defense mechanisms that keeps the genome integrity. Deficiencies in the DNA repair pathways can cause cell malfunctioning and carcinogenesis [15,16]. An individual's capacity to repair DNA damage varies due to exposure to tobacco smoke, irradiation and endogenous reactions that contain oxidants [17]. In addition, studies have shown inter-individual variations of DNA repair capacity on the risk of smoking-related cancers, such as lung cancer and HNSCC [18-20]. XRCC1 functions in the BER of genomic damage caused by exposure to carcinogens such as tobacco and alcohol [9,11]. There are more than 60 SNPs in XRCC1 gene listed in the Ensembl database, among which three coding polymorphisms at codons 194 (exon 6, Arg to Trp), codon 399 (exon 10, Arg to Gln) and codon 188 (exon 3, Arg to His) have been extensively studied [12]. These alterations of amino acids may effect the repair of DNA single-strand breaks by changing protein-protein interactions between XRCC1 and other BER proteins [14]. Association of the XRCC1 polymorphisms with different cancer types and in different populations has been previously investigated [4,8,21-24]. However, the results of a large number of studies from HNSCC patients are still confusing and not consistent.

In our study, no significant difference was observed between Gln allele frequencies in the HNSCC patients and control cases. According to the literature, the association between Arg399Gln polymorphism and cancer susceptibility remains controversial. There are various reports that support an association between Gln allele and different types of cancer [21,25-27] while others suggest lack of association [8,28-30]. The results are also conflicting for head and neck cancers. Although some reports support an association

[4,31,32], some others show lack of association [33-38], which support our results.

The allele frequency of Trp for Arg194Trp polymorphism was not significantly different in HNSCC patients with respect to control cases. There are also conflicting reports for the association of Arg194Trp polymorphism and head and neck cancer susceptibility. In contrast to reports showing positive association between Trp allele in esophegeal cancer risk [38] and head and neck cancers [32], others that suggest lack of association also exist [33,34,36,37]. These controversial results can be due to the differences in cancer type, selection criteria, sample size of the studied population, ethnic differences and other genetic factors.

In this study, we investigated the influence of smoking and chronic alcohol consumption with the risk of HNSCC and also the association with Arg194Trp and Arg399Gln polymorphisms. The results suggest that smoking and chronic alcohol consumption were both significantly associated with the development of HNSCC. However, we did not observe an association between smoking and chronic alcohol consumption and both of the XRCC1 polymorphisms in our patient group. In the literature, some of the studies suggest a significant positive association between the frequency of the variant allele for XRCC1 polymorphisms and the risk of head and neck cancer among the patients with regard to smoking [33,39]. However, our results do not parallel with these results. They are more in line with a study that shows smoking does not modify the association between XRCC1 polymorphisms and HNSCC risk among the HPV16 seropositive cases [40]. Thus, the inconsistent results of the effect of smoking and alcohol consumption can be due to selection criteria, ethnic differences, various infections and other genetic factors.

The results of this study were also compared with other studies from different populations. Recently, with increased knowledge of correlation of SNPs and clinical outcome, a number of large-scale genotype-phenotype correlation studies have been performed [14,31,34,38]. Knowledge of SNP frequencies in a population is essential for the estimation of the likelihood of inter-individual differences of treatment efficacy. Tables 6 and 7 show the allelic frequency distribution of Arg194Trp and Arg399Gln polymorphisms in healthy Turkish population cases and other populations, respectively.

In the healthy Turkish population, the incidence of Arg allele is 93% and that of the Trp allele is 7% for Arg194Trp polimorphism (Table 6). This result is in line with the other Turkish population results that have been studied previously [33,41]. Compared to other populations, there are marked differences in allele frequencies between different populations. In the Turkish population, the incidence of Trp allele is low compared to Chinese, Egyptian and Taiwanese populations [42-44], but is similar to American and Columbian populations [22,42,45]. For Arg399Gln polymorphism, the incidence of Arg allele is 57%, while Gln allele is 43% (Table 7). In the studied Turkish population Gln allele is much higher than American (Black), Taiwanese, Chinese, Saudi Arabian and Egyptian populations [35,42-44]. However, the allele frequencies are similar to American (White), Columbian, Finland, Caucasian, Italian and Indian populations [22,30,42,45-47]. Our results are also similar with the other Turkish population studies [33,41].

In conclusion, XRCC1 Arg194Trp and Arg399Gln polymorphisms do not correlated with the development of HNSCC in Turkish population. Moreover, carrying mutant alleles was not associated with risk of the disease. On the other hand, smoking and chronic alcohol consumption was associated with the risk of HNSCC but without association between Arg194Trp, Arg399Gln polymorphisms, smoking and chronic alcohol consumption in HNSCC cases. In addition, the allele frequencies of polymorphisms are in line with the other Turkish population results that have been reported previously. However, compared to different populations, there are marked differences in allele frequencies.

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