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Association of Arg194Trp and Arg399Gln Polymorphisms of XRCC1 Gene and Risk of Differentiated Thyroid Carcinoma in Iranian-Azeri Patients

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Abstract

Background: The thyroid cancer (TC) is one of endocrine malignancies which contributes to more than 50% of all deaths from endocrine cancers. Gene polymorphisms including DNA repair genes such as XRCC1 (X-ray repair cross-complementing group 1) gene are thought to modify DNA repair capacity and relate to cancer risk.
Objectives: The aim of the study was to detect the association between XRCC1 polymorphisms and increased risk of thyroid carcinoma among Iranian-Azeri patients.
Methods: This case-control study was performed on 114 differentiated thyroid carcinoma patients and 91 normal control subjects. Single nucleotide polymorphisms (SNPs) of 194 C > T and 399 G > A of XRCC1 gene were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).
Results: In the present study, polymorphism at codon 194 of the XRCC1 gene was not found in case and control groups (P = NC (not calculated). All of the case and control subjects were 194C/C. Unlike 399 G > A genotype (P < 0.001, OR = 0.184, CI = 0.09 - 0.372), there was a positive association for 399G/G (P < 0.001, OR = 3.304, CI = 1.624 - 6.780) and 399 A/A (P < 0.001, OR = 14.143, CI = 1.861 - 296.277) genotypes with differentiated thyroid carcinoma. The frequency of variant 399 A allele among the cases was slightly higher than that in controls (P = 0.269, OR = 0.798, CI = 0.535 - 1.190).
Conclusions: Based on these results, the XRCC1 399 G > A genotype could be used as a useful molecular biomarker to predict genetic susceptibility for differentiated thyroid carcinoma in Iranian-Azeri patients.

Keywords: Polymorphism, XRCC1, Thyroid Carcinoma

1. Background

Most of the cell's DNA damage is caused by endogenous and exogenous mutagenic agents, which may result in apoptosis or lead to unregulated cell growth and cancer. Therefore, DNA repair is critical for the maintenance of genome integrity. The X-ray repair cross-complementation group 1 (XRCC1) gene encodes a protein that plays an important role in the base excision repair (BER) pathway (1). The BER pathway that is responsible for repair of single strand breaks and oxidative DNA damage interacts with a complex of DNA repair proteins, such as polynucleotide kinase enzyme, DNA ligase IIIα, DNA pol-β and PARP1 (2). Numerous mutations in XRCC1 interrupt the protein function by altering catalytic domain or binding sites of the protein (3). XRCC-lacking cells have increased sensitivity to ionizing radiation, ultra-violet light, alkylating agents and hydrogen peroxide (4, 5). Several single-nucleotide polymorphisms (SNPs) in the XRCC1 gene have been identified, among them, 194C > T (rs1799782) and 399 G > A (rs25487),and deeply studied, and they caused nonconservative changes (6). Codon194 of XRCC1 gene is located in a hydrophobic linker region between two domains of DNA polymerase β and poly (ADP-Ribose) polymerase-interacting. Therefore, change from an amino acid of positively charged arginine to hydrophobic tryptophan may alter the interaction of XRCC1 with the other DNA repair proteins within the base excision repair complex (7). Arg399Gln polymorphism that is located in the conserved

residue of the poly (ADP-Ribose) polymerase-binding domain of XRCC1 alters the amino acid of Arginine to Glutamine substitution (C > T, rs25487) (2). The association between the XRCC1 polymorphisms with increased risk for various types of cancers such as lung, breast, head, neck and thyroid cancers (8-10) has been studied.

Thyroid cancer is the most common endocrine malignancy worldwide and differentiated thyroid cancer (DTC) is the most high-frequent type. Pathologically, DTC includes follicular, papillary, and Hurthle cell carcinoma (11). Exposure to ionizing radiation and a history of benign thyroid nodules seem to be related to thyroid cancer (12, 13). Generally, both environmental and individual genetic susceptibility have important roles in human cancer. The genetic susceptibility is related to genetic polymorphisms of various genes, including those involved in DNA repair (14, 15). The XRCC1 is one of these genes whose variant relationship with thyroid cancer has been less widely investigated (16).

There have been few previous reports examining XRCC1 SNPs and the risk of thyroid carcinoma (17), and these studies have reported conflicting results. Furthermore, no genetic study of XRCC1 SNPs in differentiated thyroid cancer has been performed in North West of Iran. In an effort to evaluate the genetic influence on risk of differentiated thyroid carcinoma, a genetic analysis of two polymorphisms of the XRCC1 gene (194 C > T and XRCC1 399 G > A) was performed among Azeri people of in North West of Iran.

In this case-control study, the genotype frequency distributions of the two common XRCC1 SNPs (194 C > T and 399 G > A) are compared in DTC patients and controls.

2. Objectives

The aim of the study was to detect the association between XRCC1 polymorphisms and increased risk of thyroid carcinoma among Iranian-Azeri patients.

3. Methods

3.1. Study Population

The patient group consisted of 114 patients, without regard to family history, who underwent thyroidectomy and were confirmed as having differentiated thyroid carcinoma by pathological examination (n = 114; mean age = 39. 2; Range age = 15 - 77 years). The control group consisted of 91 volunteers with no familial history of cancer. All of the participants have given informed written consent, and the study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (TUMS), which was in compliance with the Helsinki Declaration.

3.2. PCR and RFLP Analysis

Genomic DNA was extracted from peripheral blood and tissue. Two SNPs of XRCC1 gene, 194 C > T and 399G > A, were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The dimorphic sites were codon 194 (C/T, Arg/Trp, exon 6), and codon 399 (G/A, Arg/Gln, exon 10). The primers used for codon 194 were 5´-GCCCCGTCCCAGGTA-3´ and 5´-AGCCCCAAGACCCTTTCACT-3´, and for codon 399 were 5´-TTGTGCTTTCTCTGTGTCCA-3´ and 5´-TCCTCCAGCCTTTTCTGATA-3´. Polymerase chain reaction (PCR) was performed in a mixture containing 2.5 μL 10X reaction buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 μM of each primer, 0.1 - 0.5 μgDNA and 1.0 U of Taq polymerase (fermentase) in a total volume of 25 μL. PCR was carried out by thermal cycler (Sensoquest, GmbH, Germany) at 35 cycles consisting of steps: denaturation at 94°C for 3 minutes, annealing for 30 seconds at 62°C for 194 codon and 63°C for 399 codon and extension for 30 seconds at 72°C. The reaction was completed by a final extension for 7 minutes at 72°C. The first PCR products of codons 194 (491 bp) and 399 (615 bp) were electrophoresed on 2 percent agarose gel containing 5 μg/mL ethidium bromide, visualized under UV-light, and then digested with 4 U of Bisis I (HpaII) restriction enzyme (bioron, Germany) in a final volume of 10 μL at 37°C for 18 hours. Finally fragments were separated on 2 percent agarose gels containing ethidium bromide and visualized under UV-light. The amplification of codons 194 and 399 resulted in the product of 491 and 615 bp, respectively. There were two recognition sites at the positions 174 and 198 for enzyme Bisis I on the 491 bp fragment of which the second position was the polymorphic site. The wild-type allele of codon 194 generated three fragments of 293, 178 and 20 bp, and variant allele generated two fragments of 313 and 178 bp. On the 615 bp fragment, enzyme had a single recognition site and wild-type allele resulted in two fragments of 239 and 376 bp. Also, variant alleles were present as an undigested 615 bp fragment.

3.3. Statistical Analyses

Data analysis was performed using javastat online statistics package software. The Fisher's exact test was applied to compare the variables when the number of samples was equal to or less than 5. P value < 0.05 was considered as significant.

4. Results

The genotype frequency of codon 194 is 100 percent for homozygous wild-type (CC) in both groups. The genotype

frequency of XRCC1 399 GG, GA and AA were 39.5%, 48.2% and 12.5%, in the case group and 16.5%, 83.5%, 0% in the control group, respectively. The GG and AA genotypes are more common among cases than controls (P < 0.001), and GA genotype is more common among controls than cases (P < 0.001) (Table 1).

The allele frequency of XRCC1 399 G and 399A were 63.6% and 36.4%, in the case group and 58.2 and 41.8%, in the control group, respectively. There was no significant difference between groups (P > 0.05, OR = 0.798, CI = 0.535 - 1.190) (Table 1).

Table 1. XRCC1 Genotype and Allele Distribution (%) in the Samples[a,b]

SNP	Group		P Value	OR (95%CI)
	Cases (N = 114)	Controls (N = 91)		
Codon 194 (C/T)				
C/C	114 (100)	91 (100)	NC	
C/T	0	0		
T/T	0	0		
Codon 399 (G/A)				
G/G	45 (39.5)	15 (16.5)	< 0.001	3.304 (1.624 - 6.780)
G/A	55 (48.2)	76 (83.5)		0.184 (0.09 - 0.372)
A/A	14 (12.5)	0 (0)		14.143 (1.861 - 96.277)
339 G	145 (63.6)	106 (58.2)	0.269	0.798 (0.535 - 1.190)
399 A	83 (36.4)	76 (41.8)		

Abbreviations: CI, confidence interval; NC, not calculated; OR, odds ratio.
[a] Fishers' exact test (the controls genotypes were used as reference).
[b] Values are expressed as No. (%).

According to the Hardy-Weinberg equilibrium model, there was not a significant correlation between genotypes and clinicopathological parameters of Azeri patients (P > 0.05) (Table 2).

5. Discussion

DNA damage is mostly induced by endogenous and exogenous mutagenic agents. If such damage is not repaired, it could result in mutations, unregulated cell growth, genomic instability and cancer.

The role of XRCC1 gene polymorphisms in relation with differentiated thyroid carcinoma is examined in a case-control study. The effect of changes in DNA repair genes, such as XRCC1 gene has been investigated in growing studies to elucidate possible susceptibility to various types of cancers (18, 19). In the current study, the impact of XRCC1 194 C > T and 399 G > A polymorphisms were examined on the risk of differentiated thyroid carcinoma in Iranian- Azeri patients. Our results showed that all of case and control subjects were 194C/C. There was no significant association between XRCC1 194 C > T and risk of differentiated thyroid carcinoma. Previously, the study by Zhu QX et al. reported that 194 C > T polymorphism did not reveal significant association regarding this polymorphism with thyroid cancer (20). In contrast, 194C > T variant homozygote genotype was reported to be associated with increased risk of DTC (21) but was associated with decreased risk of DTC in a Korean population (22).

According to the results in Table 1, there was a positive association for 399 G/G (P < 0.001, OR = 3.304, CI =1.624 - 6.780) and 399 A/A (P < 0.001, OR = 14.143, CI = 1.861 - 296.277) genotypes compared with 399 G > A genotype (P < 0.001, OR = 0.184, CI = 0.09 - 0.372) with differentiated thyroid carcinoma. The risks of papillary thyroid carcinoma associated with XRCC1 194C > T and 399 G > A were also examined in a Chinese sample (23, 24). According to that study, the XRCC1 399Gln variant genotype was associated with increased risk of papillary thyroid carcinoma (OR: 2.71, 95% CI: 1.22 - 6.05), but the XRCC1 194Trp variant genotype was not significantly correlated (21). In contrast, Akulevich and Ho in two different studies reported that Arg399Gln polymorphism was associated with decreased risk of DTC and PTC (16). Also, Chiang et al. in another study, performed in the United States, reported that the XRCC1 399 G > A homozygous A/A genotype and variant A allele are associated with decreased risk of differentiated thyroid carcinoma (17).

In this study, the 399 A allele in the cases was slightly higher than that in controls, but it was not significant (P = 0.269, OR = 0.798, CI = 0.535 - 1.190). In contrast, functional studies of XRCC1 suggest that the 399Gln allele may be associated with multiple DNA damage phenotypes in human cells and tissues and to various types of cancers. The variant 399A allele has been associated with an increased risk of lung (25, 26), neck and head cancer (9), and possibly stomach cancer (23, 24). On the other hand, this allele has been associated with a decreased risk of bladder and esophageal cancer (27, 28). In the current study, there was no association between 399A allele and differentiated thyroid carcinoma.

To decipher the reasons for these contradictory results is difficult, but there are several factors that may impact the polymorphisms in different ways, such as variation in carcinogen exposures in different populations and different types of DNA damage in the initiation of different cancers. Moreover, an inadequate study design such as non-random sampling, limited sample size, and pitfalls of unknown confounding influences should also be considered.

Based on our results, there was no significant association between XRCC1 194 C > T genotypes and risk of differentiated thyroid carcinoma. However, the XRCC1 399 G > A genotype could be used as a useful molecular biomarker to predict genetic susceptibility for differentiated thyroid carcinoma in Iranian-Azeri patients.

Table 2. Association Between XRCC1 Polymorphism (Arg399Gln) and Pathological Characteristics[a]

Pathology	G/G	G/A	A/A	Total	P Value
Tumor grade					0.453
Stage I	8 (27.6)	16 (55.2)	5 (17.2)	29	
Stage II	18 (54.5)	15 (45.5)	0	33	
Stage III	17 (43.6)	18 (46.2)	4 (10.3)	39	
Stage IVa	2 (15.4)	6 (46.2)	5 (38.5)	13	
Pathology					0.395
PTC	28 (40)	35 (50)	7 (10)	70	
FTC	15 (44.1)	13 (38.2)	6 (17.6)	34	
MTC	2 (20)	6 (70)	1 (10)	10	
Lymph node metastasis					0.533
Positive	23 (45.1)	22 (43.1)	6 (11.8)	51	
Negative	22 (34.9)	33 (52.4)	8 (12.7)	63	

Abbreviations: FTC, follicular thyroid carcinoma; MTC, medullary thyroid carcinoma; PTC, papillary thyroid carcinoma.
[a] Values are expressed as No. (%).

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Footnotes

Authors' Contribution: None declared.

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