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Original Research

DNA repair gene XRCC1 and XRCC3 polymorphisms and their association with chronic myelogenous leukemia

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ABSTRACT

Objective: In this study, due to its importance in DNA repair in cancer, we searched for polymorphisms of XRCC1 and XRCC3 genes in Turkish chronic myelogenous leukemia (CML) patients. **Materials and Methods:** In order to determine these gene polymorphisms, the polymerase chain reaction-restriction fragment length polymorphism method was applied using genomic DNA extracted from samples. **Results:** The genotype frequencies were observed as follows: The XRCC1 Arg399Gln genotype frequencies were observed as 33.3% homozygote typical (G/G), 43.3% heterozygote (G/A) and 23.3 % homozygote atypical (A/A) in the 30 samples of patients group; the genotype frequencies of XRCC3 Thr241Met were found 50% homozygote typical (C/C), 36.7% heterozygote (C/T) and 13.3% homozygote atypical (T/T) in all samples. **Conclusion:** In this study, no statistical association was found between CML and XRCC1 Arg399Gln and XRCC3 Thr241Met polymorphisms in Turkish patients.

KEY WORDS: Chronic myelogenous leukemia, DNA repair genes, polymorphism, XRCC1, XRCC3

INTRODUCTION

Chronic myelogenous leukemia (CML) is a type of leukemia originating from hematopoietic stem cells characterized by unregulated proliferation of myeloid cells. Leukemic cells carry the chromosome, a translocation between one chromosome 9 and one chromosome 22. This chromosome has the fusion gene which consists of fragments of the breakpoint cluster region (BCR) and abelson murine leukemia (ABL) genes. The chimeric BCR-ABL oncogene encoding the p210 or p190 fusion protein exhibits a constitutively high tyrosine kinase activity.

CML is treated with tyrosine kinase inhibitors (TKI) which have dramatically improved survival rates in the last decade [1,2]. However, occasionally patients acquire resistance to TKIs and progress into blast crisis [3]. A few studies on patients with leukemia have directly focused the relationship between BCR-ABL expression and activity of proteins involved in DNA repair [4-6]. In recent years, extensive research has addressed on the relationship between polymorphisms in DNA repair genes and cancer susceptibility. Although hundreds of polymorphisms in DNA repair genes have been known [7], the functional effects of this polymorphism have not been well determined. Several polymorphisms (XRCC1, XRCC3, XPD, ERCC1, XPC, hOGG, XPF, etc.) have been determined in DNA repair genes [8,9]. Among the DNA repair genes, XRCC1 and XRCC3 have been studied most commonly [10-13].

In the present study, since DNA repair gene alterations have been shown to cause a reduction in DNA repair capacity, we hypothesized that DNA repair gene polymorphisms may be risk factor for CML. Therefore, we analyzed the polymorphisms of the XRCC1 and XRCC3 genes at rs25487 (Arg399Gln, XRCC1) and rs861539 (Thr241Met, XRCC3

gene) using a polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) method in Turkish patients.

MATERIALS AND METHODS

Demographic Profile

Thirty unrelated CML patients diagnosed clinically at Gulhane Military Medical Academy and a control group of 30 unrelated healthy volunteers were randomly selected from different geographic regions of Turkey. The consent of local ethics committee was obtained in Gülhane Military Medical Academy. The study was conducted in accordance with the guidelines of the Declaration of Helsinki.

DNA Extraction from Samples

Peripheral blood samples were obtained for DNA extraction. Genomic DNA was isolated from samples using the phenol-chloroform method [14]. DNA samples were quantified and subjected to specific PCR as described.

Determination of XRCC1 and XRCC3 Polymorphism by PCR-RFLP Method

In order to screen for Arg399Gln polymorphism of XRCC1 gene and Thr241Met polymorphism of XRCC3 gene 403 bp and 456 bp fragments containing the whole region were amplified by PCR with the following primers:

XRCC1	F: 5′-TCTCCCTTGGTCTCCAACCT-3′ R: 5′-AGTAGTCTGCTGGCTCTGG-3′
XRCC3	F: 5′-GGTCGAGTGACAGTCCAAAC-3′ R: 5′-TGCAACGGCTGAGGGTCTT-3′

Amplification was carried out on a BioRAD PCR system in a 50 μl reaction mixture containing 200 μM of deoxynucleotide triphosphates, 10 pmol each of forward (F) and reverse (R) primers, 1 U HotStarTaq DNA polymerase (Qiagen), 10X PCR buffer (Qiagen) 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 95°C for 1 min, 60°C for 1 min, 72°C for 1 min, and final extension step at 72°C for 5 min. Then the PCR product (403 and 456 bp) was digested with MspI and NlaIII (New England Biolabs, Hertfordshire, UK) and incubated at 37°C for 4 h, respectively [Figure 1]. Digestion of the PCR product by MspI yields 403 bp fragments for the presence of the A allele, and 133 and 270 bp fragments for the presence of the G allele. Digestion of the PCR product by NlaIII yields 315 and 141 bp fragments for the presence of the C allele, and 210, 141 and 105 bp fragments for the presence of the T allele. The undigested PCR product and digested products were separated on a 2.5% agarose gel electrophoresis, visualized by ethidium bromide staining under an ultraviolet illuminator, scanned and photographed using Syngene Monitoring System. Digested and undigested PCR products on an agarose gel electrophoresis are indicated in Figures 2 and 3.

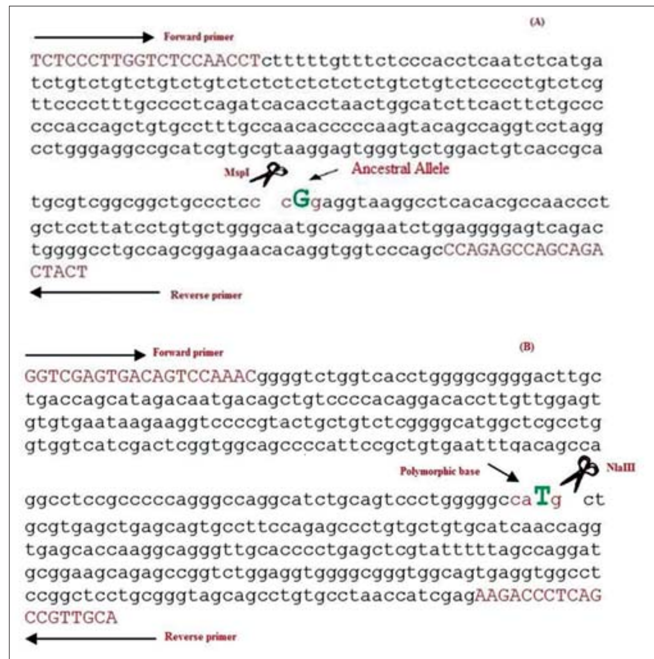

Figure 1: Schematic representation of digested PCR products (A: XRCC1; B: XRCC3)

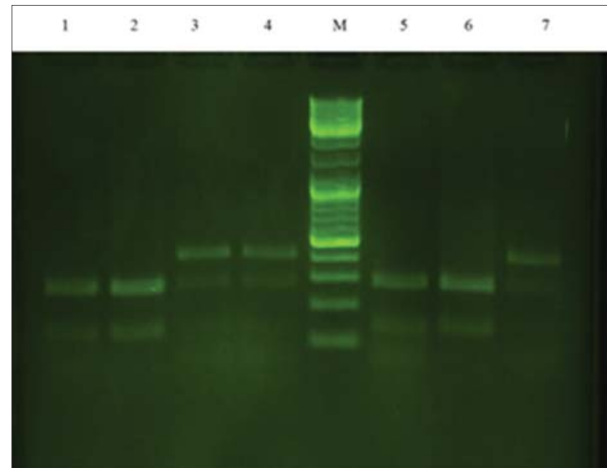

Figure 2: Digestion of PCR product by MspI in XRCC1 gene (M: 100 bp ladder, lanes 1, 2, 5, 6: homozygote typical genotype [GG], lanes 3 and 4: heterozygote genotype [GA], lanes 7: homozygote atypical genotype [AA])

Statistical Analysis

The SPSS software version 16.0, (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. The frequencies of XRCC1 and XRCC3 alleles and genotypes were obtained by direct count, and the departure from the Hardy-Weinberg equilibrium was evaluated using the chi-square test. Categorical variables were also compared using the Chi-square test and $P < 0.05$ were accepted to be significant.

RESULTS

In the first part of this study, following polymorphisms were detected in the 30 healthy subjects representing Turkish population:

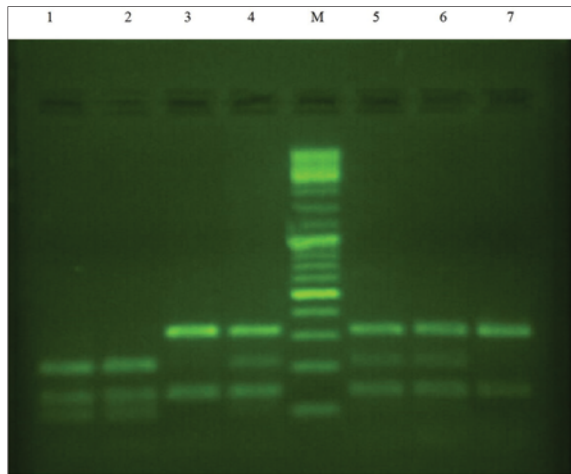

Figure 3: Digestion of PCR product by NlaIII in XRCC3 gene (M: 100 bp ladder, lanes 1 and 2: homozygote atypical genotype [TT], lanes 4, 5, 6: heterozygote genotype [CT], lanes 3 and 7: homozygote typical genotype [CC])

1. XRCC1 polymorphism when digested with MspI produced the homozygote common genotype (GG), heterozygote (GA) and homozygote uncommon genotype (AA).
2. In XRCC3 polymorphism, digestion of corresponding PCR product with NlaIII yielded the homozygote common genotype (CC), heterozygote (CT) and homozygote uncommon genotype (TT).

The XRCC1 Arg399Gln genotype frequencies in the 30 patients' samples were observed as 33.3% homozygote typical (G/G), 43.3% heterozygote (G/A) and 23.3 % homozygote atypical (A/A). The genotype frequencies of XRCC3 Thr241Met in patients' samples were as follows: 50% homozygote typical (C/C), 36.7% heterozygote (C/T) and 13.3% homozygote atypical (T/T).

The genotype frequencies of XRCC1 and XRCC3 polymorphisms were consistent with the Hardy-Weinberg equilibrium. The P values estimated with Hardy-Weinberg exact test were 0.495 and 0.408 (>0.05) for XRCC1 and XRCC3 polymorphisms, respectively [Table 1]. As a main result of the study, no statistically significant differences were observed in the alleles or in the genotype frequencies of the XRCC1 Arg399Gln and XRCC3 Thr241Met polymorphisms between the control group and the CML patients.

As shown in Table 1, the detailed distribution of allele and genotype frequency of XRCC1 Arg399Gln and XRCC3 Thr241Met of CML patients is shown. Carried mutant alleles 399Gln or 241Met were not associated with the risk of CML when compared with controls [Table 2].

DISCUSSION

In recent years, the focus on DNA repair genes single nucleotide polymorphism (SNP) function and association with human diseases has increased considerably. Articles describing the association of SNPs with cancer are also available. In the present research, we investigated a possible relation between XRCC1 and XRCC3 gene polymorphisms and CML risk in Turkish patients. Previous studies have reported associations between risk of different leukemia types (mainly AML and ALL) and DNA repair genes polymorphisms [15-18].

Table 1: Distribution of allele and genotype frequency of XRCC1 Arg399Gln and XRCC3 Thr241Met polymorphisms in CML patients

Polymorphism	Observed frequency of genotypes		Predicted frequency by hardy-weinberg equilibrium		*P* value
	N	%	*N*	%	
XRCC1					
XRCC1 GG genotype	10	33.3	9.08	30.26	0.495
XRCC1 AG genotype	13	43.3	14.85	49.5	
XRCC1 AA genotype	7	23.3	6.07	20.23	
XRCC1 G allele	33	0.55			
XRCC1 A allele	27	0.45	-	-	
XRCC3					
XRCC3 CC genotype	15	50	14.01	46.7	0.408
XRCC3 CT genotype	11	36.7	12.98	43.3	
XRCC3 TT genotype	4	13.3	3.01	10	
XRCC3 C allele	41	68.3			
XRCC3 T allele	19	31.7	-	-	

Table 2: Comparison of XRCC1 and XRCC3 polymorphism and CML risks in Turkish population

Polymorphism	Control	Patients	OR (95% CI)
XRCC1			
GG	12	10	1
GA, AA	18	20	1.333 (0.4650-3.8234)
G	32	33	1.0694 (0.5214-2.1934)
A	28	27	
XRCC3			
CC	13	15	1
CT, TT	17	15	0.7647 (0.2767-2.1137)
C	37	41	1.3414 (0.6318-2.8478)
T	23	19	

CML: Chronic myelogenous leukemia, OR: Odds ratio, CI: Confidence interval

Scarce information is present regarding the relation between DNA repair genes polymorphisms and CML. To the best of our knowledge, this is the first study focusing on the relationship between the XRCC3 codon 241 Met polymorphism and CML patients. Only one study, conducted by Deligezer *et al.* [19], reported about XRCC1 polymorphism in CML patients. They showed that the frequency of the variant allele 399 Gln was comparable in the control group and the patients. Similarly, the heterozygote and homozygote variant genotypes displayed a homogeneous distribution in both groups. Moreover, they have found that the distribution of the variant allele and subgenotypes did not significantly differ between the patient subgroups with a diagnosis age below or above 50 years [19].

Consistent with the present study, Annamaneni *et al.* [20] showed that XRCC1 polymorphisms are known to affect the function of XRCC1 and that the nature and extent of their genetic association with CML does not indicate their direct role

in the development of the disease. They suggested that XRCC1 gene might have an important role in CML progression but not in its causation [20]. The results of the present work suggest that independently Arg399Gln and Thr241Met polymorphisms do not correlate with the development of CML in the Turkish population. In addition, carriers of both of the polymorphic alleles present no elevated risk of CML.

There are a lot of studies in the literature about XRCC1 polymorphisms and their associated risk with different types of leukemia [21-25]. In a meta-analysis extracted from these studies, Zhang *et al.* [26] reported that there is no association between XRCC1 Arg399Gln polymorphism and leukemia susceptibility.

In the present study, conducted on 30 CML patients along with 30 healthy subjects representing the Turkish population, we did not find a statistically important association between CML and XRCC1 Arg399Gln or XRCC3 Thr241Met polymorphisms. However, we have to emphasize the limitation that because of lower frequency of XRCC1 and XRCC3 phenotypes, studies with a larger number of cases can give more reliable conclusions.

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