

Lack of Association between Catalase Gene C-262T Polymorphism and Systemic Lupus Erythematosus

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Article information	Abstract
<p>Article history: Received: 4 Jun 2011 Accepted: 23 Jun 2011 Available online: 30 Dec 2011</p> <p>Keywords: Systemic lupus erythematosus Catalase Polymorphism</p> <p>*Corresponding author at: Department of Clinical Biochemistry, Cellular and Molecular Research Center, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran. E-mail: sasalimi@yahoo.com</p>	<p>Background: Systemic lupus erythematosus (SLE) is an autoimmune disease with unknown etiology which affects different organs. Evidences show that SLE patients have a defect in the scavenging of oxidative stress products. One of the effective defense systems against oxidative stress damages is catalase. This study was conducted to investigate the relationship between C-262T polymorphism of catalase gene and systemic lupus erythematosus.</p> <p>Materials and Methods: This case-control study was conducted on 107 patients with SLE and 140 healthy individuals matched by age, sex and ethnicity. PCR-RFLP method was used to identify C-262T polymorphism of catalase gene. Frequency of alleles and genotypes in patients and control group was statistically analyzed using χ^2 test.</p> <p>Results: Frequency of CC, CT and TT genotypes of catalase gene were 77.6, 20.5 and 1.9 percent in SLE patients and 71.8, 27.5 and 0.7 percent in control group respectively, which was not significant.</p> <p>Conclusion: This study showed that C-262T polymorphism of catalase gene is not associated with SLE.</p>

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Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease which affects different organs including skin, heart, lungs, blood vessels, liver, kidneys, joints and nervous system [1]. The main etiology of this disease is still unknown, however both genetic and environment factors are known as effective events on its initiation and progression [2]. Genetic basis of this disease is very complex. Studies have shown that more than 100 genes can be involved in this disease [3].

The active oxygen species play an important role in the pathogenesis of lupus and lupus patients have defect in the repair of the damages resulting from oxidative stress [4-6]. One of the important enzymatic defense systems against damages caused by oxidative stress is catalase [7]. This enzyme is an important antioxidant that protects the tissue from destructive reactions of oxidative stress through breakdown of H_2O_2 produced in tissues and its conversion to H_2O and O_2 [8]. Catalase genes have 13 exons and are located on the short arm of chromosome 11 (11p13) [9]. One of the most common polymorphisms observed in catalase gene is a single nucleotide polymorphism which is created due to the replacement of C with T at position 262 in the promoter region and causes a dramatic decrease in catalase gene expression [7].

The relationship between this polymorphism and some diseases such as systemic lupus erythematosus, breast cancer, pancreatitis, lymphoma, asthma, and autoimmune diabetes has been studied [10-12]. There are rare studies about the relationship between C-262T polymorphisms of catalase gene and systemic lupus erythematosus. Furthermore no reports on this survey have been observed in Iran and other neighboring countries. Therefore, this study was conducted in Zahedan to examine the relationship between C-262T polymorphism of catalase gene and systemic lupus erythematosus.

Materials and Methods

The project was approved by the Zahedan University of Medical Sciences Ethics Committee. In this case-control study, 107 patients with SLE referred to rheumatology clinics in Zahedan in 2009 and 2010 that have been diagnosed with systemic lupus erythematosus according to ACR 1998 criteria (American Rheumatology Association). One hundred forty age, sex and ethnically matched volunteers with negative ANA test who had no systemic disease and family relation with lupus patients were selected as controls

Genotyping: Genomic DNA was extracted from peripheral blood leukocytes using DNA extraction kit (Roche, Germany). C-262T polymorphism of catalase gene was determined by RFLP-PCR method [5]. Two oligonucleotide primers, forward:

5'-AGAGCCTCGCCCCGCCGACCG-3' and reverse: 5'-TAAGAGCTGAGAAAGCATAGCT-3' based on the flanking sequences of C-262T polymorphism of catalase gene promoter were used to amplify the corresponding DNA fragments by polymerase chain reaction (PCR).

Polymerase chain reaction was performed in a 25 μ l final volume contained 25 pmol of each primer, 0.1 mmol of dNTP (Fermentas, Lithuania), 0.5 μ g of genomic DNA, 1.5 mmol/L of MgCl₂ and 2.5 μ l of PCR buffer and 1.5 unit of Taq DNA polymerase (Fermentas, Lithuania) according to the following protocol: initial denaturation at 94°C for 6min; 30 cycles of denaturation at 94°C for 1 min, annealing at 61°C for 1 min, and extension at 72°C for 2min; and final extension at 72°C for 6 minutes. The 185 bp PCR fragments were digested with 10U of SmaI restriction enzyme (Fermentas, Lithuania) for 8 h at 37°C. Digested samples were separated by electrophoresis on a 2.5% agarose gel and visualized by ethidium bromide staining.

The wild-type allele (C allele) has SmaI cleavage site and digested to 155 and 30 bp fragments, whereas the mutant allele (T allele) has not SmaI cleavage site and produce 185 bp fragment only (Fig. 1).

Finally, statistical analysis was performed with SPSS-V-11.5. Student *t* test was used to compare quantitative variables and χ^2 test was used to compare non-quantitative variables.

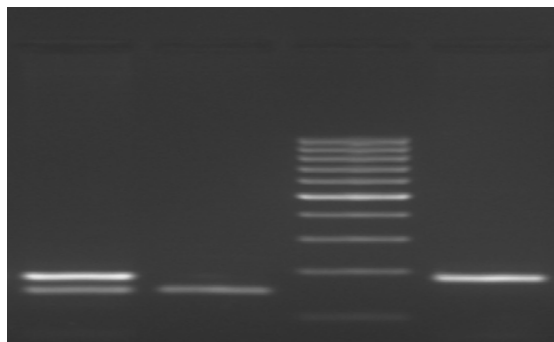


Figure 1. Genotyping of C-262T polymorphism of catalase gene. Lane 1- 30, 185, 155bp bands (genotype CT), Lane 2- 30, 155bp bands (genotype CC), Lane 3- DNA Lader 100bp and Lane 4, 185bp band (Genotype TT)

Results

Demographic data of patients with SLE and control group are shown in table 1. From 107 SLE patients 87 individuals were females (90.7%) and 10 individuals were males (9.3%) and from healthy controls 128 individuals were females (91.4%) and individuals were males (8.6%) ($p=0.5$). There was no significant difference between the mean age of control group 32.8 ± 11.5 and SLE patients 31.8 ± 7.4 ($p=0.4$).

Genotypes and alleles frequency of C-262T polymorphism of catalase gene is shown in table 2. Allele frequency of this polymorphism was in Hardy-Weinberg equilibrium.

The frequency of CC, CT, TT genotypes of catalase gene were 77.6, 20.5 and 1.9 percent in SLE patients and 70.7, 28.6 and 0.7 percent in control group respectively and it was not statistically significant. The frequency of T allele was 12 percent in the SLE patients and 15 percent in the control group and was not significantly different. We also did not observe minor T allele frequency differences between the two persian and balouch ethnic groups ($p > 0.05$).

Table 1. Demographic data of patients with SLE and control group

	SLE patients	Controls	<i>p</i> -Value
Age(years)	7.4 \pm 31.8	11.5 \pm 32.8	0.4
Sex(male/female)	97/10	12/128	0.5
Race n	Persian 49(46)	67(51)	0.4
(%)	Balouch 58(54)	73(49)	

Table 2. Genotypes and alleles frequency of C-262T polymorphism of catalase gene in patients with SLE and control group

	SLE patients	Controls	²	<i>p</i> -Value
Genotype, N(%)	CC 83(78.6)	99(70.7)	2.6	0.28
	CT 22(20.5)	40(28.6)		
	TT 2(1.9)	1(0.7)		
Allele, N(%)	C 188(88)	238(85)	0.64	0.24
	T 26(12)	42(15)		

Discussion

In the present study, the frequency of CC, CT, and TT genotypes of C-262T polymorphism of catalase gene in lupus patients and control group showed no significant difference. Also, the frequency of T allele was 12 percent in SLE patients and 15 percent in the control group and was not significantly different.

SLE is an autoimmune disease that is characterized by an abnormal immune response. This disease affects different organs such as skin, muscle, kidney and blood. Although the exact etiology of this disease is unknown, the role of genetic factors in the onset and progression of this disease and its symptoms is undeniable [6]. Some studies showed that patients with SLE has defect in scavenging and repair of the damages resulting from oxidative stress [5]. Therefore, effective proteins and enzymes against oxidative stress such as catalase and glutathione S-transferase can be proper candidates. In a linkage study conducted in 2002, Kufman et al. concluded that there is a genetic linkage between the 11p13 chromosome region that is the region which encodes catalase and lupus [14].

In another study conducted on African America families in 2003, Scofield et al. also confirmed this genetic linkage [15]. On the other hand, Forsberg et al. observed that a single nucleotide polymorphism (SNP) is involved in binding transcription factors and thereby gene transcription as C/T displacement in position -262 of

catalase gene promoter. Also, they showed that catalase activity in the serum of lupus patients was lower than the control group [13]. These results altogether present the hypothesis suggesting that C-262T polymorphism of catalase gene is associated with lupus.

Studies on C-262T polymorphism of catalase gene and SLE are rare. In 2005, Eny et al. conducted a study in Korea on the relationship between C-262T of catalase gene and SLE on 345 patients and 400 controls and no correlation was observed between this polymorphism and lupus, which is consistent with results of this study. Also in this study, frequency of rare alleles T is 3.5 percent in the control group and 2.5 percent in the patient group [5]. Whereas, the frequency of rare alleles T in our society was respectively obtained 12 percent in patient group and 15 percent in control group.

In another study conducted in America on 100 patients and 113 controls, D-Souza et al. also reported no association between this polymorphism and disease [16]. Warchol et al. in Poland evaluated the relationship between catalase gene polymorphism and lupus in 102 patients with lupus and 199 healthy controls. In this study, like the present study, no relationship was observed between genotypes of this polymorphism and the disease, but they reported that there is a relationship between genotypes of C-262T polymorphism and thrombocytopenia, leukopenia and renal disorders in lupus patients [7]. As mentioned, the results of this study are partly consistent with the three studies conducted in Korea, America and Poland, but inconsistent with genetic linkage studies.

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These differences can be due to various reasons such as racial and ethnic differences, sample size and inclusion and exclusion criteria in the study. Given that, this survey is the first study on the relationship between C-262T polymorphisms of catalase gene and lupus in Iran, it is necessary to conduct similar studies in other regions of Iran as well as studies on the relationship between this polymorphism and complications and severity of lupus. Also, considering the conducted genetic linkage studies, it is better to conduct more studies on the relationship between other polymorphisms of this gene and lupus disease. Thus, frequency of genotypes CC, CT and TT of C-262T polymorphism of catalase gene in patients with systemic lupus erythematosus and control groups showed no significant difference. Also, the frequency of rare alleles T in the patient group and control group was not significantly different.

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Conflict of Interest

No conflict.

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