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Codon 72 Polymorphism of p53 Gene and Hematologic Manifestations in Patients with Systemic Lupus Erythematosus

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Abstract

Background: Systemic lupus erythematosus is a systemic autoimmune disorder with unclear etiology. The importance of some genes in the development of systemic lupus erythematosus has been implicated. The gene polymorphism in codon 72 has attracted a lot of attention and its role in the occurrence or progression of many cancers and autoimmune diseases especially systemic lupus erythematosus has been studied. In the present study we evaluated the polymorphism of codon 72 in p53 gene among patients with systemic lupus erythematosus.

Patients and Methods: Expression of p53 gene was determined in lysed lymphocytes from patients with systemic lupus erythematosus who were admitted to Namazi Hospital, Shiraz, Iran, as well as 30 healthy individuals as the control group. The patients' information, including the epidemiological profile, disease history, disease symptoms and also the laboratory findings were extracted from the hospital records.

RESULTS: Among 77 patients with systemic lupus erythematosus, 9 (11.8%) were male and 68 (88.2%) were female. There was a significant relationship between the different allele types of p53 and systemic lupus erythematosus (p=0.033). The frequencies of Arg/Arg, Pro/Pro and Arg/Pro among normal controls were 38.8%, 28.8% and 37.5%, respectively, but among the patients, Arg/Arg, Pro/Pro and Arg/Pro genotypes frequencies were found to be 29.2%, 12.3% and 58.5%, respectively. Thus, heterozygous form of this polymorphism was shown to be associated with the disease more than the homozygous forms. There was no association between the different allele types and any of the initial manifestations of the disease and the laboratory findings.

Conclusions: The functional oncoprotein p53 with codon 72 polymorphism may play an important role in the pathogenesis and activity of systemic lupus erythematosus.

Key words: p53, systemic lupus erythematosus, polymorphism, disease activity.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease which causes inflammation in certain organs such as joints, kidneys, skin, etc. [1-3]. If signs of inflammation remain for a long time, tissues are injured and their normal function is impaired; as a result, therapeutic goal in SLE is to reduce inflammation caused by the disease [2-6]. It seems that inherited risk factors along with various random environmental factors cause abnormal immune responses among SLE patients. Factors such as hormonal imbalances during puberty, some environmental hazards such as exposure to sunlight, some viral infections and certain medications act as the trigger stimulation [3-9]; however, the exact cause of SLE is unknown [10-12]. The effect of genetic risk factors can be cited as follows [5, 13-20]: the role of specific HLA [2, 21-23], complement factor deficiency especially C2, C3, C4 [6, 24-25], complement receptor deficiency especially CR1 [7-8, 26], hormonal factors [5, 27-30], as well as impairment in activities of cell cycle and its controlling factors such as p53 [9-11, 31]

p53 controls cell cycle activities leading to apoptosis of auto reactive cells preventing the

proliferation and differentiation of these cells [10-15]. As a result, any mutations in p53 gene cause cancer and autoimmune diseases [11, 32]. p53 suppressor gene is located on the short arm of chromosome 17 in exon 11 [1-3]. DNA damage increases the level of p53 subsequently stopping the cell cycle and DNA repair or resulting in cell apoptosis [32-35].

Many studies have evaluated p53 polymorphism in which proline was replaced by arginine at codon 72 changing the function of p53 molecule making the person prone to various diseases including cancer and autoimmune diseases [11-12, 36]. In the present study we evaluated the polymorphism of codon 72 of p53 gene among patients with SLE. We determined p53 polymorphism among SLE patients and analyzed the relationship between the p53 oncoprotein and clinical activity of the disease.

Patients and Methods

In a case-control study the patients with documented SLE who were admitted in Namazi Hospital, affiliated to Shiraz University of Medical Sciences, Shiraz, Iran, from 2003 to 2005 were evaluated. Controls were recruited healthy people from same geographic area who came for check-up .The information required was extracted from the files and by visiting the patients. Informed consent was taken from the patients or their parents and all controls. Data including age, sex, onset of symptoms, first manifestation of the disease, complications related to the involvement of each organ and ANA, Ds DNA, hematologic tests were collected; then, the frequency of the patients with homozygous or heterozygous allele as well as the relationship between any of the mentioned parameters and the allele type was measured.

Procedure

After taking 10cc fresh blood, DNA was extracted using the salting-out method. DNA was also extracted from the peripheral blood of the healthy individuals, as the control group. PCR was performed to determine polymorphism of codon 72 on p53 gene.

Oligonucleotides used in genotyping of Mannose binding protein was as follow:

Arg F: TCC CCC TTG CCG TCC CAA
Arg R: CTG GTG CAG GGG CCA CCC
Pro F: GCC AGA GGC TGG TCC CCC
Pro R: CGT GCA AGT CAC AGA CTT

PCR products were analyzed using 2% agarose gel electrophoresis, with ethidium bromide staining by UV transilluminator devices. PCR products were 141bp and 177bp for arginine-containing alleles and the alleles containing proline respectively. For heterozygous samples, both PCR bands appeared while only one band of the spectrum was present for homozygous samples.

Statistical Analysis

Data were collected in forms and then transferred to SPSS software version 16 for data analysis. Non parametric variables such as number, percentage and mean + standard deviation (SD) were analyzed using descriptive statistics. Variables such as difference in proportion were tested applying chi-square test or fisher exact test when appropriate. Level of significance was set as $p \leq 0.05$. Fisher's exact test and relative risk test were used to determine the statistical significance of polymorphism association with SLE.

Results

Among 77 patients studied with the mean age of 25.61±1.24 years, 9 (11.8%) patients were male and 68(88.2%) were female. All patients

Table 1: The frequency of p53 alleles, number and percent.

Group	P53			Total
	AA	PP	AP	
SLE	19	8	38	65
	29.2%	12.3%	58.5%	100%
Control	31	19	30	80
	38.8%	23.8%	37.5%	100%
Total	50	27	68	145
	34.5%	18.6%	46.9%	100%

were Caucasian. The mean age at the onset of the first symptoms of the disease was 20.65±1.27 years. P53 alleles were positive in 66 patients. The frequency of p53 alleles in the patients with SLE was 58.5% for the Arg/Pro, 29.2% for the Arg/Arg and 12.3% for the Pro/Pro while in the control group the most frequent allele type was Arg/Arg with 38.8%; however, the frequency of Arg/Pro and Pro/Pro alleles were 37.5% and 23.8% respectively (P =0.03) (Table 1).

About 71.9% of the patients had no family history of SLE while a positive family history of SLE was found in 20.9% of the patients; positive family history of other rheumatic diseases and cancer in first-degree relatives was seen in 7% and 4.7% of the patients, respectively.

The most important initial signs and symptoms were fever in 37%, arthritis in 33%, arthralagia in 28.4% and malar rash in 22.2% of the patients (Table 2)

Among the lupus criteria for diagnosis, the most common findings were renal involvement (82.7%), anti-nuclear antibody (ANA) (81.5%), hematologic manifestation (76.5%), malar rash (74.1%), arthritis (72.7%), photosensitivity (48.1%), immunologic findings (45.5%), oral ulcer (38.3%), neurologic manifestations (seizures and psychosis) (18.5%), serositis (17.3%) and discoid rash (14.8%).

There was no significant correlation between the SLE initial manifestations, laboratory findings (ANA, Ds DNA, and Hematologic test) and different alleles of p53 gene. However, there were significant correlations between diarrhea, pulmonary involvement, photosensitivity, malar rash and p53 gene (P<0.05). There was significant correlation between diarrhea and Arg/Pro polymorphism (P=0.02), as well as pulmonary involvement and Arg/Pro polymorphism (P=0.03), photosensitivity and Arg/Arg polymorphism (P=0.03), and malar rash and Pro/Pro polymorphism (P=0.02). There was not any significant correlation between P53 allele polymorphism and morbidity of SLE.

Discussion

SLE is an autoimmune disease with complex etiology such as numerous genetic and environmental factors [37]. In absence of timely diagnosis of SLE the disease progression and many complications such as, infections, cardiovascular and renal involvement might lead to death [38-40]. According to the findings of the present study significant correlation was found between the different allele types of p53 and some clinical manifestation of SLE.

The disease incidence has a 9/1 female to male ratio [1-10]. Most patients in our study were female (88.2%) which is in line with previous findings. The disease usually occurs in women in child-bearing age (15 to 35 years) [6-12], which was also in line with our findings showing a mean age of 25.61±1.24 years among our patients. Common initial and chronic complaints have been reported as fever, malaise, joint pains, myalgias, fatigue, and temporary loss of cognitive abilities in most studies [14-18]; the same as our results.

In a study on 513 patients and 567 controls to determine the polymorphism of codon 72 of p53, there was no significant correlation between the

Table 2: Initial manifestation of lupus erythematosus among patients.

Initial sign	Frequency (%)
Fever	37
Arthritis	33.3
Arthralagia	28.4
Malar Rash	22.2
Edema	8.6
Skin	7.4
Thrombosis	7.4
Fatigue	7.4
Seizure	7.4
Anorexia	4.9
Other	33.9

patients and the control group [30, 41]. In another study in 2005 in South Korea on 90 patients with SLE who were compared with 114 controls, it was found that proline holders have more chance to develop SLE than those with arginine [29].

Our study showed no significant correlation between the initial manifestations of SLE, laboratory findings and p53. Similarly in a study in Poland which was performed on 155 patients, p53 had no significant correlation with the initial symptoms and the laboratory findings of lupus but a weak correlation was reported between Arg/Arg genotype of p53 and lupus-induced mortality (9). Our study did not show any correlation between disease morbidity and P53 polymorphism. Differences between populations, genetic and environmental factors may have influences on lupus manifestations and disease progression [9-17]. The present study showed some signs and symptoms of lupus to be significantly correlated with p53 [9]; however, other studies could not find such a correlation definitely [42-44].

Conclusions

The functional oncoprotein p53 with codon 72 polymorphism may play an important role in the pathogenesis and activity of systemic lupus erythematosus.

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