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Investigating the Association of PTPN22 Gene Polymorphism R620W with Scleroderma in Southwestern Iran in Khuzestan

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ABSTRACT

Systemic sclerosis is a chronic systemic disease with unknown variety of etiology, and has clinical chronic protests often progressive. Gene PTPN22 R620W polymorphism has been identified as a risk factor in the scleroderma disease, especially the limited one. The aim of this project is studying the relationship between gene PTPN22 R620W polymorphism with scleroderma disease. This was a clinical trial conducted on 70 patients with scleroderma referred to Golestan Hospital, Ahvaz, Iran and 70 healthy subjects with no history of genetic disorders, particularly autoimmune diseases referred to Iran Blood Transfusion Organization as control group. Diagnosis is based on physical examination and confirmation of rheumatologist. Sampling is done through Non-probability sampling convenient sampling. The PTPN22 R620W polymorphism had no significant relationship in all scleroderma patients and control group (P-value: 0.76, OR: 0.61). In addition, the gene in patients with limited scleroderma, diffuse scleroderma patients, scleroderma +ACA, -ACA scleroderma patients was not significantly more than the control group. This study showed that the prevalence of the gene PTPN22 R620W polymorphism in patients with scleroderma and its subtypes is not more than the control group.

Key words: Systemic sclerosis, ACA, Gene PTPN22, polymorphism R620W**.**

INTRODUCTION

Systemic sclerosis is a chronic systemic disease of unknown etiology, with a variety of chronic clinical protests and often progressive. In terms of epidemiology, scleroderma is an acquired sporadic disease with a global distribution that affects all races. In the United States its incidence is 19-9 per million per year. The only community-based study that was conducted on scleroderma showed scleroderma incidence as 286 cases per million. Age, gender, and ethnicity are important factors that specify the susceptibility to disease.

Scleroderma, like other connective tissue diseases, is more frequent among women with the highest incidence during childbearing age which decreases after menopause. Although scleroderma can occur at any age, the most common age of onset in both limited and diffuse cutaneous is form 30 to 50 years of age.

In terms of genetics, scleroderma has a multifactorial inheritance and non-Mendelian patterns. The amount of concordance rate of scleroderma for monozygotic twins is relatively low (4.7%), but this amount for the presence of anti-nuclear antibodies (ANA) is much higher. The fact that the 1.6% of patients with scleroderma, one of the first-degree relatives suffers scleroderma (prevalence is much higher than the general population) implies a genetic role in susceptibility to disease. To date, genetic studies have revealed that, like other multifactorial or complex diseases, several genetic loci are involved in scleroderma (1).

During the past decade, great advances have happened in understanding the genetic link between systemic scleroderma to explain the genetic predisposition observed in this disease.

More than 30 genes and gene regions have been found in the talent to suffer scleroderma including HLA and non-HLA genes that often involve dependent pathway related to safety and adjust immune function. Many other systemic autoimmune diseases have been reported in the community that indicates multiple autoimmune genes have a role in the pathogenesis. Despite great progress, only a small portion of scleroderma inheritance is known.

Genes susceptible to scleroderma

Histocompatibility complex (MHC) is the main genetic region that is involved in autoimmune diseases. The HLA allele's role in the pathogenesis remains unknown. HLA specific alleles associated with scleroderma and certain autoantibodies are known.

For example, a Case control study in scleroderma has identified its strong relationship with HLADRB1 * 1104 and DQB1 * 0301 haplotype 0501 DQA1 * and (4).

Non-HLA genes associated with scleroderma include protein tyrosine phosphatase nonreceptor22 (PTPN22) with SLE, myasthenia gravis, and Vitiligo and Addison NLRP1, IL-1; NLRP1, IL-1, and interferon regulatory factor 5 (IRF5) (5).

Gene PTPN22 is located on the short arm of chromosome 1 [1p13.3-p13.1]. Lymphoid specific phosphatase (LYP) is inactive and has been considered as an important regulator of T-CELL. International Code of this gene is UGID: 1371590 UniGene Hs.535276 (NC_000001.11).

When this conversion [1858C/T] PTPN22 occurs, in position 1858 nucleotides gene of cytosine changes to thymine, it causes the amino acid in position 620 LYP that is arginine to change to tryptophan that results in breaking down the connection between the LYP and CSK, this causes the loss of regulatory activity of T cells (6).

Given the role of PTPN22 gene in scleroderma patients, the present study aims to investigate the polymorphism R620W (rs2476601) role in the vulnerability of both healthy subjects and patients to disease, and that if this polymorphism could be involved in the prognosis of patients with scleroderma or not.

The incidence of scleroderma in Khuzestan is high. The ethnical diversity in this region is more than elsewhere in the country, and no study has been carried out in the country in this regard. Therefore, this study investigates the association of polymorphism PTPN22 R620W as a risk factor in susceptibility to suffer scleroderma in Khuzestan.

MATERIALS AND METHODS

The present study is an analytical and epidemiologic study conducted on 120 patients with scleroderma referred to Golestan Hospital, Ahvaz, Iran and 117 healthy subjects as the control group.

Diagnosis is based on physical examination and confirmation of diseases by a rheumatologist. The control group consisted of healthy people who did not have history of genetic disorders, particularly autoimmune diseases in their immediate family and after getting informed consent, they participated in the study.

Inclusion criteria included patients with scleroderma based on the examination and approval by the rheumatologist and exclusion criteria included a history of genetic disease, suffering rheumatoid disease alongside a family member suffering rheumatoid disease.

Molecular Assessment

DNA extraction from blood

Using DNA extraction kit, DNA is extracted from blood samples of patients with scleroderma and control group. In this kit, silica fibers are fixed are provided. DNA binds to silica fibers at the presence of chaotropic salt. Proteins are removed during washing and centrifugation and genomic

DNA is separated from the fibers in the presence of a low-salt solution.

Designing Primer

In this study, primer is designed by primer-3. Suggested primers for polymorphism are PTPN22 SNP (rs2476601):
F-allele T: 5- CCCCTCCACTTCCTGTAT-3
F-allele C: 5-CCCCTCCACTTCCTGTAC-3
R com: 5-TGCGCAGGCTAGTCTTG -3

Determining PTPN22 C1858T by PCR-SSCP

(Polymerase Chain Reaction) PCR is a laboratory method used for mass production of specific pieces and chosen from DNA used. The reaction based on the amplification of enzyme fragment of a piece of DNA that by using the two primers or initiator of several nucleotide supplementations 5 both threads are concerned so that the copy of DNA by polymerase enzyme is possible.

Sequence Specific Primers-Polymerase Chain Reaction (PCR-SSP) is on the basis that each allele (which here makes a serological species) matches exactly at the same area and copied and for this reason, they do not need restriction enzyme.

In order to ensure the results obtained and to delete false negative results due to the lack of correct answers, internal control actin -â is used in every PCR reaction. The gene will be amplified with the following primers will be produced that the length of product produced will be 296 bp.
ACTB F: GGCCACGGTCTCTTGTTAGA
ACTB R: ACCGTTGCCAATCTAAGTGC

Electrophoresis

In the final, electrophoresis technique is used to place the gel for placement PTPN22C1885T.

Sample size and sampling

The sample size was determined using the Fourth et al (2006) method and MedCalc statistical software. The sample consisted of 237 subjects (120 patients and 117 controls) for the two groups which were determined using a 5% margin of error and 80% power. Non-probability sampling is through convenience sampling.

Statistical Analysis

The statistical analyses of the data were performed with the statistical package of SPSS. The Chi-square test was used for correlation analysis and after Odds ratio report, logistic regression model was used for random effects.

RESULTS

This study conducted on 140 people including 70 scleroderma patients referring to the Rheumatology Clinic of Golestan Hospital in Ahvaz, Iran and attending and 70 healthy people as

Table 1: The overall result of the distribution of PTPN 22 polymorphisms R 620W gene in scleroderma patients and healthy people

Gene	Frequency in patient groupN%	Frequency in control groupN%	O.R	P-value
PTPN22	13(18.5)	19(27.5)	0.61	0.227

Table 2: frequency of PTPN 22 polymorphisms R 620W gene in limited scleroderma patients and healthy people

Gene	Frequency in patient groupN%	Frequency in control groupN%	O.R	P-value
PTPN22	9(19.5)	19(27.5)	0.65	0.24

controls. The groups of patients and control group were matched by age, sex, and ethnicity.

In the patient group, there were 22 people of Arab origin and 48 were non-Arab race and in the control group 25 people are of Arab origin and 45 were non-Arab race. Of the 70 patients with scleroderma 46 were limited and 24 were diffuse form.

1. Comparison of PTPN22 R620W polymorphism gene in patients with scleroderma and non-patient persons:

From 70 scleroderma patients, 13 (18.5%) people were had the mentioned gene and from 70 healthy subjects 19 (27%) had the mentioned gene where there was statistically significant correlation between the two groups (OR=0.61, P-value: 0.227).

2. Comparison of PTPN22 R620W polymorphism gene in patients with scleroderma and non-patients:

Out of 46 limited scleroderma patients, 9 people had the mentioned gene and out of 70 healthy individuals 19 subjects had the gene and there was no statistically significant relationship between the two groups.

3. Comparison of the frequency of PTPN22 R620W polymorphism gene in diffuse scleroderma patients and non-patients and there was no statistically significant relationship between the two groups.

Out of 24 patients with diffuse scleroderma, 4 people had the mentioned gene and out of 70 healthy individuals 19 had the mentioned gene and there was no statistically significant relationship between the two groups.

4. Comparison of the frequency of PTPN22 R620W polymorphism gene in limited scleroderma patients + ACA and non-patients and there was no statistically significant relationship between the two groups.

Out of 46 patients with limited scleroderma, 38 people had +ACA and out of these people 8 had the mentioned gene, and out of 70 healthy individuals 19 had the mentioned gene and there was no statistically significant relationship between the two groups.

Table 3: frequency of PTPN 22 polymorphisms R 620W gene in diffuse scleroderma patients and healthy people

Gene	Frequency in patient groupN%	Frequency in control groupN%	O.R	P-value
PTPN22	4(16.6)	19(27.5)	0.45	0.17

Table 4: Frequency of PTPN 22 polymorphisms R 620W gene in patients with limited +ACA scleroderma and healthy people

Gene	Frequency in patient groupN%	Frequency in control groupN%	Gene	P-value
PTPN22	8(21)	19(27.5)	0.72	0.32

Table 4: Frequency of PTPN 22 polymorphisms R 620W gene in patients with limited -ACA scleroderma and healthy people

Gene	Frequency in patient groupN%	Frequency in control groupN%	O.R	P-value
PTPN22	1(12.5)	19(27.5)	0.38	0.33

5. Comparison of the frequency of PTPN22 R620W polymorphism gene in limited scleroderma patients -ACA and non-patients.
   Out of 46 patients suffering limited PTPN22 R620W polymorphism 8 patients were negative ACA, of them one patient had the mentioned gene and out of 70 healthy people, 19 had the mentioned gene and there was no statistically significant relationship between the two groups.

DISCUSSION

Systemic sclerosis is a chronic systemic disease with unknown variety of etiology, and has clinical chronic protests often progressive.

Genetics plays an important role in the development of scleroderma, and various studies conducted in geographic areas and among different races and ethnicities, different alleles have been identified as a risk factor in scleroderma disease. In both groups in terms of ethnicity, age and gender, the individual were matched as much as possible.

All the subjects were older than 18 years. The lowest age in patients with scleroderma was 18 and maximum age was 59, where the average age in this group was 38.5. In the control group, the youngest was 18 and maximum age was 41, where the average age in this group was 32.5.

Because scleroderma is more common in women as in other autoimmune diseases, most of the participants in this study were female.

In the patient and control groups, there were 59 women (84%) and 11 males (16 percent).

A study conducted in 2002 in America included 1150 people, the results showed that PTPN22 R620W polymorphism is strongly associated with diffuse scleroderma patients (OR: 2.21) and limited positive ACA (OR: 2.03) whose results were not consistent with our results.

In a study that was conducted in 2004 on white Spanish people that included 636 patients with scleroderma (370 cases had limited and 182 had diffuse) and 1128 healthy people were as control, the results showed an association between scleroderma and PTPN22 R620W polymorphism.

(R: 1.15) in addition to this allele (11) had a strong relationship with a subset of scleroderma patients with positive ACA, and our results were not consistent with our study.
In the meta-analysis conducted by Diaz-Gallo et al (2001) on 3422 patients with scleroderma (2020 limited form and 1208 diffuse) and studied 3638 healthy individuals for Polymorphism where the results indicate the relationship of this polymorphism with the talent for scleroderma (OR: 1.15). in addition, this polymorphism has a strong connection with the sub-group of positive ACA patients (OR: 1.2). The results were consistent with our results.

In a case-control study conducted in 2012 by Ramirez et al on Colombian patients, 101 patients, it was determined that there is no relationship between PTPN22 R620W and suffering polymorphism. The results of that study were consistent with the results of our study.

Given that studying the relationship between PTPN22 R620W with scleroderma patients have not been conducted in the country, this study is very important.

Ethnic diversity of the population studied and other smaller sample size may be the main causes of the lack of consistency of the research results with the results of other studies in different countries. Findings of this study can be used to develop more efficient pharmaceutical treatments for scleroderma. In addition, considering the development of non-pharmaceutical treatments of skin related disorders during recent years, these findings can be used to develop more efficient treatments (13-16).

CONCLUSION

The results of this study indicate that the frequency of PTPN22 gene polymorphism R620W in all patients with scleroderma, limited scleroderma, diffuse scleroderma, limited

scleroderma with ACA, and patients with limited scleroderma without ACA is not more common than in healthy individuals.

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