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The susceptibility to diabetic retinopathy in type 2 diabetic patients of Iran is not affected by the M55V polymorphism of SUMO4

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ABSTRACT

Introduction: Recent reports showed that the small ubiquitin-like modifier 4 (SUMO4) M55V polymorphism is affected development of type 2 diabetes and its complications such as diabetic nephropathy and retinopathy in some populations. In this regard we intend to investigate the impact of M55V polymorphism of SUMO4 on susceptibility to diabetic retinopathy in the type 2 diabetic patients of Iran.

Methods: In current study, the control group had included 100 individuals of type 2 diabetic patients of Iran who have not retinopathy, while the patient group had included 100 individuals of type 2 diabetic patients of Iran suffering from retinopathy. We have matched all the confounding factors between patients and controls. The genotyping method was PCR-RFLP to trace a prevalent single nucleotide polymorphism at codon 55 encoding a substitution of methionine to valine (M55V) specified in the SUMO4 gene.

Results: Genotype frequency of AA, AG, and GG of SUMO4 were obtained respectively 52%, 30%, and 18% in the patient group and 44%, 36%, and 20% in the control group. The frequencies of alleles A and G of SUMO4 were 67% and 33% in the patients with type 2 diabetes having retinopathy and 62% and 38% in the patients with type 2 diabetes without retinopathy. There genotypic and allelic frequencies of SUMO4 were not significantly ($p>0.05$) different between the patient and the control groups.

Conclusions: The findings of the present study showed no correlation between M55V polymorphism of SUMO4 gene and diabetic retinopathy in type 2 diabetes patients of Iran. However, we emphasize that further studies is necessary to clarify the exact role of M55V polymorphism of SUMO4 in diabetic retinopathy in patients with type 2 diabetes.

KEY WORDS: type 2 diabetes, retinopathy, SUMO4 polymorphism, Iran.

1. INTRODUCTION

From the diabetic complications, leading to progressive injury to retina, is Diabetic retinopathy. It is a critical complication of sight-threatening in diabetes (Yau, 2012).

Diabetes is known as a disorder which is along with metabolic disorders in particular for Use and Storage of glucose that can lead to many complications. High blood glucose levels can cause injury in a wide spectrum of parts of the body such as eyes. With the passage of time, diabetes can affect the blood supplying system of the retina. Diabetic retinopathy often appeared secondary to injury to the small blood vessels supplying retina. Blood and other fluids leaking of the vessels result in swelling of the retinal tissue and also blurred vision. Both eyes are usually affected in such condition. The longer period of having diabetes, the more likely to develop diabetic retinopathy and even if left untreated, blindness may occur. There are many factors that can participate in the pathogenesis of diabetic retinopathy (Yau, 2012; Rudofsky, 2008; Tang, 2014). According to this, it seems that development and progression of diabetic retinopathy is affected by genetic susceptibility of individuals (Tang, 2014). The transcription factor of NF-κB, in most cell types can be triggered by a variety of molecular conditions such as high glucose levels (Aghdam, 2013; Huang, 2013; Chen, 2014). Also, as described by Rudofsky (2008), through a multiple logistic regression model, the SUMO4 M55V polymorphisms on the prevalence of diabetic retinopathy in type 1 diabetes individuals independent from age and duration of the disease.

Furthermore, it has been proven that the *SUMO4* gene locating in the locus of type 1 diabetes susceptibility and IDDM5, thanks to regulation of NF-κB, results in activation of transcriptional factor of heat shock deals with immune responses including both autoimmunity and inflammation (Sang, 2010; Tang, 2012). Through the substrate IκB, SUMO4 can alter immune response. This is a negative regulator of NF-κB (Gao, 2014). In unstimulated cells, IκB binds to NF-κB in cytoplasm that results in releasing a variety of stimulating factors to degradation induction of IκB via the proteasome. Secondary to degradation of IκB, NF-κB is released from cytoplasm toward nucleus and then it leads to the genes transcription involving in inflammation, immune response and programmed cell death (apoptosis) (Wada & Makino, 2013). It has been shown that the human SUMO4 protein can be conjugated to the same site of IκB (Cajee, 2012). SUMO4-modifiered IκB is resistant to degradation and cannot be ubiquitinated (Aghdam & Sheibani, 2013).

The gene of SUMO4 locating on chromosome region 6q25 encodes SUMO4 protein (Sozen, 2014). The presence of a common polymorphism in the SUMO4 gene which encodes a M55V is recently established (Gao, 2014). Recent reports have shown a relationship between the M55V polymorphism of the gene SUMO4 and the risk of type 2 diabetes, diabetic nephropathy and diabetic retinopathy in various populations (Noso, 2007; Lin, 2007); whereas in Fallah (2010), study on Iranian population no association of susceptibility to type 2 diabetes with mentioned polymorphism was observed. With this in mind, it was decided to look further into the association of this polymorphism with susceptibility to diabetic retinopathy in Iranian patients with type 2 diabetes in a larger sample size.

2. METHODS

Subjects: In the current research patients with type 2 diabetes were studied in two 100-person groups; the first one, control group without diabetic retinopathy and the other one the patient group with diabetic retinopathy. All the patients were matched in all confounding factors such as age, sex, blood glucose levels, duration of disease, and compliance to treatment with the patient group. Any significant difference was not observed between the two groups in confounding factors (Table 1). All the participants in this study had age range of 45-67 years, and at least 10 years' history of type 2 diabetes. Regarding the American Diabetes Association, 2011 Criteria on Classification and Diagnosis of diabetes, type 2 diabetes and diabetic retinopathy diagnosis was established. The present study was accepted by the Ethical Committee of Medical University of Lorestan and according to the Declaration of Helsinki the informed consents was provided. All the samples of the participating patients were collected with their written consent.

Table.1. The characterizes of diabetic patients with and without (controls) retinopathy

Group	Age*	Male	Female	FBS*
Diabetic patients with retinopathy (n=100)	60±7	50	50	272.2±28
Diabetic patients without (controls) retinopathy (n=100)	55±10	50	50	254.5±36
p value	>0.05**	-	-	>0.05**

FBS: Fast Blood Sugar (mg/dl); * Mean±SD; ** No significant difference

Genotyping: DNA samples were extracted from the peripheral leukocytes of the blood samples through the salting-out method. Then, the genotypes of the DNA samples were determined by using the PCR-RFLP method (Fallah, 2010) for amplification of the fragments of SUMO4 containing the 163A/G SNP.

PCR reactions were performed in volume of 25 µl including 2.5 µl of each primer, 50 ng/µl DNA and 12.5 µl of 10x buffer, Taq polymerase and dNTPs. The PCR-RFLP methods were used for genotyping, using restriction enzyme TSpR I (Biolab Company). The amplified products were incubated for 16h at 65°C and separated on a 3% agarose gel. Finally, the digestion patterns for different alleles were checked on an ultraviolet transilluminator and photographed (Fallah, 2010). The primer sequences, restriction enzyme and restriction digestion patterns for genotyping of SUMO4 polymorphism are assorted in Table 2.

Table.2. Primer sequences, restriction enzyme used and restriction digestion patterns for genotyping of SUMO4 polymorphism

SUMO4 polymorphism	Sequences of the primers	PCR Product Size	Restriction enzyme	Length of the Restriction fragments for different alleles
SUMO4 (163A/G)	F: 5′ TGTGAACCACGGGGATTGTCG 3′ R: 5′ TCAGTAGACACCTCCCGTAC 3′	200 bp	TSpR I	A-134 bp+66 bp G-200 bp

F: Forward; R: Reverse; bp: base pairs

Statistical Analysis: The genotypic and allelic frequencies of SUMO4 polymorphism were assayed by direct counting in the groups. The differences in genotypic and allelic frequencies of SUMO4 polymorphism were determined by Chi-Squared test between the two tested groups. Finally, $p<0.05$ was proposed as a statistically significance level after Yate's correction. The chi-square test was staffed to investigate deviations from Hardy-Weinberg equilibrium.

Ethical Considerations: The protocol of the study was reviewed and accepted by the medical research ethics committee of Medical University of Lorestan.

3. RESULTS AND DISCUSSION

The genotypic and allelic frequencies of *SUMO4* polymorphism are listed in tables 2 and 3. The frequencies of SUMO4 AA, AG, and GG genotypes got registered respectively 52%, 30%, and 18% of the type 2 diabetic patients with retinopathy and 44%, 36%, and 20% of the type 2 diabetic patients without retinopathy. No significant

differences were observed between the frequency of SUMO4 genotypes in the patient group in comparison to the control group (p>0.05) (Table 3).

Table.3. Distribution of SUMO4 genotypes in diabetic patients with and without (controls) retinopathy

SUMO4 polymorphism	Genotypes	% of patients (n=100)	% of controls (n=100)
SUMO4 (163A/G)	AA	52[a]	44
	AG	30[b]	36
	GG	18[b]	20

a: Increased frequency, b: Decreased frequency

The frequencies of *SUMO4* A and G alleles were 62% and 38% in the type 2 diabetes individuals with retinopathy and 67% and 33% in the type 2 diabetes individuals without retinopathy. There was no significant difference in frequency of SUMO4 alleles in the patient group compared with the control group (p>0.05) (Table 4).

Table.4. Distribution of SUMO4 alleles in diabetic patients with and without (controls) retinopathy

SUMO4 polymorphism	Alleles	% allele frequency in patients	% allele frequency in controls
SUMO4 (163A/G)	A	67[a]	62
	G	33[b]	38

a: Increased frequency, b: Decreased frequency

Regarding to the assessment of risk of disease, human population is a non-homogeneous factor, because of the differences existing in the genetic and environmental characteristics. Furthermore, the difference in ethnicity also supports genetic heterogeneity across population (Shahsavar, 2013). This has demonstrated that there are a number of genetic factors, contributing to the progression of diabetic retinopathy (Yau, 2012; Tang, 2014).

The results presented by Rudofsky (2008), on type 1 diabetic patients indicate a firm independent association of the genotypes of GG and AG of the SUMO4 gene in codon 55 with a reduction in prevalence of diabetic retinopathy. Their data propose that polymorphism of the SUMO4 gene not only could be dealt with the diabetes pathogenesis, but also seems to play a role in the pathogenesis of diabetic retinopathy in diabetic patients (Rudofsky, 2008). Some other studies suggest that the wild type allele could result in a lower NF-κB activity mediated by a stabilization of IκB (Gao, 2014).

In addition, we investigated the correlation of diabetic retinopathy with SUMO4 M55V polymorphism in type 2 diabetes patients of Iran. As a result, the SUMO4 frequency for AA, AG, and GG genotypes was almost the same in the two tested groups. The AG and GG genotypes frequency of SUMO4 were less common in type 2 diabetes patients with retinopathy, but it was not statistically significant (p>0.05). Thus no significant association is reported between this polymorphism and diabetic retinopathy in type 2 diabetes Iranian patients. We suggest that the reason for such paradoxical findings might be secondary to different ethnic groups of the studied cohorts, for example, the Chinese and Iranians, and thereby, it demands further researches in different ethnicities in order to identify the effect of the mentioned polymorphism in diabetic retinopathy.

In general our studies in this field have shown no association of this polymorphism with susceptibility to type 2 diabetes, diabetic nephropathy, and diabetic retinopathy in population of Iran. More ever, lack of association between M55V polymorphism of the gene SUMO4 and susceptibility to the diabetic type 2 complications in the same population might be also secondary to the small sample size.

4. CONCLUSION

Present study shows that the susceptibility to diabetic retinopathy in type 2 diabetic patients of Iran is not affected by the SUMO4 M55V polymorphism. The results illustrate that there are similarities between the SUMO4 genotypes distribution in Iranian population and other Caucasian populations in other studies. Moreover, this study followed the same conclusion with previous studies about no association susceptibility to both type 2 diabetes (Fallah, 2010) and diabetic nephropathy (Shahsavar, 2013) with M55V polymorphism of SUMO4 in Iranian population. However, we highlight that it needs to be investigated further to show the exact role of SUMO4 M55V polymorphism in type 2 diabetes and its complications.

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