Original Article

Zahedan Journal of Research in Medical Sciences

Journal homepage: www.zjrms.ir

Association of DD Genotype of Insertion/Deletion Polymorphism of Angiotensin-Converting Enzyme Gene with Systemic Lupus Erythematosus and Lupus Nephropathy

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Article information

Article history:
Received: 28 Oct 2012
Accepted: 15 Jan 2013
Available online: 12 Mar 2013
ZJRMS 2013; 15(10): 69-73

Keywords:
Systemic lupus erythematosus
Lupus nephropathy
Angiotensin-converting enzyme
Polymorphism
Iran

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Abstract

***Background*:** Systemic lupus erythematosus (SLE) is a multisystem disease with unknown etiology. We hypothesized that insertion/deletion (I/D) polymorphism of angiotensin-converting enzyme (ACE) gene may influence the development and/or progression of SLE and lupus nephritis.

***Materials and Methods*:** In a crass sectional case-control study, genomic DNA from 106 SLE patients and 103 healthy controls matched for sex, age, and ethnicity, were genotyped for the (I/D) polymorphism of ACE gene by polymerase chain reaction (PCR). Comparison of quantitative variants between two groups was assessed by student t-test and association between qualitative variables was analyzed by the chi-square or Fisher exact tests.

***Results*:** The frequency of DD genotype in SLE patients was significantly higher than control group (25.5 % vs. 14 %), and the risk of SLE was 2.2 times greater in subjects with DD genotype than the individual by DI and II genotypes (OR, 2.2 [95% CI, 1.1 to 4.4]; p=0.023). The distribution of D allele in SLE patients was significantly higher (p=0.021) compare to controls (47 and 36.4, respectively). The Risk of nephropathy in SLE patients with DD genotype was three times more than other genotypes (OR), 3 [95% CI, 1.1 to 8]; p=0.027].

***Conclusion*:** This study demonstrated that ACE DD genotype acts as a risk factor on SLE and Lupus nephropathy in an Iranian population.

Introduction

Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease which is identified by an extensive range of auto antibodies and afterwards by immunity complex sedimentation such as immunoglobulins and components of complement system that cause chronic inflammation in several organs [1].

This multisystem disease is accompanied with a wide range of clinical manifestations. The incidence of SLE is estimated to be about 40 to 400 per 100,000 individuals and it is not similar in different countries and races. Also the manifestations of SLE are different in various races [2, 3]. The pathogenesis of SLE is not completely identified; however environmental, genetic and hormonal factors can be effective in SLE onset and progression [1]. There are some evidences that show genetic factors could influence the development and progression of SLE [4]. Several candidate genes have been examined to determine the probable SLE 'susceptibility factor'. Complement component C1q [5], Tumor Necrosis Factor alfa (TNFα) [6], Interferon-gamma [7], programmed cell death 1 [8], Interleukins [9], monocyte chemoattractant protein 1[10] and Angiotensin Converting Enzyme [11, 12] are some of the candidate genes related to SLE development.

Different studies have shown probable effects of Angiotensin Converting Enzyme (ACE) gene polymorphisms in development and progression of SLE [11, 12]. This zinc metallopeptidase, has two predominant physiological functions: the cleavage of angiotensin I to the potent vasoconstrictor angiotensin II in rennin-angiotensin system (RAS) and inactivation of the vasodepressor nanopeptide bradykinin (BK) [13]. Angiotensin II (AII) as the key effecter molecule of RAS system is a vasoactive peptide and growth factor that plays important roles in vascular reactivity and tissue remodeling. Moreover AII is an effective pro-inflammatory modulator that could augment and perpetuate immune responses in renal and non-renal tissues, Therefore this molecule could mediates development and progression of renal disease in SLE [14].

Human ACE gene is 21 kb long and located in long arm of chromosome17 (17q23). The gene contains 26 exons and 25 introns expressed in many tissues as lung, endothelium, kidney, heart and testicles [13, 15]. There are several ACE gene polymorphisms, but I/D polymorphism is the most studied and clinically important one. This polymorphism consists of the insertion (I allele) or deletion (D allele) of a 287-bp repetitive sequence within intron 16 of the ACE gene [16]. Many studies reported that this polymorphism is a determining factor in ACE activity. Individuals with DD genotype have the most ACE activity in plasma. ID heterozygotes showed a moderate ACE activity while homozygotes for I allele reveal the least enzyme activity [16].

There are conflicting reports on association of the I/D polymorphism with increased susceptibility to SLE. According to some studies I/D polymorphism in intron 16 of the ACE gene might be associated with SLE disease and plays an important role in the molecular mechanism of SLE [11, 12, 17, 18].

In a few studies, I allele was found to be associated with SLE and lupus nephropathy [11, 12] whereas several other studies showed that presence of D allele can be associated with pathological manifestations caused disturbance of endothelial activity, which is considered as a probable factor in capacity and pathogenesis of vascular injury and lupus disease [19, 20]. However in other studies was not found association between this polymorphism and SLE [20, 21].

Although the frequencies and genotypes distribution of the ACE gen has been assessed in diabetes patients and general population in Iran [22, 23], to our knowledge, it has not been investigated in the SLE patients in Iran. The aim of present study was to investigate the relation of I/D polymorphism of ACE gene with SLE and lupus nephropathy in South East of Iran.

Materials and Methods

This cross sectional case-control study was conducted prospectively on 106 SLE patients recruited from Zahedan Rheumatology Clinics according to American College of Rheumatology Classification (ACR) criteria during November 2009 to June 2011. The control group consisted of 103 healthy age, sex and race matched individuals.

The project was approved by the Zahedan University of Medical Sciences Ethics Committee. At the time of admission, after written consent, a verbal interview was conducted to determine sex, age, ethnicity and SLE manifestation. Ethnicity was determined by self report. Subjects with history of high blood pressure, cardiovascular disease, diabetes, kidney and hepatic disease were excluded from the study.

DNA Analysis*:* Whole blood was collected in 2 ml Na-EDTA tubes and genomic DNA was extracted using DNA extraction kit (Roche, Germany).

The polymerase chain reaction (PCR) method was used for amplification the corresponding DNA fragments (490 bp and 190 bp for the insertion and deletion alleles respectively) as previously described. PCR products were separated by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining. Since the D allele in heterozygous samples is preferentially amplified, a second independent PCR amplification with a primer pair that recognizes an insertion-specific sequence was carried out only for DD genotypes according to Rigat et al. protocol [24, 25]. This study was conducted according to the principles expressed in the declaration of Helsinki. Ethical approvals were obtained from Zahedan University of Medical Sciences Ethics Committee.

Statistical analyses were carried out by using the SPSS-15. Quantitative data were presented as mean±standard deviation. Comparison of quantitative variants between two groups was assessed by student *t*-test. Qualitative variables were showed as frequency and percentage and association between qualitative variables was analyzed using the chi-square or Fisher exact tests. Differences with *p*-Values less than 0.05 were considered significant. Odds ratio and 95% confidence interval were calculated to evaluate the strength of the associations.

Results

Demographic SLE patients and control samples were showed in table 1. This study included 106 Iranian unrelated SLE patients and 103 age, gender and ethnicity matched control subjects. Skin manifestations, arthritis, lupus nephritis and neuropsychiatric manifestations developed in 83%, 87%, 22% and 14% respectively of patients during the course of their disease.

Allele frequencies of I/D polymorphism of ACE gene were in Hardy Weinberg equilibrium. The frequency of the three DD, ID, II genotypes were 25.5, 42.5 and 32% in SLE patients and 14, 45.5 and 40.5% in controls which were not statistically significant (p=0.084). The frequency of homozygotes for D allele (DD genotype) in SLE patients was significantly higher than control group (25.5% vs. 14%), and the risk of SLE in subjects with DD genotype was 2.2 times more than the individual by ID and II genotypes (OR , 2.2 [95% CI, 1.1-4.4]; p=0.023). The distribution of D allele was 47 percent in SLE patients and 36.4% in controls and were statistically significant (p=0.021) (Table 2).

In regard to clinical manifestations of SLE disease; the frequency of DD genotype of ACE gene was higher in patient with nephritis and the risk of nephropathy in SLE patients by DD genotype was three times more than others [95% CI, 1.1-8]; p=0.027] 3(OR) (Table 3). We did not observe any association between other SLE manifestations and I/D polymorphism of ACE gene.

Table1. Demographic characteristics of SLE patients and controls

parameter	SLE N=106	Controls N=103	*p*-Value
Age(year)	31.8±7.8	32.9±13.3	0.53
Sex (male/female)	9/97	8/95	0.5
Race N(%)			0.27
Persian	49(46)	53(51)	
Balouch	57(54)	50(49)	

Table 2. Genotype and allele frequencies of I/D polymorphisms of the ACE gene in SLE patients and controls

	Control N(%)	SLE N(%)	χ^2	*p*-Value
I/D polymorphism			5	0.084
DD	14(14)	27(25.5)		
ID	47(45.5)	45(42.5)		
II	42(40.5)	34(32)		
DD	14(14)	27(25.5)	4.7	0.023
II+ID	89(86)	79(74.5)		
D	75(36.4)	99(47)	4.6	0.021
I	131(63.6)	113(53)		

Table 3. Comparison of Genotype frequencies of I/D polymorphisms of the ACE gene in SLE patients with and without nephropathy

	SLE patients without nephropathy N(%)	SLE patients with nephropathy N(%)	χ^2	*p*-Value
I/D polymorphism			5	0.08
DD	17(20.5)	10(43.5)		
ID	37(44.6)	8(34.8)		
II	29(34.9)	5(21.7)		
DD	17(20.5)	10(43.5)	4.7	0.027
II+ID	66(79.5)	13(56.5)		

Discussion

In the present study, there was no significant difference in DD, ID, II genotype frequencies between SLE patients and controls in Iranian population; however the frequency of individuals with D allele was significantly higher in SLE patients than healthy controls. The risk of SLE was twofold higher in subjects with DD genotype. In addition distribution of D and I alleles showed significant difference in two groups. The risk of lupus nephropathy was 3 fold higher in individuals with DD genotype compare to DI and II genotypes.

Systemic lupus erythematosus is a complex autoimmune disease with wide range clinical manifestations such as arthritis, different types of rashes, serositis, cytopenia, neurological symptoms and nephropathy [1]. Although the exact etiology of SLE is unknown, but it has been revealed that a combination of genetic risk factors and environmental events could lead to disease initiation and progression [2, 3].

Lupus nephropathy is one of the most serious complications of this disease and associated with increased mortality and morbidity. It has been suggested that this complication might be related to abnormal regulation of the complex system consisting of the renin angiotensin and NO production [17, 26].

In recent years, many studies tried to identify effective genetic elements on lupus disease by case control studies. Whereas the ACE gene could be an appropriate candidate, in this study we investigated the distribution of I/D polymorphism of ACE gene in SLE patients and its association with lupus nephropathy.

Several studies have shown the association of I/D polymorphism with SLE and lupus nephropathy [11, 12, 18, 19]. Although there are some reports which showed no association between I or D allele [20, 21].

The results of our study are in contrast to the report of Sato et al. in a Japanian population. They showed that the frequency of II genotype was higher in SLE patients than controls and I allele can be considered as predictive factor for lupus [11]. Also Akai et al. reported the association of I allele of ACE gene with lupus nephritis activity in Japans [12].

In consistent to present study Pullmann et al. in Oslovaki reported that D allele frequency of ACE gene in SLE patients was significantly higher than controls (62% vs 52%) [18]. In addition they showed that DD genotype is associated with visceral injuries in lupus patients [15]. In another study in Croatia, Prkasin et al. demonstrated that D allele distribution in lupus patients is significantly higher than healthy controls [19].

However Kaufman et al. reported no association between I/D polymorphism of ACE gene and lupus in Americans [20]. Also association of this polymorphism with lupus, lupus nephritis and the vascular manifestations have not been demonstrated in the Uhm et al study in Korea; but the DD genotype was negatively associated with Raynaud's phenomenon among SLE patients [27]. In another study Molad et al. did not observe any association between this polymorphism and SLE, cardiovascular and kidney disorders [21].

Parsa et al. genotyped 644 SLE patients and 1130 family members for three ACE gene polymorphisms in United States and suggested that I/D polymorphism of ACE gene are effective in lupus and lupus nephritisis in non-Caucasians [14]. This association was not reported in Dauglas et al. study neither in the African American nor in Caucasian groups [28]. Sprovieri et al did not observe any association of polymorphism of the renin-angiotensin system genes with lupus nephropathy in Brezilian patients, however stated association of Chronic Renal Failure (CFR) progression with DD genotype [17].

Lee et al. conducted a Meta analysis on 2962 subjects in korea in 2006. Although they observed a trend for relation of the DD genotype and the D allele with SLE in Caucasian patients, the association was not statistically significant [29].

There was not any significant difference between distribution of I/D polymorphism genotypes in patients and control group in Al-Awadhi and colleagues study in Kuwait, but they pointed that DD genotype may causes predisposes to vascular disease [30].

On the other hand, Abbas et al. investigated the association of ACE gene polymorphism and serum ACE level among Egyptian SLE patients and its relation to disease activity parameters in 2012. They showed I/D polymorphism and serum ACE level could be associated with the disease activity and D allele might be used as one of the predictive factors for the activity of SLE [31].

Recently Zhou et al. performed a metaanalysis on sixteen investigations for the analysis of association between ACE I/D gene polymorphism and SLE (1959 patients with SLE and 2078 controls). They reported a significant association between D allele or DD genotype and SLE susceptibility. Also they showed DD

homozygous was associated with LN risk (OR 2.78, 95% CI 1.26-6.11, [32].

As stated the results of our study are consistent with most previous studies and in consistent with some others. These variations are predictable due to race, sample size and criteria differences. Further studies in different regions of Iran are suggested to determine the contribution of I/D polymorphism of ACE gene and other genes with lupus and lupus nephropathy.

Acknowledgements

The authors would like to appreciate the research deputy of Zahedan University of Medical Sciences for financial support of this research.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors have declared no competing interests exist. The manuscript has been seen and approved by all authors and is not under consideration for publication elsewhere.

Funding/Support

Zahedan University of Medical Sciences.

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Please cite this article as: Salimi S, Naghavi A, Zakeri Z, Nabizadeh S, Farajian Mashhadi F, Sandoughi M. Association of DD genotype of insertion/deletion polymorphism of angiotensin-converting enzyme gene with systemic lupus erythematosus and lupus nephropathy. Zahedan J Res Med Sci (ZJRMS) 2013; 15(10): 69-73.