JRHS 2014; 14(3): 218-220

JRHS
Journal of Research in Health Sciences
journal homepage: www.umsha.ac.ir/jrhs

Original Article

The L-Selectin Phe206Leu Gene Polymorphism Is Not Associated With Visceral Leishmaniasis

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ARTICLE INFORMATION

Article history:
Received: *17 April 2014*
Revised: *05 May 2014*
Accepted: *27 May 2014*
Available online: *31 May 2014*

Keywords:
L-selectins
Polymorphism
Visceral leishmaniasis

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ABSTRACT

Background: Previous studies revealed that selectins play key roles in homing of immune cells to inflamed tissues and lymphatic organs. L-selectins are expressed on immune cells and interact with P and E selectins to homing to the tissues, hence, the polymorphisms within the gene of L-selectins may are associated with alteration in its expression. Thus, the current cross-sectional analytical study has been designed to investigate the polymorphisms within L-selectin gene and their relation with visceral leishmaniasis (VL).

Methods: This study was performed on 194 samples during 2004-2012.The PCR-SSP and immunoflorescence techniques were used to evaluate the L-selectins polymorphism and anti-*Leishmania* antibody titration, respectively, in 56, 74 and 64 seropositive VL patients (group 1), seropositive healthy controls (group 2) and seronegative healthy controls (group 3).

Results: The results showed that the genotypes (P=0.711) and alleles (P=0.679) within L-selectins gene (A/C) was not differ between groups. Our results also demonstrated that the genotypes within L-selectins in group 1 (P=0.807) and 2 (P=0.441) were not associated with the titration of anti-leishmania antibody.

Conclusions: The results identified that the polymorphisms within L-selectins gene were not associated with VL and it may be concluded that these genotypes and alleles are unable to affect immune responses in VL patients.

Citation: Sardarian K, Abasi M, Solgi G, Hajilooi M. The L-selectin Phe206Leu gene polymorphism is not associated with visceral leishmaniasis. J Res Health Sci. 2014;14(3): 218-220.

Introduction

Cutaneous, mucocutaneous and visceral leishmaniasis (VL), also known as Kala Azar, are the main complications which are induced by *Leishmania* species[1]. Previous investigations revealed that VL is endemic in northwestern and southern areas of Iran.[2] Thus; the disease is a main health complication in the area[2]. It appears the altered immune responses against the parasite can be considered as an important reason for production of VL form[3,4]. Thus, it may be hypothesized that the host genetic and immunological status determines the infection outcome[5]. Ligand-selectin (L-selectin), as a main intercellular adhesion molecule, plays important roles in the regulation of immune cell`s homing to the infected tissues including parasite infections[6]. Therefore, it may be hypothesized that altered expression of this molecule may be associated with VL. Previous investigations demonstrated that the genetic variation, polymorphisms, at position +206 (Phe206Leu) is associated with expression of this molecule[7]. Interestingly, it has also been reported that Phe206Leu polymorphism within L-selectin gene is associated with several immune related diseases[8-10].

Therefore, it can be hypothesized that the polymorphism, Phe206Leu, may be associated with VL in Iranian population. Therefore, the aim of this study was to examine the Phe206Leu polymorphism in the seropositive VL patients in comparison to seropositive and negative healthy controls.

Methods

This study was performed on 194 samples during 2004-2012.This cross-sectional study was performed on three groups including; 56 seropositive VL patients (group 1), 74 seropositive healthy individual (group 2) and 64 seronegative healthy controls (group 3). The participants have been selected with the same age and sex ratios. VL diagnosis has been performed by an expert specialist using medical history, laboratory findings and clinical presentations. The criteria for diagnosis clinical presentations has been defined in our previous study[4] and were as follow; fever, severe anemia, weakness, cough, diarrhea, vomiting, fatigue, and appetite loss. The participants were selected from an endemic area for *Leishmania infantum*, East Azarbaijan (North–West of Iran)[11,12]. The participants filled out an informed consent form prior to sample collection and the study protocol has been approved by

the Ethical Committee of the Tabriz University of Medical Sciences.

A commercial kit from Bioneer Company (South Korea) was used for DNA extraction. The L-selectin Phe206Leu polymorphism protocol has been described in our previous study[8]. PCR sequence-specific primer (PCR-SSP) method was used to evaluate the polymorphism. The primer sequences for detecting the polymorphism were 5′-ATGGGCCCCAGTGTCAGT-3′ (which is used for T allele detection), 5′-ATGGGCCCCAGTGTCAGC-3′ (which is used for C allele detection) and 5′-CAAGCTCATTAGATCGTGAGC-3′ (generic primer). Internal control primer 1(HGH forward), 5'-GCCTTCCCAACCATTCCCTT-3', and internal control primer 2 (HGH reverse), 5'-TCACGGATTTCTGTTGTGTTTC-3', were used. The internal control primers were used in all reactions to amplify a 927 bp segment of the human growth hormone gene to check for successful PCR amplification. Amplification was carried out using a PCR Techne Flexigene apparatus (Roche)[8]. In a total volume of 50 µl containing 0.2 ng genomic DNA, 10 pmol allele-specific primers and 10 pmol common primers, 200 mM each dNTP, 10 mM Tris/HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase. PCR performed without DNA template represented the negative controls. The reaction was carried out as follows: initial denaturation at 94°C for 2 min, followed by 30 cycles of amplification at 94°C for 15 s, annealing at 60°C for 45 s, and extension at 72°C for 45 s; with final extension for 2 min at 72°C. The PCR reactions were performed in duplicate for every subject. In addition, the PCR reaction was repeated on 30 % of the subjects to verify the accuracy of the procedure. The amplified PCR products were analyzed by 2% agarose gel electrophoresis followed by ethidium bromide staining and ultraviolet visualization. The specific band was 339 bp. Anti-*Leishmania* antibody was measured according to our previous study[4].

The genotype data are presented in HWE. Data analysis was performed using SPSS statistical software (version 18, SPSS, Chicago, IL, USA) and a *P* value of <0.05 was considered significant. The association of L-selectin Phe206Leu polymorphisms with the disease was calculated using χ^2 analysis and the differences of quantitative data between groups was analyzed using one-way ANOVA test.

Results

Our results revealed that L-selectin Phe206Leu genotypes (A/A, A/C and C/C) were not significantly differ in group 1 when compared to either group 2 or 3 (*P*=0.711) (Table 1). Binary comparison groups did not show significant differences with each other. The results also showed that the prevalence of L-selectin Phe206Leu A and C alleles were not significantly associated with VL (*P*=0.679) (Table 1). The statistical analysis also demonstrated that the VL patients (group 1) (*P*=0.807) and healthy individuals in group 2 (*P*=0.441) carrying various L-selectin Phe206Leu genotypes did not differ regarding the titration of anti-*Leishmania* antibodies (Table 2).

Discussion

It has been documented that L-selectin is up-regulated on the immune cells in response to infectious diseases, including leishmaniasis, which results in homing of immune cells to the infected tissues[13]. Moreover, previous studies demonstrated that L-selectin plays significant roles in infiltration of immune cells to the *Leishmania* infected tissues. The studies proposed that L-selectin as a membrane adhesion molecule participates in the pathogenesis of diseases and impaired in expression of this molecule may be a risk factor for them[14]. Additionally, previous investigations demonstrated that L-selectin Phe206Leu polymorphism is associated with alteration in expression of L-selectin[8].

Table 1: The A/A, A/C and C/C genotype and A and C alleles prevalence within L-selectin gene in the studied population

L-selectin A/C polymorphism	Group 1	Group 2	Group 3	*P* value
Genotypes, n (%)				
A/A	30 (53.57)	44 (59.46)	38 (59.37)	0.267
A/C	21 (37.5)	26 (35.13)	19 (29.68)	0.554
C/C	5 (8.93)	4 (5.41)	7 (10.95)	0.646
Alleles, n (%)				
A	81 (72.32)	114 (77.02)	95 (74.22)	0.590
C	31 (27.68)	34 (22.98)	33 (25.78)	0.931

Group 1: Seropositive infected patients, Group 2: Seropositive healthy controls, Group 3: Seronegative healthy controls.

Table 2: The anti-leishmania titration in various L-selectin genotypes

Anti-leishmania antibody titration	Group 1	Group 2
L-selectin A/A genotype	3 ±0.30	3.34 ±0.10
L-selectin A/C genotype	2.76 ±0.26	3.46 ±0.13
L-selectin C/C genotype	2.60 ±1.12	3.00 ±0.01
P value	0.807	0.441

Our results revealed that the genotypes and alleles of L-selectin Phe206Leu polymorphism were not associated with VL in a sub-Iranian population. Thus, it may be concluded that the L-selectin Phe206Leu polymorphism is not associated with VL and it cannot be considered as a risk factor for VL.

To the best of our knowledge, this is the first study which has evaluated the L-selectin Phe206Leu polymorphism in VL patients. Kumar and colleagues reported that L-selectin has been down-regulated on the Monocyte of VL patients when compared to healthy controls[15]. Although, L-selectin is up-regulated in the coetaneous leishmaniasis[14] but it is down-regulated in the VL[15], hence it appears that expression levels of L-selectin are down-regulated in the VL, a chronic form of leishmaniasis. The results also revealed that the L-selectin Phe206Leu polymorphism is not associated with titration of anti-leishmania antibody in both VL patients and healthy controls. Therefore, it seems that the polymorphism is unable to change immune responses against *leishmania* in VL patients. Based on the fact that there is another polymorphism within L-selectin gene, Tre49Ser[8], thus, it may be hypothesized that the polymorphism may be associated with VL and more studies can improve our knowledge regarding the effects of L-selectin polymorphism on the *leishmania* outcome. Additionally, due to the fact that epigenetic factors including gene methylation and microRNAs play significant roles in expression of immune related molecules, hence, it may be hypothesized that further studies can be designed to explore these factors to elucidate the main mechanisms involved in the pathogenesis of VL.

Conclusions

The results identified that the polymorphisms within L-selectins gene were not associated with VL and it may be concluded that these genotypes and alleles are unable to affect immune responses in VL patients. In related to this subject, other molecules of immune system can be investigated.

Acknowledgments

We would like to thank Tabriz University of Medical Sciences for financial support of this study.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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