RESEARCH ARTICLE

Comparison of L-selectin blood level and gene polymorphism in tuberculosis patients with healthy individuals

Peyman Eini^{1,2} | Maria Shirvani³ | Mehrdad Hajilooi⁴ | Farzaneh Esna-Ashari⁵

¹Department of Infectious Diseases, Hamadan University of Medical Sciences, Hamadan, Iran

²Brucellosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran

³Department of Infectious Diseases, Kermanshah University of Medical Sciences, Kermanshah, Iran

⁴Department of Immunology, Hamadan University of Medical Sciences, Hamadan, Iran

⁵Department of Community and Preventive Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

Correspondence

Peyman Eini, Department of Infectious Diseases, Hamadan University of Medical Sciences, Hamadan, Iran.
Email: eini@umsha.ac.ir

Funding information

The Research Deputy of Hamadan University of Medical Sciences

Background: The inflammatory response to *Mycobacterium tuberculosis* bacilli influences tuberculosis (TB) progression. In this study, we aimed to identify the Phe206Leu polymorphism and serum L-selectin level in TB patients, compared to healthy individuals.

Methods: Ninety patients with a diagnosis of TB and 90 healthy controls were selected in this study. The serum L-selectin level was determined, using ELISA. L-selectin polymorphism was also evaluated using PCR. For data analysis, SPSS was used at a significance level of 0.05.

Results: According to the findings, the mean $\pm$ SD age of the participants was 57.5 ± 18.4 and 56.5 ± 17.5 years in the TB and healthy groups, respectively. The TB group showed a significantly higher serum L-selectin level (1721.1 ± 330.9) versus the healthy controls (1624 ± 279). The L-selectin Phe allele frequencies were higher than the Leu allele frequencies in the main population, whereas the patients and controls were not significantly different. Eight (0.04%) subjects had Leu/Leu genotypes, 84 (46.6%) carried Phe/Leu genotypes, and 88 (48.8%) had Phe/Phe genotypes. Our results showed that the groups were not significantly different regarding L-selectin genotypes.

Conclusion: TB patients had a significantly higher serum L-selectin level, compared to the controls. Based on the findings, the incidence of TB and L-selectin polymorphism in the Phe206Leu gene had no significant association.

KEYWORDS

gene polymorphism, L-selectin, tuberculosis

1 | INTRODUCTION

Tuberculosis (TB) is an ancient disease, infecting and coevolving with humans for thousands or even millions of years.¹ A class of closely related bacteria, known as *Mycobacterium tuberculosis* complex, is responsible for TB. Today, *M. tuberculosis* is recognized as the most important cause of TB among humans. On the other hand, *M. microti*, *M. bovis*, and *M. africanum* also belong to the *M. tuberculosis* complex causing TB. Among these species, *M. microti* has not been shown to cause TB in humans.^{2,3} Despite the development of novel diagnostic and treatment modalities for TB, millions are still affected by this condition. According to statistics, TB is among the most threatening

infectious diseases worldwide. Overall, HIV/AIDS accounts for 3 million deaths per year, TB is responsible for 2 million deaths, and malaria kills 1 million people.⁴

Inflammatory response to *Mycobacterium tuberculosis* bacilli plays the main role in the progression of TB, as demonstrated by leukocyte recruitment at the inflammation site. Selectins mediate leukocyte rolling, which is an important stage in successful leukocyte recruitment in tissues.⁵ Thus, it may be hypothesized that the host genetic and immunological status determines the infection outcome. Ligand-selectin (L-selectin), or Cd62L, as a major intercellular adhesion molecule, contributes to the regulation of immune cell homing to infected tissues.

L-selectin mediates adhesive interactions between glycoprotein counter receptors and lymphocytes in secondary lymphoid organs on high endothelial venules (required for lymphocyte homing) and in sites of chronic inflammation.^{6,7} Previous investigations demonstrated that the genetic variation, or polymorphism, at position +206 (Phe206Leu) is associated with the expression of this molecule.⁷ According to the literature, Phe206Leu polymorphism of L-selectin genes can be traced in several immune-related diseases.^{8,9} Therefore, we hypothesized that Phe206Leu polymorphism may have an association with TB infection in the Iranian population. In this study, we aimed to determine Phe206Leu polymorphism and serum L-selectin level in patients with TB, compared to healthy individuals.

2 | METHODS

This study recruited 180 subjects, referred to Hamadan Infectious Diseases Clinic and Sina Hospital in 2015. The participants were divided into two groups. The first group consisted of 90 patients with a diagnosis of TB after examination and history taking, while the other group consisted of 90 healthy individuals. The exclusion criteria were any other systemic or inflammatory diseases or risk of pulmonary diseases (such as COPD) due to occupational or habitual state.

The participants were selected to have similar age and sex distributions in the groups. TB diagnosis was confirmed by a specialist, using history taking, laboratory findings, and clinical presentations. Before sample collection, the participants filled out an informed consent form. This study was approved by the ethics committee of Hamadan University of Medical Sciences.

A commercial kit (Bioneer, South Korea) was used for DNA extraction. The Phe206Leu polymorphism protocol of L-selectin has been described in our previous study.⁸ The PCR

sequence-specific primer technique was applied to evaluate the polymorphisms. The primer sequences for polymorphism detection were 5'-ATGGGCCCCAGTGTCACT-3' (for T allele detection), 5'-ATGGGCCCCAGTGTCACT-3' (for C allele detection), and 5'-CAAGCTCATTAGATCGTGAGC-3' (generic primer). In addition, internal control primer 1 (HGH forward; 5'-GCCTTCCCAACCATTCCCTT-3') and primer 2 (HGH reverse; 5'-TCACGGATTTCTGTTGTGT-TTC-3') were used.

For confirming successful PCR amplification, a human growth hormone gene segment (927 bp) was amplified in all reactions, using the internal control primers.⁸ For amplification, a Techne Flexigene PCR device (Roche, Germany) was used in a total volume of 50 μ L, containing 10 pmol of allele-specific primers, 20–80 ng of genomic DNA, 200 mM dNTPs, 10 pmol of common primers, 0.5 U Taq DNA polymerase, 1.5 mM MgCl₂, Tris/HCl (10 mM; pH, 8.3), and 50 mM KCl. The negative controls were represented by PCR without a DNA template. The reactions were performed as follows: initial denaturation for 2 minutes at 94°C, followed by 30 cycles of amplification for 15 seconds at 94°C, annealing for 45 seconds at 60°C, and extension for 45 seconds at 72°C, with a final extension at 72°C for 2 minutes. For each subject, the PCRs were carried out in duplicate. Moreover, to confirm the procedure accuracy, in 30% of the participants, the PCR was repeated. For examining the amplified products, electrophoresis (2% agarose gel), staining with ethidium bromide, and UV visualization were carried out, respectively (specific band, 339 bp). Commercial ELISA kits (R&D Systems, MN, USA) were used for detecting L-selectin (catalog No., BBE1B [DCD540] and BBE2B). The procedures were carried out as outlined by the manufacturer. For analyzing the collected data, SPSS version 20 (SPSS, Chicago, IL, USA) was used at a significance level of 0.05. The association of L-selectin Phe206Leu polymorphism with TB was determined, using χ^2 analysis, and the differences in quantitative data between the groups were examined with Student's *t* test and ANOVA test.

TABLE 1 Demographic data of study subjects

	TB patients	Healthy controls	TB type		Pulmonary type	
			Pulmonary	Ex. pulmonary	Smear positive	Smear negative
Gender						
Male, n(%)	45 (50)	47 (52/2)	32	13	27	6
Female, n(%)	45 (50)	43 (47/8)	25	20	22	5
Total, n(%)	90 (100)	90 (100)	57 (63)	33 (36)	49 (54)	11 (12)
Age(Mean + SD)	57/52 ± 18/3	56.5 ± 17.5	61 ± 18	50 + 16	61 + 18	62 + 19

TABLE 2 Serum L-selectin level according to TB and healthy controls and TB types

L-selectin	Sex			Tb type		Pulmonary type	
	Male	Female	Total	Pulmonary	Ex pulmonary	Smear positive	Smear negative
Case	1710.35 $\pm$ 333.6	1732 $\pm$ 331.6	1721.1 $\pm$ 330.9	1733.9 $\pm$ 315.8	1716.3 $\pm$ 360.5	1735.6 $\pm$ 302	1619.2 $\pm$ 365.3
Control	1611.2 $\pm$ 296.9	1638 $\pm$ 260.8	1624 $\pm$ 279	-	-	-	-
P value	.135	.144	.035		.917		.271

3 | RESULTS

In this study, the mean $\pm$ SD age was 57.5 ± 18.4 and 56.5 ± 17.5 years in patients and healthy individuals, respectively. Fifty-seven (63.3%) patients had pulmonary TB, and 33 (36.7%) suffered from extrapulmonary TB. Furthermore, 47 (82.5%) of the pulmonary TB patients were smear positive, and 10 (17.5%) were smear negative for *Mycobacterium bacilli*. More detailed features of patients are listed in Table 1.

The serum L-selectin level (mean $\pm$ SD) was 1721.1 ± 330.9 in the TB group and 1624 ± 279 in the healthy controls; the groups were significantly different regarding L-selectin level (P). Our results also showed no significant difference between extrapulmonary and intrapulmonary TB, nor was there a significant difference between smear-positive and smear-negative pulmonary TB patients. More details are provided in Table 2.

L-selectin Phe allele frequencies were higher than the Leu allele frequencies in the main population, but no significant difference between the controls and patients was observed [136 (75.6%) vs 124 (68.9%) for Phe and 44 (24.4%) vs 56 (31.1%) for Leu in TB and healthy controls]. There was also no difference between allele frequencies in TB groups according to pulmonary and extrapulmonary TB types and smear positivity in pulmonary TB patients. Details are shown in Table 3.

We also assessed the L-selectin genotypes in the study subjects. The results showed that 8 (0.04%) subjects had Leu/Leu genotypes, 84 (46/6%) carried Phe/Leu genotypes, and 88 (48.8%) had Phe/Phe genotypes. In addition, the control and TB groups were not significantly different in terms of L-selectin genotypes. According to the results, L-selectin genotypes had no influence on the incidence of pulmonary and extrapulmonary TB or smear positivity in TB patients. More detailed results are presented in Table 4.

4 | DISCUSSION

In this study, we assessed the serum level and Phe206Leu polymorphism of L-selectin in TB patients and compared them to healthy individuals. Our results showed significantly higher serum levels of L-selectin in TB patients versus the healthy controls. In some studies, L-selectin has been shown to be upregulated on immune cells in many infectious diseases and inflammatory responses; this finding is consistent with our results.¹⁰⁻¹² Moreover, it has been shown

TABLE 3 Serum L-selectin level according to L-selectin genotypes

Genotypes	TB Patients	Healthy controls
Leu/Leu	1917 $\pm$ 357	1548 $\pm$ 68
Phe/Phe	1679 $\pm$ 330	1587 $\pm$ 258
Phe/Leu	1759 $\pm$ 328	1663 $\pm$ 306
P value	.000	.000

TABLE 4 L-selectin allele frequency and polymorphism in study subject

	TB patients	Healthy controls	P value	TB type		P value	Pulmonary type		P value
				Pulmonary	Ex. pulmonary		Smear positive	Smear negative	
Allele frequencies									
Phe n(%)	44 (24/4)	56 (31/1)	.158	27 (23.7)	17 (25.8)	.755	25 (25.5)	3 (13.6)	.113
Leu n(%)	136 (75/6)	124 (68/9)		87 (76.3)	20 (74.2)		73 (74.5)	19 (86.4)	
Polymorphism									
Leu/Leu(%)	3 (3.3)	5 (5.5)	.301	1 (1.7)	2 (6)	.532	1 (2.1)	0 (0.0)	.197
Phe/Phe(%)	49 (54.4)	39 (43.3)		31 (56.1)	18 (54.5)		23 (48.9)	2 (20.0)	
Phe/Leu(%)	38 (42.2)	46 (51.1)		25 (43.8)	13 (39.3)		23 (48.9)	8 (80.0)	

that suppression of L-selectin increases the risk of infection and can compromise cell-mediated immune reactions.¹³ However, according to some studies, L-selectin downregulates in visceral leishmaniasis (a chronic infectious disease), but increases in the cutaneous form.^{14,15}

Formation of soluble adhesion molecules is usually attributed to shedding of surface cell adhesion molecules because of cell stimulation. In the pathophysiology of inflammatory diseases (eg, atherosclerosis and cancer), these molecules have been implicated.^{16,17} According to previous studies, there is an association between autoimmune, inflammatory, and systemic diseases (eg, ischemic stroke, type-I diabetes, and acute leukemia) and increased plasma L-selectin level,¹⁸⁻²⁰ which is consistent with our results regarding TB.

Some naive and resting central memory and effector memory T cells express CD62L and regulate migration to lymph nodes.²¹ Immune responses and T-cell migration are negatively influenced by disrupted CD62L expression.^{22,23} In this study, the Leu/Leu genotype was shown to be the least frequent genotype in the whole population. This result is similar to previous studies in the same population, which reported the Phe/Phe genotype as the major homozygote genotype.^{8,12}

Based on our results, the serum level of L-selectin is higher in TB patients with Leu/Leu genotypes and healthy controls with Phe/Leu genotypes. This result is consistent with Nadi's study in a Hamadanian population with or without bronchial asthma.¹² As few individuals carry the Phe/Phe genotype, a larger sample size may be required to identify the effect of Phe/Phe polymorphism on serum L-selectin in TB patients.

Based on our review, this is the first study to assess the role of L-selectin polymorphism in TB. Our study showed no significant relationship between specific allele frequency and TB. In contrast to our results, some studies have shown a significant relationship between Leu allele frequency and risk of brucellosis, coronary artery disease, or asthma.^{8,12,24} However, Sardarian showed no relationship between L-selectin polymorphism and visceral leishmaniasis, which is more consistent with our results.²⁵ It should be noted that there is another polymorphism for L-selectin located at Tre49Ser.²⁶ We hypothesize that there may be an association between Tre49Ser polymorphism and TB infection.

ACKNOWLEDGMENTS

We extend our gratitude to the Research Deputy of Hamadan University of Medical Sciences for the financial support.

ORCID

Peyman Eini <http://orcid.org/0000-0001-5878-2883>

REFERENCES

- Hirsh AE, Tsolaki AG, DeRiemer K, Feldman MW, Small PM. Stable association between strains of *Mycobacterium tuberculosis* and their human host populations. *Proc Natl Acad Sci USA*. 2004;101:4871-4876.
- Prasad H, Singhal A, Mishra A, et al. Bovine tuberculosis in India: potential basis for zoonosis. *Tuberculosis*. 2005;85:421-428.
- Srivastava K, Chauhan D, Gupta P, et al. Isolation of *Mycobacterium bovis* & *M. tuberculosis* from cattle of some farms in north India-possible relevance in human health. *Indian J Med Res*. 2008;128:26.
- Tuberculosis Fact Sheet WHO Reviewed January 2018. <http://www.who.int/mediacentre/factsheets/fs104/en/> Accessed February 5, 2018.
- Zelensky AN, Gready JE. The C-type lectin-like domain superfamily. *FEBS J*. 2005;272:6179-6217.
- Pagán AJ, Ramakrishnan L. Immunity and immunopathology in the tuberculous granuloma. *Cold Spring Harb Perspect Med*. 2015;5:a018499.
- Chan J, Flynn J. The immunological aspects of latency in tuberculosis. *Clin Immunol*. 2004;110:2-12.
- Rafiei A, Hajilooi M, Shakib RJ, Shams S, Sheikh N. Association between the Phe206Leu polymorphism of L-selectin and brucellosis. *J Med Microbiol*. 2006;55:511-516.
- Chen HY, Cui B, Wang S, et al. L-selectin gene polymorphisms in Graves' disease. *Clin Endocrinol*. 2007;67:145-151.
- Szántó S, Gál I, Gonda A, Glant TT, Mikecz K. Expression of L-selectin, but not CD44, is required for early neutrophil extravasation in antigen-induced arthritis. *J Immunol*. 2004;172:6723-6734.
- Gupta G. L-selectin (CD62L) and its ligands. In: Gupta G, ed. *Animal Lectins: Form, Function and Clinical Applications*. New York: Springer; 2012: 553-574.
- Nadi E, Hajilooi M, Pajouhan S, Haidari M. Soluble L-Selectin as an independent biomarker of bronchial asthma. *J Clin Lab Anal*. 2015;29:191-197.
- Trinité B, Chan CN, Lee CS, et al. Suppression of Foxo1 activity and down-modulation of CD62L (L-selectin) in HIV-1 infected resting CD4 T cells. *PLoS One*. 2014;9:e110719.
- León B, Ardavin C. Monocyte migration to inflamed skin and lymph nodes is differentially controlled by L-selectin and PSGL-1. *Blood*. 2008;111:3126-3130.
- Kumar V, Bimal S, Singh SK, et al. *Leishmania donovani*: dynamics of L. donovani evasion of innate immune cell attack due to malnutrition in visceral leishmaniasis. *Nutrition*. 2014;30:449-458.
- Constans J, Conri C. Circulating markers of endothelial function in cardiovascular disease. *Clin Chim Acta*. 2006;368:33-47.
- van Kilsdonk JW, van Kempen LC, van Muijen GN, Ruiter DJ, Swart GW. Soluble adhesion molecules in human cancers: sources and fates. *Eur J Cell Biol*. 2010;89:415-427.
- Wei Y-S, Lan Y, Meng L-Q, Nong L-G. The association of L-selectin polymorphisms with L-selectin serum levels and risk of ischemic stroke. *J Thromb Thrombolysis*. 2011;32:110-115.
- Spertini O, Callegari P, Cordey A-S, et al. High levels of the shed form of L-selectin are present in patients with acute leukemia and inhibit blast cell adhesion to activated endothelium. *Blood*. 1994;84:1249-1256.
- Krętownski A, Gillespie K, Bingley P. Soluble L-selectin levels in type I diabetes mellitus: a surrogate marker for disease activity? *Immunology*. 2000;99:320-325.
- Butcher EC, Picker LJ. Lymphocyte homing and homeostasis. *Science*. 1996;272:60.
- Venturi GM, Tu L, Kadono T, et al. Leukocyte migration is regulated by L-selectin endoproteolytic release. *Immunity*. 2003;19:713-724.
- Hedrick SM, Michelini RH, Doedens AL, Goldrath AW, Stone EL. FOXO transcription factors throughout T cell biology. *Nat Rev Immunol*. 2012;12:649-661.

24. Hajilooi M, Tajik N, Sanati A, Eftekhari H, Massoud A. Association of the Phe206Leu allele of the L-selectin gene with coronary artery disease. *Cardiology*. 2006;105:113-118.
25. 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;14:218-220.
26. Tedder TF, Isaacs CM, Ernst T, Demetri GD, Adler DA, Disteché CM. Isolation and chromosomal localization of cDNAs encoding a novel human lymphocyte cell surface molecule, LAM-1. Homology with the mouse lymphocyte homing receptor and other human adhesion proteins. *J Exp Med*. 1989;170:123-133.

How to cite this article: Eini P, Shirvani M, Hajilooi M, Esna-Ashari F. Comparison of L-selectin blood level and gene polymorphism in tuberculosis patients with healthy individuals. *J Clin Lab Anal*. 2018;32:e22409.
<https://doi.org/10.1002/jcla.22409>