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ORIGINAL RESEARCH

Medicine Science International Medical Journal

Medicine Science 2019;8(1):158-61

The association between TLR4 polymorphisms and pulmonary tuberculosis

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Received 05 October 2018; Accepted 05 November 2018
Available online 11.01.2019 with doi:10.5455/medscience.2018.07.8959

Abstract

Tuberculosis (TB) is a major health problem which causes many deaths every year in the world. According to the World Health Organization (WHO) report, 1.5 million people lose their lives due to tuberculosis in every year. Although some of the infected people are showing the symptoms, in some the disease never develops, even about 90% of the affected ones are improved by the immune system's response. As in many infectious diseases, the difference between the number of infected people and the number of sick people is caused by differences in balance between the host defense and the virulence of the organism. In this case, the genetic basis of the response to infectious agents needs to be investigated in order to understand the relationship between infectious diseases and host. For this purpose; we investigated the effects of TLR4 polymorphisms (rs4986790 & rs4986791) that are involved in immune system mechanisms against susceptibility to *Mycobacterium tuberculosis* (MTB). In order to understand the association we did genotyping in 65 TB patients from Malatya. The results of the genotyping showed no significant association to the single nucleotide polymorphisms (SNPs) mentioned, for rs4986790 the p value was 0.80 and for rs4986791 p value was 0,517 for the genotypes. The population size was the limiting factor for the statistical analysis and it should be also taken into account that a number of genes are taking part in the signal transduction pathway that TLR4 is involved.

Keywords: MTB, TLR, polymorphism, genotyping, SNP

Introduction

Tuberculosis, is caused by *Mycobacterium tuberculosis* and it is the leading cause of 1.5 million deaths annually around the world. Although 1/3 of the world population is infected, development of active pulmonary tuberculosis (PTB) depends on the interaction between the bacterium and host's immune defense [1]. The disease can remain in the latent phase for a long time after infecting the affected individual. Although some of the infected people are showing the symptoms, some people never develop the disease phenotype, even about 90% of them are recovered by the immune system's response. Why some people-exposed to the pathogen- do not develop the disease is not known. In order to gain insights about the subject, the researchers focus on the host's immune response mechanisms that limit the active disease in those who are infected. Many studies performed up to date indicate that genetic factors of the host have an influence on the susceptibility to infection [2,3].

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Toll-like receptors (TLRs) are membrane-spanning proteins that detect the conserved structure of pathogenic microorganisms which are called pathogen-associated molecular patterns (PAMPs) that therefore play a critical role in immunity. The toll-like super family has 10 members which are functional in human that recognize different microorganisms and microbial products that lead to activation of the immune response and cytokines [4]. Five molecules (My-D88, TRIF, Mal, TRAM and SARM), which are TIR-domain adapters of the TLR family, interact with all members of this family. TLR1, 2, 4, 6, 8 and may be TLR9 are thought to be involved in the recognition of *Mycobacteria* [5-7].

TLR4 is encoded on human chromosome 9q32-33, and it is reported to play a significant role in triggering off the innate immune response to MTB infection. The mycobacterial ligands recognized by TLRs are lipoarabinomannan, lipomannan, phosphatidylinositol mannose, and the 19-kDa lipoprotein. After recognition of these by receptors, the TLR signal pathway is activated by binding the TIR domain to MyD88 adapter protein [6,8]. IRAK-1, Toll / IL-1 receptor domain-containing adapter protein, and TIR-domain-containing adapter-inducing IFN-β adapter protein participate in

activation of the mitogen-activated protein kinase and nuclear factor-κβ in the nucleus. This increase the levels of inflammatory cytokines, especially TNF-α, then initiate the natural immune response to bacteria [3,9-11].

TLRs are transmembrane proteins which containing leucine rich repeats (LRR) in their extracellular domains that induce a natural immune response to many pathogens. The LRR region mutations may potentially disturb phosphorylation of TLR4 altering downstream signaling of inflammatory mediator activation, ultimately contributing to disease susceptibility. Thus, individuals who have these variations in TLR4 may prone to develop TB [12]. For this reason we focused on the TLR4 polymorphisms. The two mutations Thr399Ile (rs4986791) and Asp299Gly (rs4986790) of TLR4, which is found in the ectoplasmic LRR domain, have an important role in reducing cytokine response and increasing susceptibility to various infections by affecting the TLR4 receptor [13]. We aimed to investigate the role of this two TLR4 polymorphisms in patients with pulmonary tuberculosis from Malatya.

Material and Methods

65 Turkish PTB patients and 75 age- and sex-matched healthy control individuals are the subjects of this study. Patients were recruited from the Malatya Provencial Health Directorate and molecular analyses were carried out in the Department of the Molecular Biology and Genetics. The study was performed in accordance with the guidelines of the Declaration of Helsinki and was approved by our local ethics committee (Protocol #2013/155). All participants were fully informed about the study procedures and gave written consent before the study started.

Genomic DNA was extracted from peripheral blood stored with an anticoagulant using a commercial kit (PureLink® Genomic DNA Mini Kit; Invitrogen, Carlsbad, CA, USA), according to the manufacturers' protocol. All blood and DNA samples were coded to ensure anonymity. All samples were genotyped using the TaqMan® SNP Assay (Applied Biosystems, Foster City, CA, USA): C_11722238_20 for rs4986790, and C_11722237_20 for rs4986791. We used the StepOnePlus™ Real-Time PCR System (Applied Biosystems,) for genotyping.

The data was summarized by count and percent. Hardy-Weinberg equilibrium was tested by a chi-square distribution with 1 df. Differences between groups due to allelic and genotypic distributions were analyzed by Pearson's exact or Fisher's exact tests. In comparisons the significance level was considered to be ≤0.05.

Results

65 PTB patients and 75 age matched healthy control individuals were genotyped. Two single nucleotide polymorphisms of TLR4 rs4986790 and rs4986791 were investigated and no significant association was found between the disease susceptibility and the genotype frequencies of the SNPs that were mentioned. The results of the genotyping for each SNPs investigated were summarized in tables.

The frequencies of the genotypes and alleles are given for rs4986790 for patients and controls are shown in Table 1 and gender differences of the genotypes are given in table 2. The results of the genotyping for rs4986791 are shown in Table 3 and gender related analysis is given in Table 4.

Table1. Rs4986790 Genotype and allele frequency table for patients and controls

Group	AA n (%)	AG n (%)	p	A n (%)	G n (%)	p
Control	70 (93.3)	5 (6.7)	0.805	145 (96.7)	5 (3.3)	0.809
Patient	59 (90.8)	6 (9.2)		124 (95.4)	6 (4.6)	

Table 2. Rs4986790 Genotype and allele frequency table according to gender

Group	AA n (%)	AG n (%)	p	A n (%)	G n (%)	p
Female	42 (91.3)	4 (8.7)	0.751	86 (95.6)	4 (4.4)	0.751
Male	87 (92.6)	7 (7.4)		181 (96.3)	7 (3.7)	
Female	**AA n (%)**	**AG n (%)**	**P**	**A n (%)**	**G n (%)**	**p**
Control	11 (91.7)	1 (8.3)	1.000	23 (95.8)	1 (4.2)	1.000
Patient	31 (91.2)	3 (8.8)		65 (95.6)	3 (4.4)	
Male	**AA n (%)**	**AG n (%)**	**p**	**A n (%)**	**G n (%)**	**p**
Control	59 (93.7)	4 (6.3)	0.681	122 (96.8)	4 (3.2)	0.686
Patient	28 (90.3)	3 (9.7)		59 (95.2)	3 (4.8)	

Table 3. Rs4986791 Genotype and allele frequency table for patients and controls

Group	CC n (%)	CT n (%)	p	C n (%)	T n (%)	p
Control	67 (89.3)	8 (10.7)	0.517	142 (94.7)	8 (5.3)	0.526
Patient	61 (93.8)	4 (6.2)		126 (96.9)	4 (3.1)	

Table 4. Rs4986791 Genotype and allele frequency table according to gender

Gender	CC n (%)	CT n (%)	p	C n (%)	T n (%)	p
Female	43 (93.5)	3 (6.5)	0.751	89 (96.7)	3 (3.3)	0.756
Male	85 (90.4)	9 (9.6)		179 (95.2)	9 (4.8)	
Female	CC n (%)	CT n (%)	p	C n (%)	T n (%)	p
Control	11 (91.7)	1 (8.3)	1.000	23 (95.8)	1 (4.2)	1.000
Patient	32 (94.1)	2 (5.9)		66 (97.1)	2 (2.9)	
Male	CC n (%)	CT n (%)	p	C n (%)	T n (%)	p
Control	56 (88.9)	7 (11.1)	0.713	119 (94.4)	7 (5.6)	0.720
Patient	29 (93.5)	2 (6.5)		60 (96.8)	2 (3.2)	

Discussion

TB is an infectious disease that is caused by MTB. About %30 of the world's population is carrying the pathogen and at least tenth of it develops the active form of the disease. In Turkey Tuberculosis War 2017 Report, the incidence rate of Turkey in 2015 was 0.154 per thousand and case number was 12.550. At the same time according to this report case number decreased from 173 to 102 cases in five years (2010-2015) in Malatya [14].

The molecular mechanisms how MTB cause infection are unclear that is why it is difficult to fight with the disease. The host innate immune response is the first line of defense against invading pathogens and is vital for the initial defense against MTB and activation of the adaptive immune response [15]. Many studies showed that at least some infected people exhibit a powerful immune response to the infectious agent. The first step of immune response is induced by binding of PAMPs of the invading pathogen to host pattern recognition receptors (PRRs). These PRRs include the TLRs, C-type lectin receptors (CLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and the RIG-like receptors (RLRs) [8].

As the PRR encoding genes play an important role in host immunity, variants in these genes could lead to structural and functional changes in these receptors causing an altered immune response, and influence TB disease progression. PRRs is an important component of the host response to infection with MTB, which triggers inhibitory mechanisms via TLRs.

The coordinated regulation of TLR signaling through their respective ligands might be important for controlling the extent of the host immune response to prevent the progression of MTB growth. TLR polymorphisms have shown a great impact on susceptibility to TB. Members of the population who have the certain genotype for TLRs may have affinity differences to MTB ligands therefore this may lead variations in signal transduction [15,16].

In our study we found no significant association related with the polymorphisms mentioned and the TB cases found in Malatya. These results are in accordance with the studies that are performed in Korean, Taiwan Chinese, Japan and Iranian populations [8,17]. Besides another study reported no association with Asp299Gly in Gambian population as well [18]. On the contrary Najmi et al reported a positive result in Indian populations especially for susceptibility to severe form of the disease for both of the SNPs that we also worked with [13]. On the other hand to our knowledge up to now there hasn't been any study performed in our population yet related with the association of TLR4 polymorphisms and TB.

Conclusion

As a conclusion we showed that the two polymorphisms –Asp 299Gly and Thre399Ile- of TLR4 gene were not associated with the susceptibility to PTB in our patients from Malatya, Turkey. The differences of frequencies of both genotypes and alleles between controls and patients were so close. The reason for having no association may be the number of the patients that were studied, but according to Turkey Tuberculosis War Report 2017 [14] the case numbers in Malatya was 102 patients and we could only reach 65 of them who were regularly coming to the clinic. On the other side it should also be taken into account that many molecules take part in TLR signalization pathway in the immune response of the host to outsiders. So this gives us many candidate genes to investigate the association for the susceptibility to PTB in this group of patients.

Acknowledgements
The authors would like to thank the subjects for their participation in this study. We also thank Dr. Zeynep Arıca Kazgan and Prof. Dr. Barış Otlu for their help and support. This study was supported by Scientific Projects Coordination Unit of Inonu University with grant number 2015/69.

Competing interests
The authors declare that they have no competing interest.

Financial Disclosure
This study is supported by Scientific Projects Coordination Unit of Inonu University with grant, 2015/69

Ethical approval
Prior to the study, the approval of the Ethics Committee of Inonu University (2013/155)

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