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Analysis of *IL-33* Gene Polymorphisms (rs1157505C/G and rs11792633C/T) and the Risk of Tuberculosis in Southeastern Iran

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ABSTRACT

Tuberculosis is a vagarious infectious disease that generally affects the lungs. Accordingly, in some cases, it can also affect the liver and kidney. Host genetic may affect tuberculosis caused by bacillus *Mycobacterium tuberculosis.* The main risk factors for the disease are a weakened immune system because of diabetes, some cancers, HIV/AIDS, severe kidney disease, cancer treatment, and malnutrition. Il-33 is involved in the activation of eosinophils, mast cells, basophils, and natural killer cells and the maturation of T helper type2 cells. The developments of CD4 (+) TH1 and CD8 (+) T cell responses are involved in protection against TB, IL-33 promotes the development of these cells. The purpose of the present research was to investigate the association between *Mycobacterium tuberculosis* infection and *IL-33* gene polymorphisms (rs1157505C/G and rs11792633C/T) with tuberculosis in the cases and controls from the area of high tuberculosis prevalence in Iran. In this study, 100 patients with tuberculosis disease and 91 healthy controls were included. Polymorphisms of the *IL-33* gene were genotyped using T-ARMS-PCR. The analysis of the haplotype combinations among *IL-33* polymorphisms demonstrated that the magnitude of the association was higher for the combined CC/CT genotypes. The CT genotype related to C/T polymorphism of the *IL-33* gene increased the risk of tuberculosis. The combined CG+GG genotypes related to C/G polymorphism of the *IL-33* gene also increased the risk of tuberculosis, but the difference was not statistically significant. In conclusion, *IL-33* gene polymorphisms may be considered as important contributors to tuberculosis in Iran.

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Introduction

Mycobacterium tuberculosis is the main cause of tuberculosis, a disease that affects different organs and spreads through breathing between different people (Flynn and Chan, 2001; Moosazadeh *et al.*, 2014). Tuberculosis usually influences the lungs, but may also affect other parts of the body EPTB (Raja, 2004). It was established that the people infected with HIV are more likely vulnerable to TB due to the weakness of the immune system (Getahun *et al.*, 2010). The prevalence of Tuberculosis is higher in adult males than females (Zumla *et al.*, 2013). Tuberculosis is one of the most important health problems in Iran. It has been previously reported that the human host exerted a complex response to TB. Immune system cells play a pivotal role in *Mycobacterium tuberculosis*. Zumla *et al*. also reported the association between TB, and the strong pro-inflammatory immune responses, and the harmful activity of pro-inflammatory cytokines. In humans, 'hyperinflammatory' reaction is a sign of TB progression (Zumla *et al.*, 2013). Interleukin 33, a 30 kDa protein, is the latest member of the IL-1 superfamily. The

Production and expression of IL-33 from immune cells such as mast cells, macrophages, and dendritic cells resulted in the production of cytokines and chemokines, including IL-13, IL-8, and IL-6. Non-immune cells, including smooth muscle, endothelial, epithelial cells, and fibroblasts, also produced this cytokine (Yagami *et al.*, 2010). IL-33 is mostly expressed in the Skin, gut, and lung, and expression is enriched in the brain and spinal cord (Schmitz *et al.*, 2005). Its gene is located on the short arm of chromosome 9 (9p24). The protein domains at the N- terminal part of IL-33 make this cytokine as a nuclear factor to regulate the transcription of genes. The IL-33 suppresses transcription by binding to H2A-H2B dimers at the surface of the nucleosomes (Carriere *et al.*, 2007). Also, it was thought that it could function as an 'alarmin' released following cell necrosis to alerting the immune system about the stress or tissue damage (Moussion *et al.*, 2008). Moreover, it exerts its biological effects by binding to the ST2 receptor, a member of the interleukin-1 receptor family with two isoforms as follows: transmembrane (ST2L) and soluble (sST2), which interact with il-33 that is a single ligand for this receptor. Accordingly, ST2 is a part of the Toll-like receptor (TLR)/IL1R superfamily. IL-33 induces the production of cytokine by Th2, thus can elevate the pathogenesis of Th2-related diseases such as anaphylaxis, asthma, and atopic dermatitis (Ensaf *et al.*, 2014). IL- 33 can also induce inflammatory airway response by stimulating lung ILC2s (Cayrol, 2014). Depending on the disease and model, the effects of IL-33 can be pro-inflammatory or anti-inflammatory (Miller, 2011). We aimed to determine the role of IL-33 gene polymorphisms (rs1157505C/G and rs11792633C/T) with the risk of tuberculosis**.**

Materials and Methods

Study subjects

Study subjects' baseline characteristics are shown in table 1. Two IL-33 polymorphisms were successfully genotyped in 100 tuberculosis patients (mean age= 38.83±20.31 years) and 91 controls (mean age= 38.56±16.22 years). These samples were collected from Ali-ibn Abi Talib Hospital, Zahedan, from 2012 to 2015. Detection of TB disease was performed by physical examination, medical history, test to diagnosis *M. tuberculosis* infection, and Chest X-ray The ethical committee at the Medical University of Zahedan approved this study.

Blood samples of cases and controls were collected in tubes containing EDTA. DNA was extracted from 500µl of blood samples using total DNA Extraction Kit tests 50 (Cat. No. EX6001.Sinaclon, Iran). The Tetra Arms technique, a fast and straightforward detection technique, was used to identify the genetic diversity of the gene under study.

PCR experiments were performed in a total volume of 25 µL. The PCR mixture consisted of 50 ng of genomic DNA, 10 pM of each primer, and premix master mix (including *Taq*, dNTP, PCR buffer, and Mg^{2+}). The PCR reactions were subjected to initial heating at 95°C for 5 min, followed by 35 cycles at 95°C for the 30s, annealing temperature at 63°C (C/G SNP), 61°C(C/T) for 20s, extension at 72°C for 30s and final extension 72°C for 10 min. The PCR products were electrophoresed using a 2% agarose gel, stained with DNA green viewer, and visualized under ultraviolet illumination. The sequences and annealing temperature of primers are shown in Table 2. Statistical analysis was performed using SPSS statistical software ver. 20 and Epical version 3.2. The association between polymorphisms and susceptibility to tuberculosis was examined by estimating odds ratios (OR) and 95% confidence intervals (95% CI). Categorical data were analyzed by Pearson's x2.

Results

No significant differences in allele frequencies of rs1157505C/G and rs11792633C/T were observed between cases and controls groups, with OR= 0.8, 95% CI= 0.468-1.3, *p*= 0.42, and OR= 1.06, 95% CI=0.69-1.64, *p*=0.77, respectively (Table 3). In this study, we investigated two SNPs in the IL-33 gene, *(*rs1157505C/G and rs11792633C/T) and discovered one genotype of rs11792633C/T SNP of IL-33 gene, CT genotype, which was significantly associated with tuberculosis at univariate analysis, with OR=1.94, 95% CI=1.025-3.66, *p*-value=0.042 (Table 4).

Table1. The socio-demographic characteristics of the case and control groups.

Variables	Cases (N=100)	Controls (N=91)	*P*-value
Sex			
Females	54	55	0.37
Males	46	36	
Age	38.83±20.31	38.56±16.22	0.92
BMI	18.83±3.95	20.6±4.74	0.006
Educational level			
Illiteracy	70	18	
Primary	17	49	
School			<0.001
Guidance	8	19	
High school	2	2	
AD	3	2	
BA	-	-	
≥ MA	-	1	
Job-status			
Unemployed	7	5	
Housekeeper	46	46	
Worker	12	2	
Farmer	2	0	0.13
Rancher	1	1	
Self-employment	23	30	
Employee & Retired	3	4	
Student	6	3	

Table 2. The sequences and annealing temperatures of the primers.

SNPs	Primer name	Sequence (5'→ 3')	Product size (bp)	Annealing temp (°C)
IL-33rs1157505 C/G	Fi(C)	5'-GCCTCCAATTCCTGGGCTCAAGCAATCTTC-3'	139(C)	63
	Ri(G):	5'-CCAGCTGCTTGGGAAGCTGAGATCGC-3'	109(G)	
	Fo	5'-ATCTTGCTCTGTAGCGCAGGCTGGAGCA-3'	192	
	Ro	5'-TTCCAGCCTGGCAACAAAGCAAAGACCC-3'		
IL-33rs11792633 C/T	Fi(C)	5'-CCCAGAGTCCACACTCAGTATTAGGCAGGC-3'	176(C)	61
	Ri(T)	5'-TAGTCAGCATCACATGGGAACGTGATCGA-3'	193(T)	
	Fo	5'-TGCTTGTCCTACTAGATGCTAGCCCCCACA-3'	310	
	Ro	5'-GCATGAGTTTTGGTGGAAACATTCAAACCA-3'		

Table 3. Genotype and allele frequency of *IL-33* geneC/G and C/T polymorphisms in Tuberculosis patients and healthy controls.

Genotype	Cases (%) N= 100	Controls (%) N= 91	OR	CI (95%)	P-value
A) Genotypes and allele Frequency of IL33 C/G					
CC	76(76%)	73(80.23%)	-	-	Ref
CG	11(11%)	8(8.79%)	0.75	0.288-1.99	0.57
GG	13(13%)	10(10.98%)	0.8	0.331-1.94	0.62
CG+GG	24(24%)	18(19.78%)	1.28	0.64-2.55	0.48
C	163(81.5%)	154(84.61%)	1	-	Ref
G	37(18.5%)	28(15.39%)	0.8	0.468-1.37	0. 42
B) Genotypes and allele Frequency of IL33 C/T					
CC	56(56%)	43(47.25%)	-		Ref
CT	27(27%)	38(41.75%)	1.94	1.025-3.66	0.042
TT	17(17%)	10(11%)	0.78	0.325-1.87	0.57
CT+TT	44(44%)	48(52.74%)	0.71	0.398-1.24	0.23
C	139(69.5%)	124(68.14%)	1	-	Ref
T	61(30.5%)	58(31.86%)	1.06	0.69-1.64	0.77

Moreover, the rs11792633C/T polymorphism has no significant association with tuberculosis in the same genotype after adjustment for binary logistic regression (OR=2.2, 95% CI= 0.875-5.47, *p*= 0.09), which shown in table 4. As given in Table 5, the CC+CT genotype of IL-33 gene significantly increased the risk of tuberculosis (OR= 0.48, 95% CI= 0.236-1.01, *p*= 0.054). The analysis of gene-gene interaction between IL-33(rs1157505C/G and rs11792633C/T) did not find significant data (Table 5).

Table 4. Association of rs1157505C/G and rs11792633C/T variations and Tuberculosis after adjustment for binary logistic regression.

Genotype	Unadjusted			Adjusted*		
	OR	95%CI	P-value	OR	95%CI	P-value
IL33 C/G						
CC (ref)	1	-	-	1	-	-
CG	0.75	0.288-1.99	0.57	0.37	0.067-2.003	0.25
GG	0.8	0.331-1.94	0.62	0.91	0.224-3.65	0.88
CG+GG	1.28	0.64-2.55	0.48	2.379E9	-	0.99
IL33 C/T						
CC (ref)	-	-	-	-	-	-
CT	1.94	1.025-3.66	0.042	2.2	0.875-5.47	0.09
TT	0.78	0.325-1.87	0.57	0.4	0.1-1.49	0.17
CT+TT	0.71	0.398-1.24	0.23	0.84	-	1

Table 5. Association of different genotypic combinations of *IL-33* gene and Tuberculosis.

Genotype	Cases (%)	Controls (%)	OR	95%CI	P-value
CC/CC	44(44%)	34(37.36%)	-	-	Ref
CC/CT	19(19%)	30(32.96%)	0.48	0.236-1.01	0.054
CC/TT	13(13%)	9(9.89%)	1.11	0.427-2.91	0.82
GC/CC	3(3%)	4(4.39%)	0.58	0.121-2.76	0.49
GC/CT	6(6%)	3(3.29%)	1.54	0.36-6.63	0.55
GC/TT	2(2%)	1(1.09%)	1.54	0.134-17.76	0.72
GG/CC	10(10%)	5(5.49%)	1.54	0.483-4.94	0.46
GG/CT	1(1%)	5(5.49%)	0.155	0.017-1.38	0.09
GG/TT	2(2%)	0	-	-	0.9

Discussion

The present study analyzed for the first time for SNPs of IL-33 encoding gene among Iranian tuberculosis patients. Clinical evidence showed that the host response to M. tuberculosis plays a vital role in the clinical signs of people exposed to this microorganism (Schluger and Rom 1998). Tissue damage, inflammation, and infection caused by an environmental agent triggers the release of il-33 from the cells. This stimulation has been linked to the induction of IL-33. For example, a pulmonary challenge with LPS containing house dust mite can lead to an epithelial-dependent release of IL-33 protein in the lungs of mice (Smith, 2010). IL-33 plays an important role in the development and pathogenesis of tuberculosis by reducing macrophage-derived foam cell formation, support of Th2 cells, and endothelial cell function (Kurowska-Stolarska *et al.*, 2011). In the asthmatics and idiopathic pulmonary fibrosis patients (Tajima *et al.*, 2003), the concentration of sST2 is elevated, that is showing that various human diseases are associated with the IL-33-sST2 signaling pathway (Oshikawa *et al.*, 2001). The up-regulation of soluble receptor in pathological conditions provides indirect evidence for activation of pathway related to disease; however, the possible roles of *IL-33* gene in tuberculosis remain poorly understood up to now. Many research projects have indicated that the variation in the *IL-33* gene is related to Allergic rhinitis (Sakashita *et al.*, 2008) and asthma (Gudbjartsson *et al.*, 2009) disorders. According to a study performed by Azazi *et al*., high levels of il-33 and its receptor sST2 in the serum of patients with bronchial asthma, indicate their importance as therapeutic targets in asthma (Azazi *et al.*, 2014). Moreover, the results of a study by Lee *et al.* have shown that there is an association among the elevated pleural IL-33 levels and TB pleurisy (Lee *et al.*, 2013). IFN-γ and TNF, which locally increased in the pleural space of patients with the tuberculous pleural effusion, can induce *IL-33* expression. Findings of the study by Lee *et al*. indicated that IL-33 is more relevant to the TPE pathophysiology than other types of pleural effusions (Lee *et al.*, 2013). It is still unclear how genetic diversity may influence the IL-33 function to increase or decrease the risk of TB. However, the present study had several limitations. In the present study, which was a case-control, all the contributors were Iranian, and confounding variables including ethnicity were excluded. Indeed, the subjects should be from diverse racial and ethnic backgrounds that may influence the results of the study. So, no test was performed to determine the practical

consequences of genetic variability in the cases, such as the levels of IL-33 in tissue or plasma, and also the relation among polymorphisms in the *IL-33* gene and tuberculosis are conjectural. Finally, the results of the current study are based on a few numbers of cases and must be viewed as preliminary. Therefore, further studies on large-scale and diverse populations are needed to clarify the exact association among *IL-33* gene polymorphisms and the development of TB.

Conclusion

The study concluded that genetic variation of rs11792633C/T in the IL-33 gene maybe have a central role in developing tuberculosis in Southeastern Iran and supplied more contribution toward new chance to consider prevention, pathogenesis, and treatment of tuberculosis.

Conflicts of interest

The authors declare that they have no conflict of interest.

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