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Interleukin-10 -1082A>G polymorphism and susceptibility to pulmonary Tuberculosis in Lur population of Iran

Shahsavar, F.[1], Azargoon, A.[2*] and Sheikhian, A.[1]
[1]Associate Professor, Department of Immunology, Lorestan University of Medical Sciences, Khorramabad, Iran
[2]Associate Professor, Department of Internal Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran
[*]Corresponding author e-mail: alireza.azargoon@gmail.com
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Abstract. Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* is one of the major causes of death. Cytokines play a major role in immune defense against such infectious agents. Polymorphisms in the genes that encodes various cytokines have been associated with tuberculosis susceptibility. In this study we investigated whether *IL-10* -1082A>G, -819T>C and -592A>C polymorphisms have any association with the susceptibility to pulmonary TB in the Lur population of Iran. *IL-10* polymorphism genotyping was performed by the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method in 100 pulmonary TB patients and 100 healthy controls of Lur population. The genotypic frequencies of *IL-10* -819T>C and -592A>C polymorphisms did not vary significantly between TB patients and healthy controls. Only, in *IL-10* -1082A>G polymorphism, a significantly increased frequency of genotype AG was observed among patients compared with controls (74% in the patients vs. 58% in the controls, P=0.0252, OR=0.485, CI=0.4307-0.5988). The allelic frequencies of *IL-10* -1082A>G, -819T>C and -592A>C polymorphisms did not have significant difference between the pulmonary TB patients and the healthy controls. Our results demonstrate that the *IL-10* -1082A>G polymorphism may be a valuable marker to predict the risk for the development of TB in the Lur population of Iran.

INTRODUCTION

Tuberculosis (TB) which is a chronic infectious disease is caused by different species of Mycobacteria, specially *Mycobacterium Tuberculosis* (MTB). This disease is a found all over the world with 9 million new cases per year and approximately 2 million deaths annually (WHO, 2014). Almost one third of the world population is infected by this bacterium, while only 5 to 10% are infected with active form of TB. Sensitivity to this disease is variable in the different populations, so contact with this microorganism does not always lead to active infection. Furthermore, the disease course is variable in the different people (Azad *et al.*, 2012).

These differences could be the result of host factors and genetic sensitivity of different people against this disease (Abel *et al.*, 2014; Png *et al.*, 2012). Among the different genetic factors playing some role in the pathogenicity of the disease, is *KIR3DS1* gene and its combination with *HLA-B BW4 Ile80* ligand (Shahsavar *et al.*, 2012; Mousavi *et al.*, 2011). Moreover, human and mouse studies on MTB infection have demonstrated involvement of different loci such as *Toll like receptors (TLRs)* in the susceptibility or resistance to TB (Velez *et al.*, 2009a; Velez *et al.*, 2009b; Shahsavar *et al.*, 2016; Zhang *et al.*, 2013). Th1/Th2 balance is known to play a key role in controlling MTB infection (He *et al.*, 2010). The production of proinflammatory cytokines such as TNFα is

essential for host resistance against MT infection (Shahsavar *et al.*, 2016).

IL-10, which is expressed by activated monocytes/macrophages, natural killer (NK) cells, dendritic cells (DCs), mast cells, B cells, and regulatory T cell subsets, is known to have macrophage-deactivating properties and attenuating the Th1-driven proinflammatory response by down-regulating the production of several cytokines (Mousavi *et al.*, 2009). O'Leary *et al.*, 2011 demonstrated that in macrophages, IL-10 may prevent phagosome maturation, thus leading to MTB persistence in humans. Several studies have also reported high levels of IL-10 production in TB patients (Barnes *et al.*, 1993; Verbon *et al.*, 1999). Furthermore, in mouse models, over-expression of IL-10 may affect the recurrence of latent TB but shows little effect on susceptibility to primary infection (Turner *et al.*, 2002). These results indicate that the *IL-10* gene and its protein product, IL-10, play a critical role in susceptibility to and pathogenesis of TB (Gao *et al.*, 2015).

The *IL-10* gene maps to chromosome 1q31-32. The *IL-10* promoter is highly polymorphic, and three single nucleotide polymorphisms (SNPs) at positions -1082, -819, and -592 within the promoter region have been shown to correlate with IL-10 production (Lyer & Cheng, 2012).

To date, many genetic epidemiology studies have assessed the association between *IL-10* gene polymorphisms and the risk of TB in different populations (Gao *et al.*, 2015). According to the considerable differences in the distribution of *IL-10* gene in the races and nations on one hand and the association of *IL-10* gene polymorphisms with the pulmonary TB on the other hand, we were encouraged to concentrate on the role of innate immunity to TB to discover whether the common *IL-10* gene polymorphisms have any association with susceptibility to pulmonary TB in the Lur population resident in Lorestan province. Therefore the susceptibility to pulmonary TB infection was assessed by studying of *IL-10* -1082A>G, -819T>C and -592A>C polymorphisms in the TB group and the results were compared with healthy control group.

MATERIALS AND METHODS

Patients and controls

We used case-control study design to perform our investigation. The case orgroup was comprised of 100 unrelated TB patients referred to health centers of Khorramabad city in Lorestan province and was selected by having a positive result of sputum microbe culture. The control group was comprised of 100 unrelated individuals with identical race and geographic region who were asymptomatic, had normal radiologic x-ray images of their chest and their PPD test was negative. All participants were third-generation natives of people lived in the selected geographic region. Ethnic information about the place of birth of the patients and their parents and grandparents was obtained to be confident on their racial origin. The patient group was equalized with the control group. The blood samples were collected after obtaining of written informed consent. The Lorestan University of Medical Sciences ethics committee approved the competency of this study.

Genotyping

We extracted the patients and controls DNA samples by employing QIAmp (Qiagen, Germany) kit. Extraction was performed according to the manufacturer's instructions. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method suggested previously by Wu *et al.*, 2008 was used to determine *IL-10* -1082A>G, -819T>C and -592A>C polymorphisms from patients' and controls' genomic DNA. The list of forward and reverse primer sequences (Qiagen, Germany), restriction enzymes (Biolabs, USA) and digestion patterns for different genotypes are shown in the Table 1.

The amplification was carried out by using MasterCycler (BioRad, USA) in a volume of 25µl containing 50 ng genomic DNA, 0.2 µM primer, 0.2 mM dNTPs, 2.0 mM

Table 1. Primer sequences, restriction enzymes used and restriction digestion patterns for genotyping of *IL-10* polymorphisms

IL-10 polymorphisms	Sequences of the primers	PCR Products Size	Restriction enzymes	Fragments size
IL-10 (-1082A>G)	F: 5' gacaacactactaattctcctttggga 3' R: 5' gtgagcaaactgaggacagaaat 3'	315 bp	Bsl I	AA:278 bp AG:278 bp+253 bp+25 bp GG:253 bp+25 bp
IL-10 (-819T>C)	F: 5' gacaacactactaattctcctttggga 3' R: 5' gtgagcaaactgaggacagaaat 3'	315 bp	Ssp I	TT:315 bp TC:315 bp+291 bp+24 bp CC:291 bp+24 bp
IL-10 (-592A>C)	F: 5' ggtgagcactacctgactagc 3' R: 5' cctaggtcacagtgacgtgg 3'	412 bp	Rsa I	AA:412 bp AC:412 bp+236 bp+176 bp CC:236 bp+176 bp

$MgCl_2$ and 0.625 units of Taq polymerase in 1x reaction buffer. Amplification conditions used were as follows: Denaturation initiated at 9°C for 5 min and was followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C to 61°C for 30 s, extension at 72°C for 40 s, and a final extension at 72°C for 10 min. Then the PCR products were incubated to digest at 37°C for 16h with restriction enzymes. The electrophoresis of PCR products was accomplished on 3% agarose gel consisting of 0.5 mg/ml ethidium bromide. Finally, the products were visualized by UV illumination.

Statistical analysis

The genotypic and allelic frequencies of *IL-10* -1082A>G, -819T>C and -592A>C polymorphisms were determined by direct counting in the TB population and healthy control population. All polymorphisms were consistent with values predicted by Hardy-Weinberg equilibrium in the both patient and control groups. The differences in the genotypic and allelic frequencies of *IL-10* -1082A>G, -819T>C and -592A>C polymorphisms were determined by the Chi-Square and Fisher's exact tests between TB population and healthy control population. Overall, $P<0.05$ was suppposed to be statistically significant after Yates correction. The odds ratio (OR) was calculated by the cross-product ratio and exact confidence intervals (CI) of 95% were calculated.

RESULTS

The study subjects comprised of 100 healthy controls with mean age of 30.21 years (±2.55 SD) and 100 pulmonary TB patients with mean age of 39.65 years (±3.87 SD). Among the healthy controls, 50 people were males and 50 people were females, and among the pulmonary tuberculosis patients, 40 people were males and 60 people were females (Table 2).

The genotypic and allelic frequencies of *IL-10* -1082A>G, -819T>C and -592A>C polymorphisms were listed in the Tables 3 and 4. The genotypic frequencies of *IL-10* -819T>C and -592A>C polymorphism were not significantly different between the pulmonary TB patients and the healthy controls. Only, in *IL-10* -1082A>G polymorphism, a signicantly increased frequency of genotype AG was observed among patients compared with controls (74%

Table 2. Characteristics of tuberculosis patients group and healthy controls group

	Tuberculosis patients group (n=100)	Healthy controls group (n=100)
Age*	39.65±3.87	30.21±2.55
Male	40	50
Female	60	50

* mean±SD

Table 3. Distribution of *IL-10* genotypes in pulmonary tuberculosis patients and healthy controls

IL-10 Polymorphisms	Genotypes	Associated phenotypes	% of tuberculosis patients (n=100)	% of healthy controls (n=100)
IL-10 (-1082A>G)	AA	Low	24	32
	AG	Intermediate	74*	58
	GG	High	2	10
IL-10 (-819T>C)	TT	–	46	52
	TC	–	44	38
	CC	–	10	10
IL-10 (-592A>C)	AA	–	30	36
	AC	–	68	60
	CC	–	2	4

*Significant difference after correction (P=0.0252, OR=0.485, CI=0.4307-0.5988)

Table 4. Distribution of *IL-10* alleles in tuberculosis patients and healthy controls

IL-10 Polymorphisms	Alleles	% allele frequency in tuberculosis patients	% allele frequency in healthy controls
IL-10 (-1082A>G)	A	61	61
	G	39	39
IL-10 (-819T>C)	T	68	71
	C	32	29
IL-10 (-592A>C)	A	64	66
	C	36	34

in the patients vs. 58% in the controls, P=0.0252, OR=0.485, CI=0.4307-0.5988) (Table 3).

The allelic frequencies of *IL-10* -1082A>G, -819T>C and -592A>C polymorphism were not significantly different between the pulmonary TB patients and the healthy controls (Table 4).

DISCUSSION

IL-10 which is a T cell regulatory cytokine plays a central role during chronic and latent stages of pulmonary TB. The IL-10 production is high during the infection promoting reactivation of TB. The excessive production of this cytokine results in failure to control the infection (Turner *et al.*, 2002). Recent studies have reported the increased production of IL-10 in patients with active disease (Joshi *et al.*, 2015; Gao *et al.*, 2015). In these studies, *IL-10* -1082A>G, -819T>C and -592A>C polymorphisms were widely determined as the candidate genes of susceptibility to TB infection. The results suggested that the *IL-10* -1082A>G polymorphism is associated with increased TB risk in Caucasians, while *IL-10* -819C/T and IL-10 -592A/C polymorphisms are important in Asians.

In this study, we showed the association of *IL-10* -1082A>G, -819T>C and -592A>C polymorphism in the susceptibility to pulmonary TB in the Lur population of Iran. The *IL-10* -819T>C and -592A>C polymorphisms had no significant difference between pulmonary TB patients and the healthy control group. Only, *IL-10* -1082A>G polymorphisms were found to be significantly

associated with patients versus healthy control. Also, earlier studies in the Hong Kong, Chinese (Tso *et al.*, 2005), Colombian (Henao *et al.*, 2006), Spanish, Turkish and Cambodian populations (Delgado *et al.*, 2002) have also shown the same relationship.

The GG genotype of *IL-10* -1082A>G was shown to be significantly associated with the disease in Colombian population as addressed by Meenakshi *et al.*, 2013, and Henao *et al.*, 2006, whereas in the present study and also in Tunisian (Ben-Selma *et al.*, 2011), West African (Thye *et al.*, 2009), Macedonian (Trajkov *et al.*, 2009) Gambian (Bellamy *et al.*, 1998), Spanish (Lopez-Maderuelo *et al.*, 2003) and Korean (Shin *et al.*, 2005) population, it was not associated. The results of our study indicated that AG genotype of *IL-10* -1082A>G polymorphism is significantly associated with pulmonary TB. The frequency of AG genotype which is 74% in our study was found to be similar to the frequency of the genotype in whole population of Iran (82.5%) which was reported by Amirzargar *et al.*, 2006.

Allele frequency of the IL-10 gene in our population was not significantly different which is in accordance to the result of a study done in Tunisian population (Ben-Selma *et al.*, 2011). In contrast to our results, other recent reports by Mosaad *et al.*, 2010 and Akgunes *et al.*, 2011 reported significant association with TB susceptibility. However, only one allele was associated with the disease in Italian population (Scola *et al.*, 2003).

Furthermore, the former studies have demonstrated that overproduction of IL-10 can lead to TB reactivation (Turner *et al.*, 2002; Joshi *et al.*, 2015). Hence despite of paradoxical findings, the association of *IL-10* polymorphisms with TB seems undeniable. However, we can indicate other *IL-10* polymorphisms, the race of studied population and the size of studied samples as the reasons of these controversial results.

In conclusion, the findings of this study indicated that the IL-10 -1082A>G polymorphism may be a valuable marker to predict the risk of pulmonary TB development of in the Lur population of Iran. Association of IL-10 polymorphisms with pulmonary TB in the Lur population of Iran is similar to what previously reported in Caucasians. We suggest that more studies with larger sample size should be carried out to verify the role of IL-10 -especially IL-10 -1082A>G- polymorphisms in the pathogenesis or development of the disease in the future.

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