Cytokines Genes Polymorphisms in Iranian Patients with Pulmonary Tuberculosis

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ABSTRACT

Background: Pulmonary tuberculosis (PTB) has recently become a major problem in developed countries especially in immune compromised HIV infected individuals. Cytokines, their genes and receptors have been implicated in the protective immunity, pathophysiology and development of tuberculosis. **Material & Methods:** In the present study the genotype frequencies of a number of polymorphic genes coding for cytokines or for cytokine receptors have been investigated in a case control study including a group of 40 Iranian PTB patients and 40 healthy individuals. The allelic polymorphism of cytokines SNPs were analyzed according to the protocols of the cytokine component designed for the 13th IHW by the Heidelberg University group. Using PCR-SSP method the following cytokine genes have been determined: IL-1α (T/C –889), IL-1β (C/T +3962), IL-1R (C/T pstI 1970), IL-1RA (T/C mspaI 1100), IL-4RA (G/A +1902), IL-12 (C/A –1188), TGF-β (C/T codon 10, G/C codon 25), TNF-α (G/A –308, G/A –238), IL-2 (T/G –330 G/T +166), IL-4 (T/G –1098, T/C –590, T/C –33), IL-6 (G/C –174, G/A nt 560), IL-10 (G/A –1082, C/T –819, C/A –592). **Results:** From IL-1R cluster (pro- inflammatory cytokines) a positive significant association was found at position pstI 1970 C/T polymorphism where the C allele was over presented in the PTB patients (60% vs. 37.5%, P = 0.04). A significant negative association at codon 10 TGF-β C/T polymorphism has also been shown in our patients, where the T allele was not detected in the patients but 10% of the control subjects expressed this allele (Fisher exact test, P = 0.05). At this codon allele T (Leucine substitution) is associated with high TGF-β production. For TNFα an insignificant tendency was found at position -308 A/G polymorphism where the G allele carried by 80% of cases and 65% of controls (P = 0.07). At position -238 a negative association was found at the GA polymorphism (10% vs. 25%, P = 0.07). For IL-6 an insignificant positive association at position -174 C/G

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polymorphism, G allele (57.5% vs. 37.5, P = 0.07) was found. At the other cytokine genes no specific association were found. **Conclusion:** In conclusion it is suggested that C allele at position pstI 1970 of IL-1 cluster increases and T allele at codon 10 of TGF-β decreases in PTB patients.

Key words: Cytokine, PCR-SSP, Polymorphism, Tuberculosis

INTRODUCTION

A recent study by Hussain et al. using a diluted whole blood assay has shown that PTB patients significantly suppressed T cell derived IFN but had higher monocyte derived IL-6 and IL-10 production in response to culture filtrate proteins in comparison with endemic healthy controls (3). In a TNF-α (-238 and -308) gene polymorphism analysis that was carried out by Selvaraj et al. in an Indian population, they suggested that TNF-α/β gene variants are not associated independently with susceptibility to PTB (4). Manderuelo et al. have investigated IFN-γ and IL-10 gene polymorphism in an Spanish population, and have shown that homozygous AA individuals at position IFN-γ (+874) had a 3.75 fold increased risk of developing tuberculosis, in contrast IL-10 polymorphism did not affect susceptibility to tuberculosis (5). Bellawy et al. have investigated IL-1 cluster gene in a group of Gambian PTB patients and suggested that susceptibility to tuberculosis in Gambian patients may be partly determined by a region in the IL-1 gene cluster on chromosome 2 (6). TGF-β codon 10 polymorphism was investigated in 110 healthy control and 101 tuberculosis patients by Niimi et al. They did not find any significant differences between TGF-β genotypes of healthy controls and tuberculosis patients (7). Polymorphism of the TNF-α gene at -308 position has been investigated in a group of patients with infiltrative tuberculosis from Bashkorstan population of Russia, they found that the frequency of TNF2 allele in tuberculosis patients was significantly higher than that of controls (P = 0.001)(8). Dolores et al. investigated the relationship of the single base change polymorphic variants identified in the first intron of the IFN-γ (+874) and in the promoter region of IL-10 gene (-1082 T/A) with cytokine production by peripheral blood mononuclear cells and tuberculosis susceptibility in Spanish population, they found that in individuals homozygous for the IFN-γ (+874) A allele had a 3.75 fold increased risk of developing tuberculosis (P = 0.001) (9). The frequencies of the functional polymorphisms at TNF-α (-308) and IL-10 (-1082) genes were analyzed by ARMS-PCR in a group of Sicilian patients with chronic lung tuberculosis (CTB) by Letizia Scola et al. (10). They reported a reduction of -308 GG TNF homozygous individuals in CTB affected subject group. In the present study the genotype frequencies of a number of polymorphic genes coding for cytokines or for cytokine receptors have been investigated in a case control study including a group of 40 Iranian PTB patients and 40 healthy individuals.

METHODS

Of 40 sputum positive PTB patients and 40 healthy blood donors, 5 ml whole blood was collected. Genomic DNA was extracted from the samples by a modified salting out method. The allelic polymorphism of cytokines SNPs was analyzed according to the protocols of the cytokine component designed for the 13th IHW by the Heidelberg University group. Using PCR-SSP method the fallowing cytokine genes have been determined, IL-1α (T/C –889), IL-1β (C/T +3962), IL-1R (C/T pstI 1970), IL-1RA (T/C mspaI 1100), IL-4RA (G/A +1902), IL-12 (C/A –1188), TGF-β (C/T codon 10, G/C codon 25), TNF-α (G/A –308, G/A –238), IL-2 (T/G –330 G/T +166), IL-4 (T/G –1098, T/C –590, T/C –33), IL-6 (G/C –174, G/A nt 560), IL-10 (G/A –1082, C/T –819, C/A –592).

RESULTS

Mean age of the patients was 45 15 years including 19 females and 21 males. No MDR positive cases were observed in the patient group. Genotype frequencies were calculated in PTB patients and control subjects, the results are shown in table 1. As it is shown, a positive significant association was found at position pstI 1970 C/T polymorphism of IL-1R gene where the C allele was over presented in the PTB patients (60% vs. 37.5%, P = 0.04). A significant negative association at codon 10 of TGF-β C/T polymorphism was also observed, where the T allele was not detected in the patients but 10% of the control subjects expressed this allele (P = 0.05). For TNFα an insignificant tendency was found at position -308 A/G polymorphism where the G allele carried by 80% of cases and 65% of controls (P = 0.07). At position -238 a negative association was found at the GA polymorphism (10% vs. 25%, P = 0.07). For IL-6 an insignificant positive association at position –174 C/G polymorphism, G allele (57.5% vs. 37.5, P = 0.07) was found. At the other cytokines genes loci no specific associations were found.

DISCUSSION

In IL-1 cluster (pro-inflammatory cytokines) a positive significant association was found at pstI 1970 C/T polymorphism where the C allele was over presented in the PTB patients (60% vs. 37.5%, P = 0.04). An insignificant increase in the TT genotype of -880 T/C polymorphism at IL-1α locus was observed (25% vs. 12.5%). A previous report in Gambian PTB patients has confirmed a positive association with IL-1 cluster genes and this is compatible with our data. A significant negative association at codon 10 of TGF-β C/T polymorphism has also been shown in our patients, where the T allele was not detected in the patients but 10% of the control subjects had this allele (Fisher

Table 1. Cytokine gene polymorphism in Iranian PTB patients and normal controls.

Cytokines	Position	Genotype	PTB %	NP %	P value
IL-1α	-889	CC	19(47.5%)	19(47.5%)	OR = 2.53
		TC	11(27.5%)	14(35%)	P = 0.15
		TT*	10(25%)	5(12.5%)	
IL-1β	-511	CC	12(30%)	9(22.5%)	
		TC	17(42.5%)	20(50%)	
		TT	11(27.5%)	8(20%)	
	+3962	CC	17(42.5%)	14(35%)	
		CT	22(55%)	23(57.5%)	
		TT	2(5%)	0	
IL-1R	PstI 1970	CC*	24(60%)	15(37.5%)	OR = 2.5
		CT	15(37%)	19(47.5%)	P = 0.04
		TT	2(5%)	3(7.5%)	
IL-1RA	MspaI 1100	CC	3(7.5%)	0	
		TC	12(30%)	17(42.5%)	
		TT	26(65%)	21(52.5%)	
IL-4RA	+1902	AA	31(77.5%)	27(67.5%)	
		GA	5(12.5%)	6(15%)	
		GG	4(10%)	5(12.5%)	
IL-12	-1188	AA	26(65%)	23(57.5%)	
		CA	11(27.5%)	12(30%)	
		CC	3(7.5%)	3(7.5%)	
TGF-β	Codon10	CC	15(37.5%)	5(12.5%)	Fisher exact
		CT	26(65%)	18(45%)	Test
		TT*	0	4(10%)	P = 0.05
	Codon25	CC	2(5%)	1(2.5%)	
		CG	2(5%)	2(5%)	
		GG	36(90%)	37(92.5%)	
TNF-α	-308	AA	0	1(2.5%)	OR = 2.54
		GA	8(20%)	12(30%)	P = 0.07
		GG*	32(80%)	26(65%)	
	-238	AA	0	0	OR = 0.33
		GA*	4(10%)	10(25%)	P = 0.07
		GG	36(90%)	28(70%)	
IL-2	-330	GG	3(25%)	2(5%)	
		GT	16(40%)	17(42.5%)	
		TT	15(37.5%)	15(37>5%)	
	+160	GG	22(55%)	20(50%)	
		GT	16(40%)	18(45%)	
		TT	3(7.5%)	0	
IL-4	-1098	GG	0	0	
		GT	22(55%)	20(50%)	
		TT	19(47.5%)	18(45%)	
	-590	CC	6(15%)	5(12.5%)	
		TC	35(87.55)	32(80%)	
		TT	0	0	
	-33	CC	22(55%)	22(55%)	
		CT	18(45%)	16(40%)	
		TT	0	0	
IL-6	-174	CC	4(10%)	3(7.5%)	OR = 2,25
		GC	13(32.5%)	20(50%)	P = 0.07
		GG*	23(57.5%)	15(37.5%)	
	nt565	AA*	4(10%)	3(7.5%)	Fisher exact test
		GA	13(32.5%)	20(50%)	P = 0.05
		AA	23(57.%)	15(37.5%)	
IL-10	-1082	AA	7(17.5%)	5(12.5%)	
		GA	31(77.5%)	33(82.5%)	
		GG	2(5%)	0	
	-819	CC	19(47.5%)	20(50%)	
		CT	20(50%)	18(45%)	
		TT	2(5%)	0	
	-590	AA	2(5%)	0	
		CA	20(50%)	18(45%)	
		CC	18(45%)	20(50%)	

exact test, P = 0.05). At this codon the T allele (Leucine substitution) is associated with high TGF-β production. For TNFα an insignificant tendency was found at position-308 A/G polymorphism where the G allele carried by 80% of cases and 65% of controls (P = 0.07). At this position A allele is associated with high TNFα production and G allele is associated with low level TNFα production. At position –238 a trend of negative association was found at the GA polymorphism (10% vs. 25%, P = 0.07). Sevaraj et al. did not confirm TNF association with PTB in Indian population. For IL-6 an insignificant positive association of G allele (57.5% vs. 37.5, P = 0.07) at position –174 C/G, was found. At the other cytokine genes loci no specific association were found. In conclusion it is suggested that pro inflammatory cytokines and TGF-β decrease in PTB patients, however further studies with a larger sample size for better understanding of cytokine gene polymorphism in PTB patients is necessary.

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