

Transforming Growth Factor-Beta 1 Gene Polymorphism in Tuberculosis Patients

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ABSTRACT Tuberculosis (TB) is a curable infectious disease that kills around half million individuals every year in India. Cytokines play a vital role in the pathogenesis of this deadly disease. Transforming growth factor-beta 1 (TGF- β 1) is one of the important anti-inflammatory cytokine found to be elevated in TB patients. Single nucleotide polymorphisms (SNP) in the promoter of TGF- β 1 cytokine gene are known to alter the production of this important cytokine. Given its immunosuppressive nature increased production of this cytokine is implicated towards susceptibility to TB. The C/T polymorphism in TGF- β 1 promoter at -509 site is strongly associated with circulating levels of this cytokine and T allele is shown to be associated with its high production. Thus, in the present study we looked for association of TGF- β 1 (C-509T) SNP with TB. A total of 245 subjects (145 TB patients and 100 normal healthy controls) were recruited for study. The -509 polymorphism was studied using Amplification Refractory Mutation System- Polymerase Chain Reaction (ARMS-PCR). The distribution of C/T alleles in TB patients and normal healthy controls did not reveal any statistically significant association of this polymorphism with TB ($\chi^2 = 0.04$; $p = 0.85$).

INTRODUCTION

Approximately two billion individuals globally are infected with *Mycobacterium tuberculosis*, yet only 10% of them develop clinical tuberculosis (TB). This can be attributed to the host genetic factors, which play an important role in development of clinical disease following infection with this deadly pathogen (Awomoyi et al. 2004). A growing body of evidence indicates that both the risk of acquiring infection and risk of developing severe complications are related to genetic variability at cytokine loci (McNicholl et al. 2000). In this regard, it has been shown that polymorphisms in genes encoding for different cytokines are associated with TB (Lopez-Maderuelo et al. 2003).

Transforming growth factor-beta 1 (TGF- β 1) has important anti-inflammatory effects (Kehrl et al. 1986) and is increased in serum of patients with advanced TB (Fiorenza et al. 2004). Monocytes from TB patients secrete increased

amount of TGF- β 1 upon *in vitro* stimulation with mycobacterial antigen (Olobo et al. 2001). Langerhans giant cells and epithelioid cells in TB granulomas express increased levels of TGF- β 1 mRNA suggesting local production of it may result in deactivation of macrophages leading to immunopathology of the disease (Toosi et al. 1995). In TB, TGF- β 1 increases intracellular growth of *M. tuberculosis* whereas neutralizing antibody to TGF- β 1 reduces intracellular growth (Hirsch et al. 1994). Thus, it is conceivable that allelic variations in the promoter of TGF- β 1 gene resulting in altered protein production might be responsible for differential TB pathogenesis among individuals. The C-509T polymorphism in TGF- β 1 promoter is associated with circulating levels of this cytokine. Additionally, the T allele is shown to be associated with high production of TGF- β 1 (Grainger et al. 1999). The present study was conducted to evaluate the association of C-509T allele polymorphism and susceptibility to tuberculosis in Punjabi population of North India.

MATERIALS AND METHODS

Study Population: The study population consisted of 145 TB patients (confirmed by

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physician) and 100 normal healthy control subjects. Informed consent was taken from all the individuals participating in the study. Information related to name, age, sex, ethnic origin and family history was collected from the patients. Venous blood samples from patients were collected in storage vials containing EDTA as an anticoagulant, visiting Department of TB and Chest, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar; TB and Chest hospital, Government Medical College, Amritsar; TB and Chest Wing, Civil Hospital, Gurdaspur, Punjab.

DNA Extraction and Genotyping TGF- β 1 (C-509T) Polymorphism: DNA was extracted from peripheral blood using modified inorganic method (Miller et al. 1988). Extracted DNA was quantified using UV-Spectrophotometry. The polymorphism for TGF- β 1 C-509T allele was genotyped using Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) method developed in our laboratory. Briefly, two complementary reactions were established for each allele consisting of target DNA; allele specific ARMS primers (RC for C allele and RT for T allele) and the common primer (CF). A 349 base pair region (nt - 488 to - 836 from the transcription start site) in the TGF- β 1 gene promoter was targeted for amplification. The characteristics of primer sequences are shown in table 1. To assist in the genotyping of -509 polymorphism, the penultimate base in the primers RC and RT was mutated from A to G. The optimized reaction conditions consisted of 40 ng of genomic DNA in a reaction volume of 30 μ l, containing 0.16 μ M of each primer, 30 μ M of each dNTP, 10 μ M Tris-HCl (pH 9.0), 1.5 μ M MgCl₂, 50 μ M KCl, 0.01% gelatin and 0.3U of Taq DNA polymerase. Amplification was carried out for 35 cycles, each cycle consisting of denaturation at 94°C for 30s, annealing at 61°C for 20s, extension at 72°C for 20s and finally a 3 min extension at 72°C. The amplified PCR products were analyzed by agarose gel electrophoresis on a 2% agarose gel stained with ethidium bromide. Known positive and negative controls were included in each batch of amplification.

Statistical Analysis: Allelic and genotypic frequencies in TB patients and normal healthy control population were compared using 2x2 contingency tables and chi-squared (χ^2) statistics. Statistical significance was defined at the standard 5% level.

RESULTS

A total of 245 subjects (145 TB patients and 100 normal healthy subjects) were genotyped for C-509T TGF- β 1 polymorphism, by ARMS-PCR. A representative gel photograph showing various genotypes of C-509T polymorphism are shown in figure 1. The sample was labeled CC or TT genotype, if a 349 bp band was observed only with C or T allele specific primer, respectively. However, if bands were observed with both allele specific primers then the sample was labeled with CT genotype. A known positive and negative control was included in each set of amplification. For example, for a C allele specific primer a known CC genotype worked as positive control and TT genotype served as a negative control, similarly a T allele specific primer will not amplify target DNA having CC genotype but give positive results with other two (CT, TT) genotypes. Tuberculosis and control populations were found to be in Hardy-Weinberg equilibrium with chi square values of 1.14 and 0.78, respectively. Table 2 shows distribution of TGF- β 1 polymorphism in studied populations. The frequency of various genotypes in both the groups was almost similar. TB patients had little more heterozygotes (49.7 vs 42.0%) while the normal controls had a little higher frequency of CC genotype (43.0 vs 40.0%). However, the difference in allele frequencies were statistically non significant between normal healthy control subjects and TB patients ($\chi^2 = 0.04$; p value = 0.85).

DISCUSSION

The extent to which progressive clinical TB is associated with genetic host factors remains

Table 1: Characteristics of C-509T TGF- β 1 polymorphism and primer sequences used for Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) method.

Location Allele	Primers	Sequence	Product Size
TGF- β 1; Constant	Antisense (CF)	5'CTACGGCGTGAGTGCTGAG 3'	349 bp
TGF- β 1C; ARMS	Sense (RC)	5'AAGGGGCAACAGGACACCTGGG 3'	
TGF- β 1T; ARMS	Sense (RT)	5'AAGGGGCAACAGGACACCTGGA 3'	

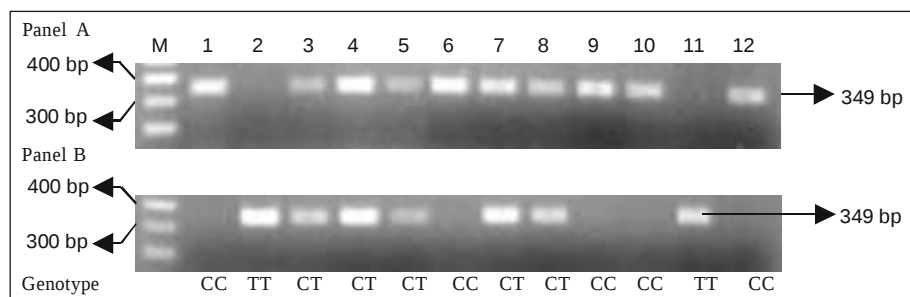


Fig. 1. A representative agarose gel photograph of the TGF- β 1 (C-509T) polymorphism. The panels A and B depict the DNA samples amplified with C allele specific and T allele specific, primer pairs, respectively. Lane M represents 100 bp ladder. Lane 11 and 12 correspond to control samples of TT and CC genotypes, respectively. Lanes 1 to 10 show random samples amplified. The genotypes of samples are given at the bottom of each lane.

Table 2: Genotype distribution and allele frequencies for TGF- β 1 (C-509T) SNP in studied population.

Population (n)	Genotype frequencies (%)			Allele frequencies
	CC	CT	TT	C/T
TB patients (145)	58 (40.00)	72 (49.70)	15 (10.30)	0.65/0.35
Controls (100)	43 (43.00)	42 (42.00)	15 (15.00)	0.64/0.36

χ^2 based on allele frequency (degrees of freedom = 1), (TB patients Vs Controls) = 0.04 p value = 0.85

unidentified in spite of large number of reports, which indicates association of clinical features of disease to host background (Scola et al. 2003). There is ample data to document that TGF- β 1 protein plays a vital role in the pathogenesis of TB. It has been shown to down regulate production of proinflammatory cytokines, deactivate macrophage function against *M. tuberculosis* and decrease responsiveness of T-cells to IL-2 (Kehrl et al. 1986; Shalaby et al. 1988; Wahl et al. 1988; Musso et al. 1990). Studies also demonstrate prominent role for TGF- β 1 in modulating production of IFN- γ in patients with pulmonary TB (Hirsch et al. 1996) which is an important macrophage activating cytokine during infection with *M. tuberculosis* (Kamijo et al. 1993; Huang et al. 1993). TGF- β 1 is also known to suppress TNF- α production in human and murine macrophages (Espevik et al. 1987) which is required early to limit *M. tuberculosis* multiplication and is a crucial factor in elimination of TB (Olobo et al. 2001). In our study, we tried to find out the association between T allele of C-509T polymorphism and susceptibility to tuberculosis, as it is associated with high circulating levels of this cytokine (Grainger et al. 1999). However, results obtained from this study do not show any statistically significant

difference for TGF- β 1 C-509T polymorphism between TB patients and normal healthy controls in our population.

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