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Association of TNF-α, IL-10 and IL-6 promoter polymorphisms in pulmonary tuberculosis patients and their household contacts of younger age group

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ABSTRACT

Single nucleotide polymorphisms (SNPs) of cytokine genes have been found to be involved in the clinical outcome of Tuberculosis. The present study was aimed to identify the high risk genotypes in Tuberculosis patients and their household contacts. A total of 490 subjects were studied which includes 150 active pulmonary tuberculosis patients (APTB), 190 household contacts (HHC) and 150 healthy controls (HC). The SNPs of TNF-α (-308A/G), IL-10(-1082G/A) and IL-6(-174G/C) were performed by ARMs PCR. The IL-10 GA genotype showed significant association in APTB and HHC and was 2.3 times higher risk in APTB and 3.7 times in HHC compared to HCs. The A allele was found to be significantly associated with the risk of disease. The CC genotype of IL-6 was found to be significantly associated in APTB and an insignificant positive association in HHCs. The multifactor dimensionality reduction (MDR) analysis indicated that the genotypes of IL-6 were showing high risk with GA genotype of IL-10. In conclusion the gene interaction may be useful for identification of genotypes as biomarkers to distinguish high risk individuals.

1. Introduction

Globally one-third of the human population is infected with tuberculosis (TB) and only 10% of them develop the active disease. Approximately 2 billion people were estimated to be infected with *Mtb* and remain asymptomatic [1,2]. The infected individuals may develop the disease later in their life (Reactivation of TB) or may remain in latent form. The disease transmission was observed to be high among the household contacts than the casual contacts. Among the household contacts, younger age and absolute or relative immunodeficiency states are at higher risk of acquiring infection from their index case [3,4]. The difference between infection rate and incidence rate may be caused by smoking history, physical condition, genetic factor and socio-economic factors. The highest incidence of TB is in Asia (58%), followed by Africa (27%), the Eastern Mediterranean Region (8%), the European Region (4%), and the Region of the Americas (3%) [5].

The occurrence of TB depends on different ethnic groups, regions and populations, implying host genetic factors might play an important role in disease susceptibility. Among the host genetic factors single nucleotide polymorphisms (SNPs) of cytokine genes are one of the major factors in determining the susceptibility. The cytokine gene polymorphisms have been shown to be involved in the susceptibility, the severity and clinical outcomes of several diseases, including infectious diseases.

The cytokine genes can induce a predominantly Th1 or Th2 type immune response upon antigen stimulation. Among Th1 cytokines, IFN-γ and TNF-α were identified as the most important agents of the antimycobacterial cytokine cascade. Th2 type includes IL-4, IL-10, TGF-β and other cytokines. IL-6 is a macrophage derived proinflammatory cytokine. These cytokines would be involved in shifting the state of latency to the stage of clinical tuberculosis [6]. TNF-α has been implicated as a key protective cytokine involved in macrophage activation and granuloma formation [7]. In latent TB infection (LTBI), it is important in controlling persistent infection and in residing organisms within granulomas [8]. The most widely studied SNP in the TNF- α gene is the A to G substitution at position −308. IL-10 is a T regulatory cytokine and is considered primarily an inhibitory cytokine, important to the adequate balance between inflammatory and immunopathological responses [9]. The analysis of the IL-10 gene polymorphisms involved in the development of infectious diseases suggests that this polymorphism has a critical role in the immunity and progression of inflammation. Human genetic studies examined the key polymorphisms in the *IL-10* gene, at loci 1082, 819, and 592 [10]. The −1082 polymorphism of IL-10 promoter was reported to be associated

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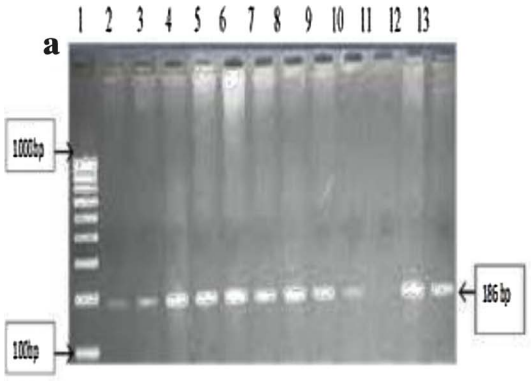
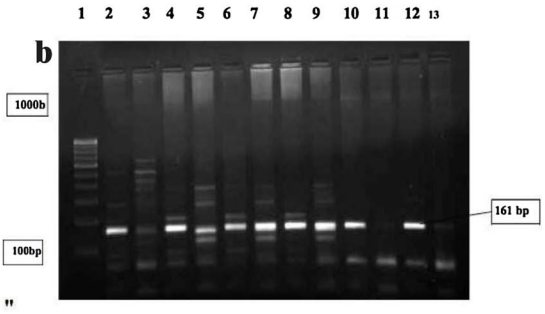
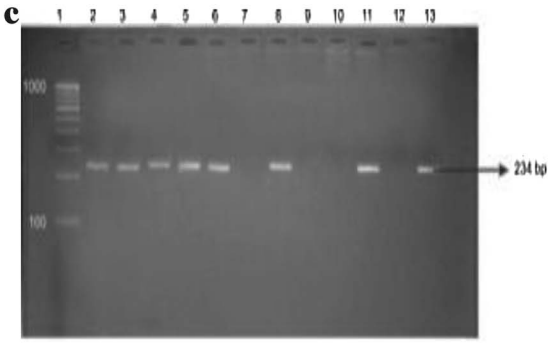


Lane 1- 100 bp ladder
Lane 2-3; 4-5; 6-7; 8-9;12-13 shows the bands in both wells indicating heterozygous GA genotype(186bp)
Lane 10-11 shows the band in single lane indicating homozygous GG genotype



Lane 1 – 100bp Ladder
Lane 2, 3 and 8, 9 shows the bands in both wells indicating GA genotype (161bp)
Lane 10, 11 and 12, 13 shows the band in single lane indicating GG genotype (161bp)



Lane 1 100- 1000bp ladder
Lane 2,3 and 4, 5 shows the bands in both wells indicating GC genotype(234 bp)
Lane 6,7 and 8, 9 shows the band in single lane indicating GG genotype (234 bp)
Lane 10,11 and 12,13 CC genotypes shows the band in single lane indicating CC genotype (234 bp)

Fig. 1. Gel images of TNF- α, IL-10 and IL-6 genes. a. Representative agarose gel electrophoresis illustrating PCR products for the TNF-α (−308A/G) promoter polymorphisms. b. Representative agarose gel electrophoresis illustrating PCR products for the IL-10 (−1082G/A) promoter polymorphism. c. Representative agarose gel electrophoresis illustrating PCR products for the IL-10 (−1082G/A) promoter polymorphism IL-6 (−174G/C) gene polymorphism.

with TB susceptibility and production of IL-10 has been associated with anergy in tuberculosis [11]. IL-6, which has both pro- and anti-inflammatory properties, is produced early during mycobacterial infection and at the site of infection. A rare genetic variation of *IL-6* gene, (-174G/C), was observed to be significantly associated with TB disease in Chinese Han population [12]. The mechanism underlying the genetic variations that influence the susceptibility or resistance to tuberculosis may lead to better understanding tuberculosis pathogenesis and the development of novel strategies for prevention and treatment of tuberculosis [8].

The present study was aimed to identify the susceptible or resistant genotypes of TNF-α (-308A/G), IL-10(-1082G/A) and IL-6(-174G/C) associated with latency in tuberculosis patients and their household contacts.

2. Materials and methods

2.1. Study groups

The study was carried out at free chest clinic and PPM-DOTS center, Mahavir Hospital and Research Center. Based on the data analysis over 10years (1998–2008) at the center it has been observed that most of the tuberculosis patients treated were in the age group of 15–25 yrs. Hence the present study included the subjects within the range of 15–25 yrs of age. A total of 490 cases were enrolled during 2009–2012 of which 150 were active pulmonary tuberculosis patients (APTB), 190 Household contacts (HHC), and 150 Healthy controls (HC). All patients had positive acid-fast bacilli (AFB) smear microscopy. The bacterial sputum gradation was based on the number of Acid Fast Bacilli (AFB) observed on the slide under microscope as per the Revised National Tuberculosis Control Program (RNTCP) guidelines. The household members of the patient of same age group have been taken as contact who were asymptomatic to TB. Healthy controls with 15–25 yrs age and no history of TB were included.

2.2. Ethical statement

All the study protocols were reviewed and approved by the institutional ethical committee of Bhagwan Mahavir Medical Research Center, Hyderabad, India. Prior written informed consent was taken from all the participants enrolled in the study, and in case of minors, the written consent was taken from their guardians.

Informed consent, clinical details and personal history was obtained from all the subjects included in the study.

2.3. DNA isolation

A 2 ml of whole blood was collected in EDTA vaccutainers from all