Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.4, No.27, 2014

Gene Polymorphism of Tumor Necrosis Factor-Alpha in a Sample of Iraqi Pulmonary Tuberculosis Patients

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Abstract

The study aimed to investigate the association between tumor necrosis factor (*TNF* gene) alleles and pulmonary tuberculosis (PTB) in a sample of Iraqi patients, in which a total of 94 patients were investigated, in addition to 80 age, gender and ethnicity matched controls. All subjects were genotyped by polymerase chain reaction with sequence specific primers (SSP-PCR) method at two positions of *TNF* gene; -308 and -238 (TNF_{-308} and TNF_{-238}), which were presented with three genotypes (GG, GA and AA) at both positions. At position -308, a significant ($P = 6.9 \times 10^{-5}$) decreased frequency of GG genotype was observed in PTB patients compared to controls (60.6 *vs*. 87.5%), and the preventive fraction of such difference was 0.68. In contrast, the genotype GA was significantly ($P = 1.3 \times 10^{-4}$) increased in patients (38.2 *vs*. 12.5%), and the associated relative risk and etiological fraction were 4.34 and 0.30, respectively. The corresponding TNF_{-308} alleles (*G* and *A*) also showed variations between patients and controls. Allele *G* was decreased (79.8 *vs*. 93.8%), while *C* allele was increased (20.2 *vs*. 6.2%) in patients, and both differences were significant at a P of 1.5×10^{-4}. However, at position -238, neither TNF_{-238} genotypes nor alleles demonstrated a significant difference between patients and controls. The present results suggest that the GG genotype and *G* allele of *TNF* gene at position -306 may be associated with a protection against PTB in Iraqi population.

Keywords: Tumor necrosis factor; Gene polymorphism, PCR; Pulmonary tuberculosis.

1. Introduction

Tuberculosis (TB) remains a major global health problem, causing ill-health among millions of people each year. The latest estimates revealed that there were 8.6 million new *Mycobacterium tuberculosis* infected cases in 2012 and 1.3 million TB deaths (WHO, 2013). In Iraq and based on 2012 estimates, the incidence of TB was 45 per 100,000 populations per year, but as a result of deteriorating socioeconomic conditions during the last decade, the incidence is expected to rise (WHO, 2014). The infection usually takes place in lungs (pulmonary TB, which is the most common), and begins as an alveolar inflammatory reaction that progresses to a typical delayed type granulomatous reaction (Santucci *et al*., 2011).

The well-established observation that only 10% of the population infected by M. tuberculosis will develop TB has led to an intense search for factors that determine its development in individuals; therefore a genetic component that confers resistance or susceptibility has been suspected (Schurr, 2011). Genetic studies demonstrated a significant hereditable component in variations observed between individuals in their response to *M. tuberculosis*. Evidence includes twin studies that showed a higher concordance rate in monozygotic twins than in dizygotic twins (Qu *et al*., 2011), and racial differences in susceptibility to infection by the pathogen have also been documented (Nahid *et al*., 2011). Candidate gene approach and association studies have identified various host genetic factors that affect TB susceptibility, especially those genes that control immunological functions (Möller and Hoal, 2010).

Host immune response against TB is regulated by interactions between lymphocytes (T helper and cytotoxic cells) with antigen-presenting cells (APCs) and cytokines secreted by these cell types. Upon infection, phagocytes are activated to produce pro-inflammatory cytokines; including tumor necrosis factor-α (TNF-α) (Yang *et al*., 2006). TNF-α Plays a key role in the initiation, regulation, and perpetuation of the inflammatory response, and it is also required for induction of apoptosis in response to mycobacterial infection (Mangangcha *et al*., 2011). So, the production of pro-inflammatory cytokines is essential for host resistance against MTB infection. The TNF gene cluster is located within the class III region of the highly polymorphic major histocompatibility complex (MHC) on human chromosome 6p21 (Ben-Selma *et al*., 2011), and polymorphisms

in *TNF* gene have been associated with susceptibility to TB in different ethnic groups but the results have been inconclusive (Delgado *et al*., 2002; Oral *et al*., 2006).

The present work was conducted with the aim to determine the type of association between *TNF* gene polymorphism (genotype and allele frequencies) at two positions; -308 and -238 (TNF_{-308} and TNF_{-238}) and PTB in a sample of Iraqi patients. To our best knowledge this is the first report on single nucleotide polymorphisms (SNPs) of *TNF* gene in Iraqi PTB patients.

2. Subjects, Materials and Methods

2.1 Subjects

Ninety four Iraqi Arabs patients with PTB were enrolled in the study. They were referred to the Institute of Chest and Respiratory Diseases in Baghdad for diagnosis and treatment during the period May-October 2012. Patients included were clinically and radiologically diagnosed for PTB and confirmed by conventional sputum smear and culture for *M. tuberculosis*. Among the patients, 70 were males (mean age ± S.E. = 43.5 ± 1.7 years) and 24 were females (mean age ± S.E. = 36.6 ± 2.6 years). A control sample of 80 clinically healthy individuals with no signs, symptoms or history of previous mycobacterial infection was also included. They were blood donors and matched patients for gender (60 males and 20 females) and ethnicity (Iraqi Arabs). The male age mean was 40.2 ± 2.8 years, while for females, it was 38.3 ± 3.5 years. An informed consent was obtained from each participant.

2.2 Blood Collection and DNA Isolation

Three milliliters of venous blood were collected in tubes containing ethylene diamine tetra acetic acid (EDTA) as anti-coagulant and kept frozen until use. Genomic DNA was extracted from frozen whole blood using Blood gDNA Miniprep kit (Promega, USA). Extracted DNA was quantified by spectrophotometery, checked for purity and stored at -20ºC until further analyses.

2.3 Determination of *TNF* Gene Polymorphism

The genetic polymorphism of *TNF* gene was determined at two positions; -308 and -238 (TNF_{-308} and TNF_{-238}) by polymerase chain reaction with sequence specific primers (SSP-PCR) using Heidelberg University cytokine genotyping kits. Amplification was carried out using a PCR 9600 thermal cycler (Applied Biosystems, Foster City, CA, USA) under the following conditions: initial denaturation at 94ºC for 2 minutes; denaturation at 94ºC for 15 seconds; annealing+extension at 65ºC for 1 minute (10 cycles); denaturation at 94ºC for 15 seconds; annealing at 61ºC for 50 seconds; extension at 72ºC for 30 seconds (20 cycles). The presence or absence of PCR products was visualized by 2% agarose gel electrophoresis. Each of primer mixes contained an internal positive control, which was a primer that amplified a part of human C-reactive protein (CRP) gene that produces a 440-bp amplicon.

2.4 Statistical analysis

Alleles and genotypes of cytokines were presented as percentage frequencies, and significant differences between their distributions in PTB patients and controls were assessed by two-tailed Fisher's exact probability (P). In addition, relative risk (RR), etiological fraction (EF) and preventive fraction (PF) were also estimated to define the association between an allele or a genotype with the disease. The RR value can range from less than one (negative association) to more than one (positive association). If the association was positive, the EF was calculated, while if it was negative, the PF was given. (Ad'hiah, 1990). These estimations were calculated by using the WINPEPI computer programs for epidemiologists. The latest version of the WINPEPI package (including the programs and their manuals) is available free online at http://www.brixtonhealth.com.

3. Results

At position -308, a significant ($P = 6.9 \times 10^{-5}$) decreased frequency of GG genotype was observed in PTB patients compared to controls (60.6 *vs*. 87.5%), and the PF of such difference was 0.68. In contrast, the genotype GA was significantly ($P = 1.3 \times 10^{-4}$) increased in patients (38.2 vs. 12.5%), and the associated RR and EF were 4.34 and 0.30, respectively. The corresponding TNF_{-308} alleles (*G* and *A*) also showed variations between patients and controls. Allele *G* was decreased (79.8 *vs*. 93.8%), while *C* allele was increased (20.2 *vs*. 6.2%) in

patients, and both differences were significant at a P of 1.5 x 10^{-4} (Table 1). However, at position -238, neither TNF_{-238} genotypes nor alleles demonstrated a significant difference between patients and controls (Table 2).

Table 1. Observed numbers and percentage frequencies of *TNF* genotypes and alleles at position -308 in pulmonary tuberculosis patients and controls.

Genotype or Allele	Patients (No. = 94)		Controls (No. = 80)		RR	EF or PF	P	95% C.I.
	No.	%	No.	%				
GG	57	60.6	70	87.5	0.22	0.68	6.9 x 10^{-5}	0.10 - 0.48
GA	36	38.2	10	12.5	4.34	0.30	1.3 x 10^{-4}	2.00 - 9.46
AA	1	1.06	0	0	-	-	-	-
G	150	79.8	150	93.8	0.26	0.69	1.5 x 10^{-4}	0.13 - 0.55
A	38	20.2	10	6.2	3.80	0.15	1.5 x 10^{-4}	1.83 - 7.89

RR: Relative Risk; EF: Etiological Fraction; PF: Preventive Fraction; P: Two-sided Fisher's Exact Probability; C.I.: Confidence Interval.

Table 2. Observed numbers and percentage frequencies of *TNF* genotypes and alleles at position -238 in pulmonary tuberculosis patients and controls.

Genotype or Allele	Patients (No. = 94)		Controls (No. = 80)		RR	EF or PF	P	95% C.I.
	No.	%	No.	%				
GG	84	89.3	69	86.2	1.34	0.23	N.S.	0.54 - 3.32
GA	8	8.5	10	12.5	0.65	0.04	N.S.	0.25 - 1.73
AA	2	2.1	1	1.2	1.72	0.01	N.S.	0.16 - 19.02
G	176	93.6	148	92.5	1.19	0.15	N.S.	0.52 - 2.72
A	12	6.4	12	7.5	0.84	0.01	N.S.	0.37 - 1.92

RR: Relative Risk; EF: Etiological Fraction; PF: Preventive Fraction; P: Two-sided Fisher's Exact Probability; N.S.: Not Significant; C.I.: Confidence Interval.

4. Discussion

TNF-α is produced by macrophages, dendritic cells and T cells when stimulated or infected with *M. tuberculosis*. In a murine model, the protective role of TNF-α in immunity against M. *tuberculosis* has been well documented. In mice deficient in TNF-α or the 55-kDa TNF-α receptor, *M. tuberculosis* infection resulted in rapid death, with a higher bacterial burden than that observed in control mice. Furthermore, in the absence of TNF-α or the 55-kDa TNF-α receptor, the granulomatous response was deficient following acute *M. tuberculosis* infection in murine models (Yim and Selvaraj, 2010).

For TNF gene at position -308, a significant negative association with the GG genotype or *G* allele was observed, and the protective effect of such genotype reached 68%, while the heterozygous genotype GA proved to be a susceptibility genotype with RR of 4.34 and EF of 0.30. Both findings highlight the importance of such position of *TNF* gene in susceptibility to or protection against PTB development in Iraqi patients. Although results of associations between TNF polymorphisms and susceptibility/resistance to TB have been already studied in different ethnic populations and showed ethnic-specific pattern, they are still questionable due to the different observations that were made. The present results for association between TNF_{-308} polymorphism and TB came to be sustained by results obtained from Bashkortostan and Sicily (Bikmaeva *et al*., 2002; Scola *et al*., 2003), but they are apparently in a disagreement with those data from Thailand (Vejbaesya *et al*., 2007), Iran (Amirzargar *et al*., 2006), Colombia (Henao *et al*., 2006), India (Selvaraj *et al*., 2001; Kumar *et al*., 2008; Sharma *et al*., 2010), Turky (Ates *et al*., 2008), China (Wu *et al*., 2008), Canada (Larcombe *et al*., 2008), Korea (Oh *et al*., 2007) and Tunisia (Ben-Selma *et al*., 2011). The present study also could not confirm the positive (susceptible) association of TNF_{-238} *G* allele or GG genotype with TB in Iranian patients (Amirzargar *et al*., 200); an observation that also shared by study from Turkey (Ates *et al*., 2008). The apparent inconsistency between these studies could be due to ethnic-specific genetic variations that greatly influencing host immunity to TB and/or samples size of studied populations causing differential susceptibility to tuberculosis. It is also possible that other more distal promoter elements are involved. However, the present results suggest that the GG genotype and *G* allele of *TNF* gene at position -306 may be associated with a protection against PTB in Iraqi population.

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