The Role of Interleukin-10 and Interferon-Gamma Single Nucleotide Polymorphisms in Multi-Drug Resistant Tuberculosis

Wedad M. Abdelraheem¹, Nagwa M. Zenhom³, Ebtessam E. Hassan³, Aliaa S. Abd El Fatah⁴

ABSTRACT

Background: Multi-drug resistant tuberculosis (MDR-TB) represents a major public health problem on a global scale.

Objectives: This study aimed to determine the association of Interleukin-10 (IL-10) rs.1800896 and Interferon-Gamma (IFN-γ) rs.2430561 single nucleotide polymorphisms (SNP) with MDR-TB.

Methods: 60 tuberculosis patients (30 patients with and 30 patients without MDR-TB) and 30 healthy subjects as controls participated in this study. Serum IL-10 and IFN-γ levels were measured by ELISA. SNPs of IL-10 -rs.1800896 and IFN-γ rs.2430561 genes were detected using allele-specific amplification.

Results: IL-10 serum level was significantly higher in TB patients with MDR (57.3 ± 14.8 pg/l) than TB patients without MDR (41.5 ± 8.4 pg/l). Serum IFN-γ was significantly lower among TB patients with MDR (41.7 ± 15.8 pg/l) compared to TB patients without MDR (83.7 ± 25.05 pg/l). The AA genotype of IL-10 rs.1800896 and the TT genotype of IFN-γ was significantly higher in the control group than in patients groups. Also, The AA genotype of IFN-γ rs.2430561 was significantly higher in patients with MDR than in patients without MDR-TB.

Conclusions: Regarding IL-10 GG and AG genotypes were considered a risk from MDR TB (OR = 11.54 and 4.1 for GG and AG respectively). Regarding INF-γ TT and AT genotypes considered protective for MDR TB (OR = 0.09 and 0.17 for TT and AT respectively).

KEY WORDS

IFN-γ, IL-10, MDR-TB, SNP

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis (M. tuberculosis). Humans are the natural reservoir for M. tuberculosis. M. tuberculosis infects about 2 billion individuals worldwide, only 5-10% of them develop active TB.

TB is exacerbated by the emergence of multidrug- and extremely drug-resistant (MDR and XDR) M.tuberculosis strains which represent a major public health problem on a global scale. In 2013, approximately 210,000 people died from MDR-TB, and there were about 80,000 new cases of MDR-TB worldwide.

IL-10, one of the anti-inflammatory cytokines, seems to play an important role during the chronic/latent stage of TB, with increased production playing a potentially central role in promoting reactivation of TB.

One of the most important SNPs in the promoter region of the IL-10 gene is -1082A/G (rs.1800896) polymorphism that may affect the transcription of IL-10.

IFN-γ is an important pro-inflammatory that is secreted by natural killer (NK) cells and T cells; its production plays a critical role in macrophage activation in controlling M. tuberculosis infection.

There is a single nucleotide polymorphism at the +874T/A (rs.2430561) position in the first intron of the human IFN-γ gene. IFN-γ +874T/A SNP is located within a binding site for NFκB (the transcription factor that can induce the expression of interferon-γ) and NFκB specifically binds to the DNA sequence containing T allele. Indeed, it has been shown that the T and A alleles most likely correlate with high and low expression of IFN-γ, respectively.

TB occurs predominantly in parts of the world such as Africa and South Asia. The occurrence of TB at different rates among particular races and families indicate a genetic predisposition to TB susceptibility. However, the contributions of some specific genetic variations in the human genome to host susceptibility or resistance to TB drugs remain unknown. Therefore, this study aimed to determine the associa-
DNA was confirmed by Bio-Photometer (Genova, UK) and DNA was Genomic DNA Purification Kit (Thermo Scientific, INC). Quantity of column method according to the manufacturer’s instructions using a DNA extraction instructions.

kits (Thermo Fisher Scientific, INC) according to the manufacturer’s patients before the start of treatment and controls using standard ELISA Measurement of Serum IL-10 and IFN-γ serum level of IL-10 and IFN-γ. ples were separated and kept frozen at -20℃ for the determination of was collected in sterile EDTA-containing tubes for DNA extraction, and aseptic conditions and divided into 2 portions; 1.5 mL of whole blood

MATERIALS AND METHODS

Study Population

60 tuberculosis patients (26 males and 34 females) were confirmed by conventional bacteriological methods (smear or culture positive) and 30 healthy subjects (10 males and 20 females) as controls participated in this study.

All the TB patients were recruited for this study from El Minia Chest Hospital in the period from October 2016 to June 2018. The inclusion criteria for the control group were the absence of pulmonary diseases, autoimmune diseases and family history of TB disease and, for the patients were the absence of HIV infection or a history of immuno-compromised conditions.

The patients were assigned to two groups: the first group - 30 patients with MDR-TB (Resistant to first-line anti-tuberculous drugs), the second group - 30 patients with non-MDR -TB. Written informed consent was obtained from all individuals. The study was approved by the Ethical Committee of Minia University, Faculty of Medicine.

Blood samples

Blood samples were taken from each participant under complete aseptic conditions and divided into 2 portions; 1.5 mL of whole blood was collected in sterile EDTA-containing tubes for DNA extraction, and the rest was then centrifuged at 3000 rpm for 10 minutes. Serum samples were separated and kept frozen at -20°C for the determination of serum level of IL-10 and IFN-γ.

Measurement of Serum IL-10 and IFN-γ

Serum cytokine (IL-10 and IFN-γ) levels were measured for all patients before the start of treatment and controls using standard ELISA kits (Thermo Fisher Scientific, INC) according to the manufacturer’s instructions.

DNA extraction

Genomic DNA was extracted from EDTA whole blood using a spin column method according to the manufacturer’s instructions using a Genomic DNA Purification Kit (Thermo Scientific, INC). Quantity of DNA was confirmed by Bio-Photometer (Genova, UK) and DNA was stored at -20°C.

Molecular methods

SNPs of IL-10 -1082 G/A (rs.1800896) and IFN-γ +874T/A (rs.2430561) genes were detected using allele-specific amplification, which was assessed by SYBR Green qPCR (Thermo Scientific, US). For each gene, two separate reactions were performed. Each reaction contained one of the two allele-specific forward primers and a generic primer similar to that first described by Perrey et al.11.

Sequences of primers used were as follow:12) IL-10 rs.1800896 (G allele primer: 5'-CTA CTAAGG CTT CCT TGG GAG -3', A allele primer: 5'-AG TGC CAA CTG AGA TTT GG -3') IFN-γ rs.2430561 (A allele primer: 5'-TTC TTA CAA CAC AAA ATC-3', T allele primer: 5'-TTC TTACAA CAC AAA ATC AAA TCT-3', Generic primer:5'- TCA ACA AAG CTG ATA CTC CA ATC -3').

Each PCR reaction was performed in a 25-μl volume containing 100 ng of genomic DNA (5 μl), 2x SYBR Green master mix (12.5 μl), 0.3 μM each primer (1 μl), 50x reference dye low (0.5 μl) and PCR-grade water (up to 25 μl). An ABI 7500 instrument (Applied Biosystems, USA) was used for PCR amplification and fluorescence curve analysis.

Statistical Analysis

Continuous data are expressed as the mean and standard deviation for normally distributed variables. Categorical data are presented in the form of number and percentage. All statistical analyses were performed using the SPSS program for Windows (version 20 statistical software; Texas instruments, IL, USA). Pearson’s chi-square test was used to determine the significance of genotype distribution in all of the study subjects. The odds ratios (ORs) and corresponding p-values for the samples were analyzed by logistic regression analysis. A two-tailed p-value

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control group N = 30</th>
<th>TB patients N = 30</th>
<th>p1</th>
<th>p2</th>
<th>p3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10 Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>17</td>
<td>3</td>
<td>5</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>AG</td>
<td>9</td>
<td>13</td>
<td>11</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>GG</td>
<td>4</td>
<td>14</td>
<td>14</td>
<td>0.002</td>
<td>0.002</td>
</tr>
</tbody>
</table>

| IFN-γ Genotype |
| γ | 60 | 60 | 60 |
| A | 43 | 19 | 21 | 0.008 | 0.003 | 0.3 |
| G | 17 | 41 | 39 | 0.004 | 0.007 | 0.3 |

| Allele | 60 | 60 | 60 |
| A | 21 | 30 | 43 | 0.001 | 0.003 | 0.009 |
| T | 39 | 30 | 17 | 0.06 | 0.002 | 0.006 |

| p1 | (between control and TB without MDR), p2 (between control and TB with MDR), p3 (between TB with MDR and TB without MDR)

Table 1: Demographic characters of the study population

<table>
<thead>
<tr>
<th>Demographic characters</th>
<th>Control group N = 30</th>
<th>TB patients N = 30</th>
<th>p1</th>
<th>p2</th>
<th>p3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean ± SD)</td>
<td>37.9 ±</td>
<td>39.8 ±</td>
<td>42.9 ±</td>
<td>0.5</td>
<td>0.08</td>
</tr>
<tr>
<td>Family history of TB</td>
<td>0 (0%)</td>
<td>5 (16.6%)</td>
<td>8 (26.7%)</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Smokers</td>
<td>5 (16.7%)</td>
<td>10 (33.3%)</td>
<td>15 (50%)</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Non smokers</td>
<td>21 (70%)</td>
<td>20 (66.7%)</td>
<td>10 (33.3%)</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>4 (13.3%)</td>
<td>0</td>
<td>5 (16.7%)</td>
<td>c</td>
<td>c</td>
</tr>
</tbody>
</table>

| Sex | Female | 20 (66.7%) | 15 (50%) | 19 (63.3%) | b | b | b |
| Male | 10 (33.3%) | 15 (50%) | 11 (36.7%) | |

| Residence | Urban | 19 (63.3%) | 20 (66.7%) | 20 (66.7%) | b | b | b |
| Rural | 11 (36.7%) | 10 (33.3%) | 10 (33.3%) | |

| Genotype of IFN-γ rs.2430561 and IL-10 rs.1800896 single nucleotide polymorphisms with the susceptibility to TB and MDR-TB.

| Allele | 60 | 60 | 60 |
| A | 43 | 19 | 21 | 0.008 | 0.003 | 0.3 |
| G | 17 | 41 | 39 | 0.004 | 0.007 | 0.3 |

| Allele | 60 | 60 | 60 |
| A | 21 | 30 | 43 | 0.001 | 0.003 | 0.009 |
| T | 39 | 30 | 17 | 0.06 | 0.002 | 0.006 |
of < 0.05 was considered statistically significant.

RESULTS

Demographic characteristics of the TB patients and controls

The demographic characteristics of the TB patients and controls are shown in Table 1s regard age, sex, residence, family history of TB, and smoking.

Serum IL-10 and IFN-γ level

Statistically significant increase of serum IL-10 level was observed in TB patients with MDR (57.3 ± 14.8pg/l) than TB patients without MDR (41.5 ± 8.4pg/l) and controls (11.07 ± 5.08pg/l), (p = 0.001). Serum IFN-γ was significantly higher among TB patients without MDR (83.7 ± 25.05pg/l) compared to TB patients with MDR (41.7 ± 15.8pg/l) and controls (11.07 ± 5.08pg/l), (p = 0.001). This is supported by Pasquinnelli et al. and Rolandi et al. who stated that The degree of reduction in IFN-γ production by peripheral blood mononuclear cells (PBMC) is a marker of disease severity in patients with TB. Besides, IFN-γ secretion is lower in patients with the most severe manifestation of tuberculosis, which may clarify its role in counteracting the effect of M. tuberculosis infection and inactivation of infected macrophages.

IL-10 and IFN-γ polymorphisms in TB patients and controls

SNPs at position -1082 G/A in the promoter region of the IL-10 gene and at position +874T/A of IFN-γ gene were genotyped using alleles specific amplification by SYBR Green real-time PCR.

Table 2 shows the distribution of different alleles and genotypes of IL-10 and IFN-γ polymorphisms in TB patients and controls.

Logistic regression analysis of IL-10 and IFN-γ genotypes

Regarding IL-10 GG and AG genotype was considered a risk from MDR TB (OR = 11.54 and 4.1 for GG and AG respectively). Regarding INF-γ TT and AT considered protective for MDR TB (OR = 0.09 and 0.17 for TT and AT respectively) as shown in table 3.

Serum IL-10 and IFN-γ levels for different genotypes

A statistically significant increase of serum IL-10 levels was observed with the GG genotype. Serum IFN-γ was significantly higher with the TT genotype as shown in table 4.

DISCUSSION

The prevalence of multidrug-resistant tuberculosis is increasing and emerging health burden. So understanding pathogenesis and factors affecting resistance to one or more drug are critically important for successful treatment.

Our results show that smoker patients are susceptible to multidrug resistance to anti-tuberculosis drugs (p = 0.001). It may be attributable to the various effects of tobacco smoking on pulmonary macrophages and lymphocytes that have a fundamental role in cellular immunity, and also Feng et al. reports that cigarette smoke exposure increased the bacterial burden and inhibit the pulmonary T-cell response in mice infected with M. tuberculosis.

Moreover, certain studies examined the link between smoking and MDR-TB, and they identified an association of smoking with acquired MDR-TB.

We observed an increase of IL-10 serum level in T.B patients groups than the control group (p = 0.001). This may suggest its role in T.B infection, moreover, the mean of IL-10 was higher among T.B patients with MDR than T.B patients without MDR (p = 0.001) that may be related to the effect of IL-10 in the development of drug resistance in T.B patients.

This is in agreement with Jamil et al. and Lago et al. who reported that "low level of IL-10 favors the immune response against Mycobacterium, while high levels are associated with disease progression and recurrence". This controversy may be related to sample size, different ethnicity or genetic susceptibility. Also, it was higher in T.B without MDR group than T.B patients group with MDR (p = 0.003) that may shed the light toward its important immunological role in response to anti-tuberculosis drugs.

In the current study IL-10 -1082 A allele is common among control group than TB patients groups (p = 0.008 and 0.003). Also, the AA genotype was significantly higher in the control group than in the patient group (p = 0.0001) thus A allele is protective while genetic susceptibility to infection is increased with G allele polymorphism. Also, IL-10 GG and AG genotype were considered a risk from MDR TB (OR = 11.54 and 4.1 for GG and AG respectively).

These results were consistent with a study carried by Asgharzadeh et al. who reported that IL-10 -1082 A allele was more frequent in the control group(p = 0.001, OR = 2.183) than in the patient group. However this is in contradiction with the results of a study which was done on Nigerian populations, and the authors concluded that genotype GG of G1082A polymorphism in the IL-10 gene is immunogenic and lymphocytes that have a fundamental role in cellular immunity, and also Feng et al. reports that cigarette smoke exposure increased the bacterial burden and inhibit the pulmonary T-cell response in mice infected with M. tuberculosis.

This controversy may be related to sample size, different ethnicity or even genetic heterogeneity.

In our study +874 A allele and AA genotype in IFN-γ gene is common in both TB patients groups more than control group (p=0.06 and 0.03) and so A allele is considered a risk for infection, moreover INF-γ TT and AT considered protective for MDR TB (OR = 0.09 and 0.17 for TT and AT respectively, emphasize IFN-γ role in helping response to anti-TB drugs.

This is supported by a large meta-analysis study on 8,574 individuals with TB and 9,011 controls. The main discovery of their study was that the T-allele of the IFN-γ +874 T/A(rs2430561) polymorphism showed a protective effect for TB susceptibility, which was consistent with the observation that the presence of the T-allele correlates with
high IFN-γ expression and increased resistance to *M. tuberculosis* infection, whereas the A-allele correlates with low expression\(^1\). IFN-γ +874 polymorphism was found to have a significant association with TB in different studies with different ethnic populations in Brazilian\(^2\), South African population\(^3\), Chinese\(^4\), Spanish population\(^5\) and Italian\(^6\).

In Egypt, Mosaad et al. in a study included 110 children with tuberculosis and 118 healthy control children reported that IFN-γ +874 genotype AA was significantly higher in patients with TB (p = 0.015) than in controls\(^7\).

Semen level of IFN-γ was higher in both TB patients groups, especially in TT genotype and mainly in patients without MDR more than the group of MDR which magnifies the fundamental protective role against infection and also protection from MDR.

Therefore, the identification of host genes responsible for susceptibility and resistance to TB should provide a significant contribution to understanding the pathogenesis of the disease and may lead to the development of new prophylaxis and treatment strategies.

Acknowledgement

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References


