Archives of Razi Institute, Vol. 77, No. 4 (2022) 1505-1510

DOI: 10.22092/ARI.2022.357620.2073

Original Article

Relation between Serological Findings and Expression of IL-1β and IL-6 genes in Patients Infected with *Mycobacterium Tuberculosis* in Iraq

Majid Jameel, Y[1*], Kassem Khalil, Z[1]

1. Department of Optometry, Medical-Technical Institute, Al-Mansour Middle Technical University, Baghdad, Iraq

Received 26 January 2022; Accepted 30 April 2022
Corresponding Author: yousor.alazzawi@gmail.com

Abstract

Mycobacterium Tuberculosis (TB) is one of the serious bacterial infections that cause diseases and may lead to death. In this study, 178 individuals were examined for TB infection at Baghdad TB center during the period from 15th January to 1st October 2021. Out of 178 participants, 73 were shown to be positive for TB infection, while 105 showed negative results. According to the results, there was no significant variation between infected males and females with TB in comparison to the control group (P>0.05). The results showed that the mean age of the patients for both males and females was in the range of 2–65 years. Additionally, there were significant differences in patients with TB compared to the control group in terms of the weight loss of 8.82 ± 6.75 Kg, red blood cell (RBC) count (3.43 ± 0.56) × 10^6/µl, white blood cell (WBC) count (3.12 ± 1.57) × 10^6/µl, platelet count (1.03 ± 0.56) × 10^6/µl, and hemoglobin level (6.66 ± 1.34) g/dl. A total of 30 TB patients and 50 normal individuals were genotyped to detect the IL-1β rs 114534 gene. The polymerase chain reaction (PCR) was used for exon amplification in region 5 of the *ILB1* gene in the TB patients by using specific primers. The finding showed that there was an amplified product of 249bp located in chromosome 2q13-14. A total of 30 TB patients and 50 normal individuals were also genotyped to detect the *IL-6* rs 1800795 gene. The PCR was used for amplification of the IL-6 gene in TB patients by using specific primers. The finding showed that there was an amplified product of 431 bp located in chromosome 7p15-p2. The expression of the *ILB1* gene was investigated in TB patients and healthy controls by using qPT-PCR. Results showed that there was a high Ct value for patients and controls with a high Ct value of templates, preoperational to the total ribonucleic acid (RNA) concentration and gene expression. The expression of the *IL-6* gene was investigated in TB patients and healthy controls by using qPT-PCR. Our findings revealed a high Ct value for patients and controls with a high Ct value of templates, preoperational to the total RNA concentration and gene expression.

Keywords: IL-1β, IL-6 genes, *Mycobacterium Tuberculosis*

1. Introduction

Mycobacterium Tuberculosis (MTB) is a bacterium that may lead to severe infection (*Tuberculosis*) and consequently lead to death. Active *Tuberculosis* (TB) may show typical symptoms such as bloody sputum, chronic coughs, fevers, night sweats, and weight loss as well as other symptoms which may be seen in the extra-pulmonary conditions (1). The conventional method to detect MTB in a clinical sample e.g. sputum, pus, or tissue biopsy takes about two to six weeks. So, for rapid MTB detection, several techniques were developed like ELISA, real-time polymerase chain reaction (RT-PCR) Gen-Probe amplification, and latex agglutination (1, 2). At present, the detection of MTB is based on the AFS and culturing methods which have been used for a long time (3). While these methods

were continuously improved and refined, they still have strict limitations. The positive predictive value of the AFS smears is more sensitive compared to other methods (4). Furthermore, because of the funding, training difficulties, and staffing of the most rapid and sensitive culture method, this method and other staining methods are not used by all laboratories (5). The Western blot is based on the specific mouse IgG anti-M. Tuberculosis monoclonal antibody (TB-55 mAb) was utilized to identify the target mycobacterial circulating antigen of 55-kDa molecular weight in the sera of the infected patients with confirmed TB with no bands detected in the sera of the healthy persons. Antigens of the target TB were isolated and characterized as proteins. They are composed of 15 amino acids which 46.4% of these amino acids are hydrophilic, while 24.6% of them are hydrophobic (6). The tuberculin skin test has limited the specificity and false-positive results can occur as a result of the prior Bacillus Calmette–Guérin vaccinations or infections with non-tuberculous mycobacteria (7). The serological tests using various TB antigens used for detection of MTB infection were rapid but lacked the required sensitivity. New techniques, such as the nucleic acid amplification method were developed which can give false-positive results despite their specificities. Over the conventional methods, the immunologic (QuantiFERON and T-SPOT.TB) tests measure IFN-gamma production by TB-specific T-lymphocytes after encountering *M. tuberculosis* antigens (8).

Therefore, this study aimed to investigate the relation between serological findings and expression of IL-1β and IL-6 genes in patients infected with Mycobacterium Tuberculosis.

2. Materials and Methods

2.1. Clinical Workflow at the Patient Recruitment Site

A total of 178 participants were recruited at the Baghdad TB center from 15th January to 1st October 2021. Baghdad TB center follows World Health Organization general guidelines for TB diagnostics, directly observed treatment short-course, and patient care. Out of 178 participants, 73 were shown to be positive for TB infection, while 105 were negative. Patients positive by acid-fast bacilli (AFB) sputum smear microscopy for at least one sample, were considered positive (AFB+) and their anti-TB treatment (ATT) was immediately initiated. For AFB- cases, broad-spectrum antibiotics (Amoxicillin 500 mg and Co-trimoxazole combined with Trimethoprim 100mg) were prescribed for two weeks followed by another round of AFB microscopy and Chest X-ray (CXR). If the CXR was suggestive and the clinical symptoms were consistent with pulmonary TB persist, the patient was considered an AFB- pulmonary TB patient and ATT was initiated. In this study, for all pulmonary TB cases, sputum samples were cultured (Lowenstein-Jensen (LJ) and MGIT) for confirmation of TB.

2.2. Diagnostic Tests

Sputum samples were processed for AFB microscopy (Ziehl-Neelsen staining) in two Microbiology Laboratories at Baghdad TB center, Iraq. Cultures were performed on two independent sputum samples from the patients who were prescribed ATT at these two diagnostic laboratories. At AIMC, sputum cultures were performed by two methods: 1) on solid LJ media, and 2) on liquid MGIT-960. At GDCH, only solid culture on LJ media was performed. To detect the MTB IgA antibodies, the indirect ELISA method was used.

2.3. Control Group

Blood samples of the healthy individuals (n = 105) of mixed genders were taken from the same geographical area as the TB patients; these individuals had no history of active TB, no pulmonary symptoms, and no known medical conditions (such as infection, malignancy, or metabolic disease). This group consisted of random young individuals to represent the general healthy population in comparison to TB patients.

2.4. Blood Sampling

As previously described, blood samples (5 ml) were collected into a Vacutainer tube (EDTA, catalog # 367899; BD, Franklin Lakes, NJ) through venipuncture, then all the blood parameters were

assessed, and finally plasma was collected and frozen in aliquots at −80°C until usage. By the way, patients were de-identified (there was no personal information).

2.5. Molecular Analysis

The DNA isolation from culture samples was done by the CTAB method. The polymerase chain reaction (PCR) was performed to amplify the IL-1β gene and IL-6 gene sequence of 249 and 431 base pairs, respectively, using DNA extracted from culture.

The primers used in this study for IL-1β and IL-6 genes were F: AAGCAGCGTATTGTCGAGTAGAT and R: CGTCTCTTTCATTCCCACATTT on one hand; and on the other hand, F: CAGAAGAACAGATGACTG and R: GTGGGGCTGATTGGAAACC, respectively. Primers used with RT-PCR for *ILB1* were F: AACAGATGAAGTGCTCCTTCCAGG and R: TGGAGAACACCACTTGTTGCTCCA; and for IL-6 gene was F: CTCCTTCTCCACAAGCGCCTTC and R: GCGCAGAATGAGTTGTC.

2.6. Statistical Analysis

The statistical analysis of data was done by using SPSS V.24 program. For determination of significance levels, the t-test and Monte Carlo test at 5% and 1% were used, respectively.

3. Results

As demonstrated in table 1, there was no significant difference in the mean age of TB patients (35.29 ±17.355 years) compared to the control group (37.36±17.087 years) (P>0.05).

Table 1. The mean age of the positive and negative TB infected individuals

TB	n	Mean (Age/Year)	Standard Deviation	Standard Error	t-test (P-value)
Positive	73	35.29	17.355	1.505	P=0.279
Negative	105	37.36	17.087	1.176	Non sign.
Total	178				(P>0.05)

The recorded data showed that 33 (45.2%) of male participants were positive for TB while 43 (40.1%) of the males participants were negative for TB (P>0.05). The results revealed that 73 (54.8%) of female participants were positive for TB while 105 (58.9%) of the females participants were negative for TB (Table 2).

Table 2. Distribution of TB infection according to gender

Gender		TB		χ^2 test (P-value)
		Positive	Negative	
Male	N	33	43	P=0.534 Non sign. (P>0.05)
	%	45.2%	40.1%	
Female	N	40	62	
	%	54.8%	58.9%	
Total	N	73	105	
	%	100.0%	100.0%	

Table 3 showed that the age range (2–65) and mean age of patients for both males and females were as 7.33 ± 3.94 years. Moreover, there were significant differences in patients with TB compared to the control group in terms of the weight loss (8.82 ± 6.75 Kg), red blood cell (RBC) count (3.43 ± 0.56) × 10^6/µl, white blood cell (WBC) count (3.12 ± 1.57) × 10^6/µl, Platelet count (1.03 ± 0.56) × 10^6/µl, and Hemoglobin level (6.66 ± 1.34) g/dl. A highly significant difference was found between male and female TB patients in mean age groups of 2–65 years (7.33 ± 3.94 years), (P<0.01). In addition, there were significant differences in patients with TB in terms of the weight loss (8.82 ± 6.75 kg), RBC count, (3.43 ± 0.56) × 10^6/µl, WBC count, (3.12 ± 1.57) × 10^6/µl, platelet count, (1.03 ± 0.56) × 10^6/µl, and hemoglobin level, 6.66 ± 1.34 g/dl, (P<0.01).

Table 3. Results of TB according to the clinical signs and symptoms among the studied patients

Clinical signs & symptoms	Mean± SD	χ^2 test (P-value)
Male/female	13.33±15.61	No Sign. (P>0.05)
Age 2–65 year	7.33 ± 3.94 yr	Sign. (P<0.01)
Weight loss	8.82 ± 6.75 kg	Sign. (P<0.01)
RBC count	(3.43 ± 0.56) × 10^6/µl	Sign. (P<0.01)
WBC count	(3.12 ± 1.57) × 10^6/µl	Sign. (P<0.01)
Platelet count	(1.03 ± 0.56) × 10^6/µl	Sign. (P<0.01)
Hemoglobin level	6.66 ± 1.34 g/dl	Sign. (P<0.01)

RBC: red blood cell, WBC: white blood cell

A total of 30 TB patients and 50 normal individuals were genotyped to detect the *ILB1* rs 114534 gene. The PCR was used for exon amplification in region 5 of the IL-1β gene in TB patients by using the specific primers. The findings illustrated in figure 1 showed that there was an amplified product of 249bp located in chromosome 2q13-14.

A total of 30 TB patients and 50 normal individuals were genotyped to detect the *IL-6* rs 1800795 gene. The PCR was used for amplification of the IL-6 gene in TB patients by using the specific primers. The findings illustrated in figure 2 showed that there was an amplified product of 431 bp located in chromosome 7p15-p2.

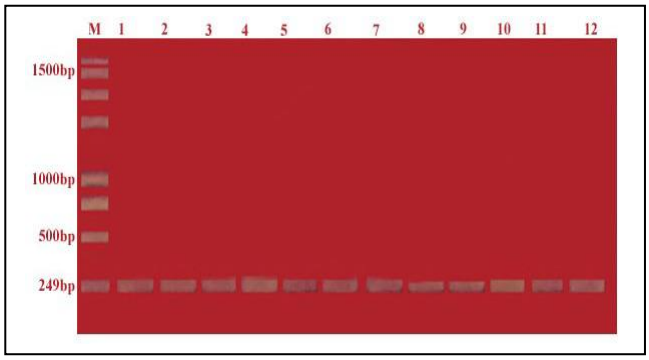

Figure 1. Amplified products of IL-1β gene in exon region 5 after electrophoresis on 1% agarose gel

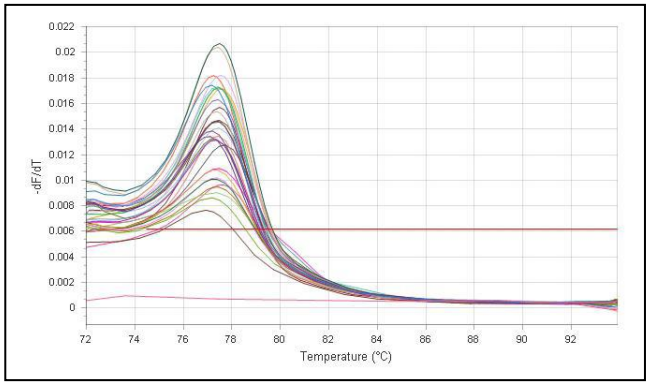

Figure 3. Melting curve analysis of the real-time PCR* for IL-1 β expression
*PCR: polymerase chain reaction

The expression of the IL-1β gene was investigated in TB patients and healthy controls by using qPT-PCR. Results showed a high cycle threshold (Ct) value for patients and controls with a high Ct value of templates, preoperational to the total ribonucleic acid (RNA) concentration and gene expression (Figure 3).

The expression of the *IL-6* gene was investigated in TB patients and healthy controls by using qPT-PCR. Results showed a high Ct value for patients and controls with a high Ct value of templates, preoperational to the total RNA concentration and gene expression (Figure 4).

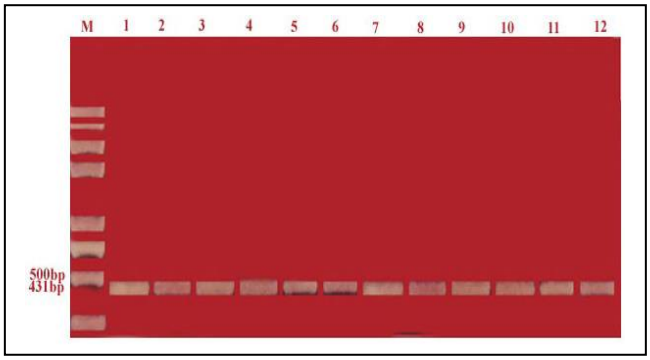

Figure 2. Amplified products of *IL-6* gene in exon region 5 after electrophoresis on 1% agarose gel

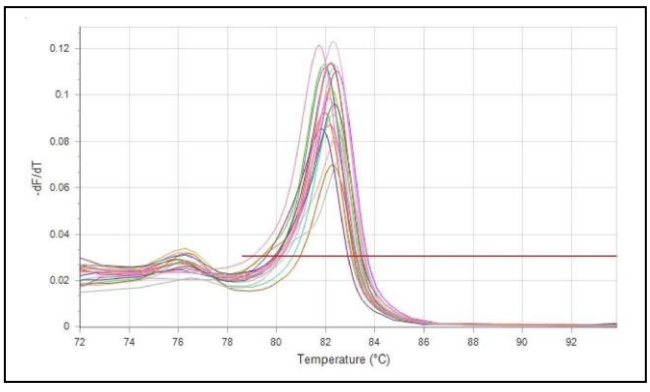

Figure 4. Melting curve analysis of the real-time PCR for *IL-6* expression
*PCR: polymerase chain reaction

4. Discussion

Mycobacterium Tuberculosis is one of the serious bacterial infections that cause disease in the human body that may lead to death. According to the results, there was no significant variation between infected males and females with TB in comparison to the control group. These results disagreed with Hertz, Dibbern (6) who reported that men are more susceptible to TB infection which may be due to smoking, higher physical activity, poorly ventilated workplaces, or gender differences of the body physiology of men and women.

The mean age of the male and female TB patients was 2–65 years. Balaky, Mawlood (1) stated that the younger cases were most susceptible to infection with TB at their elder ages. The reason may be attributed to the physical structure of these youngsters, their living conditions, or the cold areas they live in Iraqi Kurdistan.

Furthermore, there were significant differences in patients with TB in terms of weight loss. According to Warmelink, Nick (9), for those treated, there was especially a weight loss that may reach half of the body mass with pallor in color and changes that occur in the affected person's body. Additionally, there was a decrease in RBC with a decrease in hemoglobin levels and an imbalance in WBC from the normal limits in TB infection. These results matched with Gil-Santana, Cruz (4) who reported that there was anemia that was induced by infection with TB, and this anemia may be very severe, as well as there was an imbalance in the levels of WBC due to infection with these dangerous bacteria.

The results of *ILB1* gene expression showed that most of the infected cases were positive for TB patients a clear gene expression was recorded, and this was evident that infection could be determined through this gene. These findings agreed with Sousa, Cá (10) who reported that secretion of IL-1β was a good surrogate of the differences observed and accordingly a good way to classify strains as probable drivers of different TB severities. Furthermore, it was demonstrated that MTB isolates that induced low levels of IL-1β production could evade macrophage cytosolic surveillance systems (11).

Regarding IL-6, there was an increase in the levels of this cytokine in TB infections. Kumar, Moideen (8) reported an increase in the secretion of this cytokine in patients with TB. Moreover, the gene expression of this gene confirmed that IL6 was useful in determining the incidence of pulmonary tuberculosis (12).

Authors' Contribution

Study concept and design: Z. K. K.
Acquisition of data: Z. K. K.
Analysis and interpretation of data: Y. M. J.
Drafting of the manuscript: Y. M. J.
Critical revision of the manuscript for important intellectual content: Z. K. K.
Statistical analysis: Z. K. K.
Administrative, technical, and material support: Y. M. J.

Ethics

The study protocol was approved by the medical ethics board of the Al-Mansour Middle Technical University, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Balaky STJ, Mawlood AH, Shabila NP. Survival analysis of patients with tuberculosis in Erbil, Iraqi Kurdistan region. BMC Infect Dis. 2019;19(1):1-8.
2. Cao X-J, Li Y-P, Wang J-Y, Zhou J, Guo X-G. MPT64 assays for the rapid detection of Mycobacterium tuberculosis. BMC Infect Dis. 2021;21(1):1-15.
3. Dunn JJ, Starke JR, Revell PA. Laboratory diagnosis of Mycobacterium tuberculosis infection and disease in children. J Clin Microbiol. 2016;54(6):1434-41.
4. Gil-Santana L, Cruz LA, Arriaga MB, Miranda PF, Fukutani KF, Silveira-Mattos PS, et al. Tuberculosis-

associated anemia is linked to a distinct inflammatory profile that persists after initiation of antitubercular therapy. Sci Rep. 2019;9(1):1-8.

5. Gong W, Wu X. Differential diagnosis of latent tuberculosis infection and active tuberculosis: A key to a successful tuberculosis control strategy. Front Microbiol. 2021:3126.
6. Hertz D, Dibbern J, Eggers L, von Borstel L, Schneider BE. Increased male susceptibility to Mycobacterium tuberculosis infection is associated with smaller B cell follicles in the lungs. Sci Rep. 2020;10(1):1-9.
7. Khan FY. Review of literature on disseminated tuberculosis with emphasis on the focused diagnostic workup. J Fam Community Med. 2019;26(2):83.
8. Kumar NP, Moideen K, Banurekha VV, Nair D, Babu S, editors. Plasma proinflammatory cytokines are markers of disease severity and bacterial burden in pulmonary tuberculosis. Open forum infectious diseases; 2019: Oxford University Press US.
9. Warmelink I, Nick H, van der Werf TS, van Altena R. Weight loss during tuberculosis treatment is an important risk factor for drug-induced hepatotoxicity. Br J Nutr. 2011;105(3):400-8.
10. Sousa J, Cá B, Maceiras AR, Simões-Costa L, Fonseca KL, Fernandes AI, et al. Mycobacterium tuberculosis associated with severe tuberculosis evades cytosolic surveillance systems and modulates IL-1β production. Nat Commun. 2020;11(1):1-14.
11. Zhou F, Xu X, Wu S, Cui X, Fan L, Pan W. Protein array identification of protein markers for serodiagnosis of Mycobacterium tuberculosis infection. Sci Rep. 2015;5(1):1-10.
12. Zhou F, Xu X, Cui X, Pan W. Development and Evaluation of a Fusion Polyprotein Based on HspX and Other Antigen Sequences for the Serodiagnosis of Tuberculosis. Front Microbiol. 2021:4128.