

Original Paper

Immunological Study for Tuberculosis Among Sample of Children and Young Iraqi Patients

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ABSTRACT

Mycobacterium tuberculosis is highly successful as an intracellular pathogen that has developed strategies to survive even in the presence of high immune pressure. Blood were collected from a total of 65 patients confirm with tuberculosis (TB), who attended the Institute of Chest and Respiratory Diseases/ Medical City in Baghdad during the period between January-2026 till the end of July-2026, and 15 apparently healthy individual as control group, from both genders with age rang (1-25) year. The Immunological study was carried out to estimate the level of TNF- α and IFN- γ in patients and control group by ELISA method using causabiokit. The effect of age and gender on TNF- α and IFN- γ levels of patients were studied. The outcome of TNF- α showed an elevation in its value among TB patients (49.66 ± 6.24), in comparison with control group (10.952 ± 3.07), also the level of IFN- γ in patients was found high with mean value (38.27 ± 10.82), in comparison with healthy control group (3.069 ± 0.84). it was appeared that elevation of TNF- α and IFN- γ values significantly not effected by age and gender.

KEYWORDS: *Mycobacterium tuberculosis*, TB, Blood, ELISA, IFN- γ , TNF- α .

1. INTRODUCTION

Tuberculosis (TB) is one of the infectious diseases, which is spread from one individual to another by air droplet through cough, sneezing and talking. A cough, weight loss, hungriness, fever, drenching night perspiring and extreme tiredness or lack of energy are the main common symptoms of this disease. *Mycobacterium tuberculosis* (MTB) is the main causative agent of TB [1,2]. TB has existed for millennia and remains a major global health problem. It causes ill-health in millions of people each year and in 2015 was one of the top 10 causes of death worldwide, ranking above HIV/AIDS as one of the leading causes of death from an infectious disease [3].

In Iraq, TB is a serious health problem. Iraq is one of seven high-burden countries in the Eastern Mediterranean Region, accounting for 3% of regional cases, with an incidence of 42/100,000 and about 15,000 new cases annually. The case detection rate is only 54% due to security challenges and displacement. However, incidence declined from 45 to 23/100,000 between 2013-2023, reaching 21/100,000 in 2023, reflecting NTP/WHO efforts.

Studying TB in Iraqi children and youth is crucial as it affects productive age groups, reflects household transmission, and helps reduce economic burden under conditions of overcrowding and low detection.

Tuberculosis, a granulomatous disorder, in which the cellular immune response plays an important role by produced different cytokines have been shown to play principal roles in the defense against mycobacteria infection [4].

Cytokines play a critical role in the defense against bacterial infections, both directly, by the inhibition of bacterial replication, and indirectly, through determination of the prevalent Th1/Th2 pattern of host immune response [5,6]. Th1 cells produce the cytokines IFN- γ and TNF- α which are known to be protective through the activation of functions in macrophage cells, that can control intracellular *Mtb* [7]. Therefore, this study aimed to evaluate some immune factors related to TB, such as TNF- α and IFN- γ , in patients

2. MATERIALS AND METHODS

I. Patients and Control

Sixty-five patients with tuberculosis, including both males and females, were enrolled in this study. The patients ranged in age from 1 to 25 years and attended the Institute of Chest and Respiratory Diseases, Baghdad Medical City, Baghdad, Iraq, during the period from January 2026 to the end of July 2026. The diagnosis of tuberculosis was established based on clinical manifestations, radiological findings, and laboratory investigations, including sputum examination and/or other appropriate microbiological tests, according to the diagnostic criteria adopted by the treating physicians. The control group consisted of fifteen apparently healthy volunteers with no clinical evidence or previous

history of tuberculosis. All participants were enrolled according to the inclusion and exclusion criteria established for the study.

II. Samples collection

Five milliliters of blood were collected from each individual participating in the study using disposable syringes, after obtaining informed consent from the patients or their parents/guardians for children. The blood was centrifuged and the serum was separated, placed into a clean test tube and kept at -20°C for estimation of some cytokines including interferon gamma ($\text{IFN-}\gamma$) and tumor necrosis factor alpha ($\text{TNF-}\alpha$).

III. Immunological tests

The level of $\text{IFN-}\gamma$ and $\text{TNF-}\alpha$ was measured for all patients and control individually by Enzyme-Linked ImmunoSorbent Assay method using causabio kit.

which is a biochemical assay that uses antibodies and an enzyme-mediated color change to detect the presence of antigen (proteins, cytokines) in a given sample (sera)[8].

IV. Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage and Least significant difference –LSD test (or T-Test) was used to significant compare between means in this study. In addition, Receiver Operating Characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of $\text{TNF-}\alpha$ and $\text{IFN-}\gamma$ in terms of area under the curve (AUC), sensitivity, specificity, PPV and NPV values

3. RESULTS

Detection of $\text{TNF-}\alpha$ Level in Patients and Control

The level of $\text{TNF-}\alpha$ was estimated in patients and control groups, the outcome have shown an elevation in their mean value among TB patients (49.66 ± 6.24), in comparison with healthy control group (10.952 ± 3.07) (Figure 1) with high significant differences ($P= 0.0001$) as shown in Table 1.

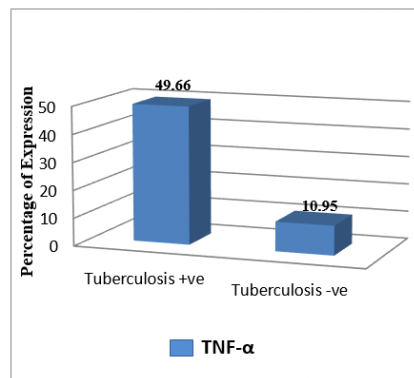


Figure (1): The $\text{TNF-}\alpha$ percentage in the patients and control groups

In this study the levels of $\text{TNF-}\alpha$ was estimated in Positive predictive value (PPV) and Negative predictive value (NPV) groups, the outcome has shown an elevation in their percentage among Positive predictive value (89.9%), in comparison with Negative predictive value (42.9%) (Figure 2), with Highly significant difference ($P<0.01$), as shown in Table (2).

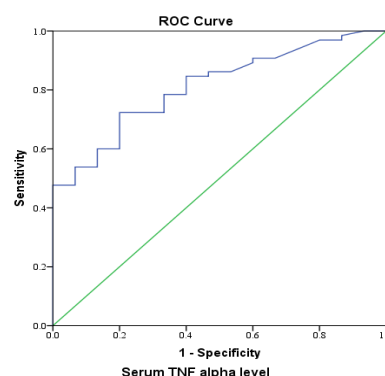


Figure (2): ROC curve; validity tests for serum TNF alpha level depends on clinical diagnosis
Effect of Age Group in TNF- α Level in Patients

The mean value of TNF- α concentration was (54.45 ± 14.20) ; (48.81 ± 12.81) and (48.59 ± 7.82) among patients group which were age less than 16, 16-20 and 21-25 years respectively with no significant differences ($P=0.944$) as shown in Table 3.

Effect of Gender in TNF- α Level in Patients

The mean value of TNF- α concentration was 46.63 ± 7.09 among males in comparison with 54.50 ± 11.70 among females as shown in Table 4, with no significant difference ($P=0.551$).

Detection of IFN- γ Level in Patients and Control

The level of IFN- γ in patients was elevated with mean value among TB patients (38.27 ± 10.82), in comparison with healthy control group (3.069 ± 0.84) (Figure 3) with high significant differences ($P= 0.0001$) as shown in Table 5.

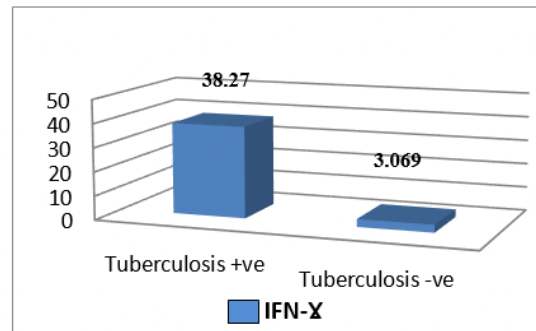


Figure (3): TNF- α percentage in the two groups of casesEffect of Age Group in IFN- γ Level in Patients

In this study the levels of IFN- γ was estimated in Positive predictive value (PPV) and Negative predictive value (NPV) groups, the outcome has shown an elevation in their percentage among Positive predictive value (94.5%), in comparison with Negative predictive value (48%) (Figure 4), with Highly significant difference ($P<0.01$), as shown in Table (6).

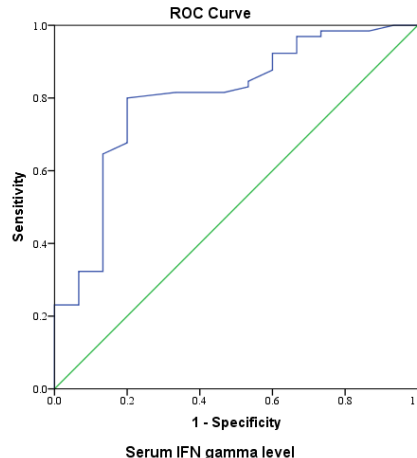


Figure 4: ROC curve; validity tests for serum IFN gamma level depends on clinical diagnosis

Effect the Age in IFN- γ Level of TB Patients

The mean value of IFN- γ concentration was 26.41 ± 9.25 in age less than 16, 25.67 ± 11.59 in age group of 16-20 and finally 18.91 ± 6.46 in age group of 21-25, with no significant difference ($P=0.0851$) as shown in Table 7.

Effect of Gender in IFN- γ Level in Patients

The mean value of IFN- γ concentration was 17.65 ± 7.99 among males and 29.25 ± 12.62 among females with no significant difference between them ($P=0.0966$) as shown in Table 8.

Table 1: The Level of TNF- α in Patients and Control group.

The group	No.	Mean $\pm$ SE of TNF- α
Control	15	10.952 $\pm$ 3.07
Patients	65	49.66 $\pm$ 6.24
T-Test	---	11.853 **
P-value	---	0.0001
O.R.	---	1.873

** (P<0.01).

Table 2: The Level of TNF- α of Positive predictive value and Negative predictive value

Serum TNF- α level	
Sensitivity	81.5%
Specificity	60%
Positive predictive value (PPV)	89.9%
Negative predictive value (NPV)	42.9%
Accuracy	77.5%
Area Under the curve (AUC)	0.812
Cutoff value	7.5
P-value	0.00HS

HS=Highly significant difference (P<0.01).

Table 3: Effect of Age on TNF- α Level of Patients.

Age group (year)	Mean $\pm$ SE TNF- α
Less than 16	54.45 $\pm$ 14.20
16-20	48.81 $\pm$ 12.81
More than 20	48.59 $\pm$ 7.82
LSD value	34.265 NS
P-value	0.944

NS: Non-Significant.

Table 4: Effect of Gender on TNF- α Level of Patients.

Gender	Mean $\pm$ SE TNF- α
Male	46.63 $\pm$ 7.09
Female	54.50 $\pm$ 11.70
LSD value	26.210 NS
P-value	0.551

NS: Non-Significant.

Table 5: The Level of IFN- γ in Patients and Control group.

The group	No.	Mean $\pm$ SE of IFN- γ
Control	15	3.069 $\pm$ 0.84
Patients	65	38.27 $\pm$ 10.82
T-Test	---	8.316 **
P-value	---	0.0001
O.R.	---	1.794

** (P<0.01).

Table 6: The Level of IFN- γ of Positive predictive value and Negative predictive value

Serum TNF- α level	
Sensitivity	80%
Specificity	80%
Positive predictive value (PPV)	94.5%
Negative predictive value (NPV)	48%
Accuracy	80%
Area Under the curve (AUC)	0.8
Cutoff value	2.4535
P-value	0.00HS

HS=Highly significant difference (P<0.01).

Table 7: Effect of Age on IFN- γ Level of Patients

Age group (year)	Mean $\pm$ SE
	IFN- γ
Less than 16	26.41 $\pm$ 9.25
16-20	25.67 $\pm$ 11.59
More than 20	18.91 $\pm$ 6.46
LSD value	16.528 NS
P-value	0.0851

NS: Non-Significant.

Table 8: Effect of Gender on IFN- γ Level of Patients

Gender	Mean $\pm$ SE
	IFN- γ
Male	17.65 $\pm$ 7.99
Female	29.25 $\pm$ 12.62
LSD value	15.701 NS
P-value	0.0966

NS: Non-Significant

4-DISCUSSION

Tumor necrosis factor alpha is a critical immune mediator in protection against and pathology of tuberculosis [9]. It is produced by macrophages, monocytes and dendritic cells in response to mycobacteria or its products. It has immunoregulatory properties and brings about the activation of macrophages granuloma formation, T-cell stimulation and regulation of chemokine induction, thus controlling infections [10]. There is evidence to suggest that TNF- α is necessary at the beginning of the inflammatory process in order to limit the multiplication of mycobacteria [11]. High levels of TNF- α were reported in chronic TB accompanied by the evaluated release of TNF- α receptors resulting in fever, necrosis and weight loss. The TNF- α has both protective and immunopathologic effects [12]. The results of This study were in agreement with many researchers, who reported that TNF- α level was high when they examined the pleural fluid, sera or monocyte culture of patients with TB before or after the onset of the treatment, when compared with control [11,13]. Beig [14], was previously found that increased levels of expression of TNF- α after 2 months of intensive anti-TB therapy were significantly associated with poorer outcomes on chest radiography. This result may indicate that there is an association between increases of TNF- α level and TB clinical deterioration early in treatment. Other researchers found that, quick recovery is associated with a rapid decrease of TNF- α in plasma [15]. On contrast other study by Auda [16] in Iraq, observed that, no significant difference (p=0.198) was found in serum TNF- α concentration between TB patients (mean= 8.09 pg/ml) and control group (mean=5.62 pg/ml). Regarding the relationship between age and TNF- α levels in patients, the results of the current study revealed that the increase in TNF- α levels was not significantly associated with age, same results were observed by Abdul-Hussein [17] in Iraq. Effect of Gender in TNF- α Level in Patients. In terms of the effect of gender on TNF- α levels in patients, the result of the current study was in agreement with [16] in Iraq, who investigated that TNF- α concentration in male TB patients nearly similar with that in female. As a conclusion, it was found male and female are similar in the production of TNF- α when they get infection with TB. Tumor necrosis factor alpha level in TB patients may associate with recovery of the patient after treatment, and no significant association was found between the levels of TNF- α in TB patients' sera and their age and gender.

Interferon-gamma is one of the representatives of cytokines involved in the clearance of intracellular pathogens. In this study, IFN- γ is significantly increased in concentration levels in sera of TB patients compared with the control, this result supported by hypothesis which has believed: The amount of mycobacterial replication is controlled by many host immune factors: Effector T cells and macrophages participate in controlling of mycobacterial infection. IFN- γ and TNF-

α produced by T cells are important macrophage activators [4]. The results of this study in agreement with other study In Iraq conducted by Abbas[18], who stated that sera IFN- γ level in TB patients and control groups (422.624 ± 33.824 and 75.816 ± 0.209 pg/ml) respectively with significant differences ($P \leq 0.05$). The results of the present study revealed that IFN- γ concentrations were not significantly associated with age, which is consistent with Abdul-Hussein [17] in Iraq and Sinaga [19], who found that IFN- γ concentrations were non-significantly increased among different age groups. Similarly, IFN- γ concentrations did not differ significantly according to gender, in agreement with the findings of Bothamley [20] and Abdul-Hussein [18] in Iraq.

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