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ARTICLE

A Comparative Study of Interleukin 1beta (IL-1β) Levels in Pulmonary and Extra-pulmonary Tuberculosis Patients Among Iraqi Population

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Abstract

Background: **The immune response to tuberculosis infection involves the activation of many immune cells and the production of cytokines such as IL-1β. IL-1β is thought to be crucial for the immune system's reaction to TB. Production of IL-1β from infected lung cells by *M. tuberculosis* draws and activates immune cells including macrophages and T cells to the infection location. Then, these immune cells go to work containing and getting rid of the germs.**

Aim of the study: **To assess the impact of Interleukin 1beta (IL-1β) in Iraqi patients with pulmonary and extrapulmonary tuberculosis.**

Patients and methods: **The patient's group includes seventy-five tuberculosis-infected individuals, While the control group includes twenty-five healthy apparent individuals. The blood and sputum samples were collected from each group. A sputum sample was used for acid-fast stain while blood was used for measurement of IL-1β by ELISA technique according to the construction method of the kit from (Sunlong-Biotech company).**

Results: **The present investigation demonstrated a statistically significant reduction in IL-1β levels (18.841 ± 2.722) among tuberculosis patients as compared to the control group (20.638 ± 1.972), with a P value (P > 0.021). The level of IL-1β P value (P > 0.253) does not significantly differ between pulmonary and extrapulmonary tuberculosis.**

Conclusion: **This study concludes that individuals with low levels of IL-1β may be more susceptible to tuberculosis infection, while the level of this interleukin hasn't a role if the infection is pulmonary or extends to extrapulmonary tuberculosis.**

Keywords: Interleukin 1beta (IL-1β), *Mycobacterium tuberculosis*, Tuberculosis

1. Introduction

The biggest cause of curable illness-related mortality is active tuberculosis (TB), which affects 2 billion people worldwide [1,2]. Although *Mycobacterium tuberculosis* infects one-third of the world's population, just five percent of infected people experience disease progression in the first year of infection and a further five percent do so later in life. It has been established that a variety of host genetic variables play important roles in TB susceptibility through the use of candidate genes and association studies [3].

Dendritic cells, monocytes, and macrophages all produce the crucial proinflammatory cytokine known as IL1β when there is an infection or inflammation. There is evidence that the amount of IL1β protein generated is influenced by IL1β gene variants [4,5]. The immune system's response to mycobacteria is significantly impacted by IL1β, which may also boost resistance to early infection. Human TB has been linked to IL1β polymorphisms [6].

Monocytes and activated macrophages both produce IL6. It is a crucial immunoregulatory factor that can lower IL1β and TNF-alpha production [7].

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According to earlier studies, IL6 levels in TB patients were higher than in healthy controls [8].

Mycobacterium tuberculosis is the bacterium that causes tuberculosis (TB), a bacterial infection. The immune response to tuberculosis infection involves the activation of many immune cells and the production of cytokines such as IL-1β. It is thought that the immune response to tuberculosis (TB) requires IL-1β. When *M. tuberculosis* infects lung cells, IL-1β is released, luring immune cells such as T cells and macrophages to the infection site and stimulating them. Then, these immune cells go to work containing and getting rid of the germs. However, IL-1β during TB infection can also contribute to tissue damage and inflammation. Granulomas, which are structures that grow around *M. tuberculosis* to confine the infection, can occasionally result from excessive IL-1β production. Granulomas are capable of treating TB, but they can also harm the lungs and hasten the onset of TB disease [9]. The primary in vivo cellular sources of IL-1β during a clinical *M. tuberculosis* infection are believed to be innate immune cells. After breathing in *M. tuberculosis*, dendritic cells, monocytes, and inflammatory macrophages all generate increased IL-1 [10]. IL-1β is a well-known cytokine that promotes inflammation and causes both regional and systemic reactions. IL-1β has a complex dual function in long-lasting infections [11]. In the early phases of infection, the production of IL-1β is required for the proper maturation of antimicrobial immunity that is adaptive [12]. Chronic IL-1β production, however, promotes granulocyte retention in the lung, causes the release of additional mediators of inflammation including prostaglandin E2, and activates metalloproteinases that damage tissue [13].

To further understand this relationship, the study will look at the levels of Interleukin 1beta (IL-1β) in patients with pulmonary and extrapulmonary tuberculosis in Iraq.

2. Patients and methods

This case-control study comprised a total of 75 individuals with tuberculosis; these patients ranged in age from 7 to 74 years old and were made up of 36 males and 39 females. The control group includes 25 healthy apparent individuals (11 males, 14 females) whose ages range (from 12 to 60 years).

All the samples of patients were collected from patients conducted at the chest and respiratory diseases center in Ramadi City. All the samples were collected under the supervision of specialized doctors during the period from August 2022 to January 2023.

Sputum and 3 ml of Blood samples were collected from patients and the control group. Sputum samples were used for Acid-fast staining while blood samples were placed in a gell tube and centrifuged to collect serum for measurement of IL-1 β by ELISA technique according to the instruction method of the kit from Sunlong-Biotech company.

The National Center of Tuberculosis, Iraqi Ministry of Health and university of Anbar approval committee gave their approval for this study and all the participant of this study gave informed written consent before process of samples taking.

2.1. Statistical analysis

The data was statistically analyzed using SPSS for Windows, version 26 (SPSS Inc., Chicago, Illinois, United States). The information was shown as a mean and standard deviation (SD). The parameters under study were checked to see if they adhered to a Gaussian distribution using the Shapiro-Wilk normality test.

3. Results

Regarding the 75 tuberculosis patients, there was a statistically significant difference in the ages of the males and females (p-value >0.004). The ages of the 25 male and female members of the control group did not differ significantly (p-value = 0.9), as in Table 1.

In the patients group, the number of patients with pulmonary tuberculosis was 44⁄75 while the patients with extrapulmonary tuberculosis were 31⁄75, It was distributed to both genders as in Table 2.

There is a decrease in the level of interleukin-1 beta in tuberculosis patients **mean + SD** (18.841 ± 2.722), compared to the control group **mean + SD** (20.638 ± 1.972), According to statistics, there was a significant difference ($P < 0.021$) as in Table 3.

Additionally, the *P*-value ($P > 0.253$) indicated that there was no statistically significant difference in interleukin-1 beta levels between patients with pulmonary and extrapulmonary tuberculosis as in Table 4.

4. Discussion

The model multifunctional cytokine is interleukin (IL)-1 (IL-1α and IL-1β). IL-1 impacts almost all cell types, unlike lymphocyte and colony-stimulating factors. Numerous biological effects of IL-1 have been connected to it, such as the development of

Table 1. Patients and controls are divided up based on age and gender.

Age groups	Group of patients (no.75)		Group of Control (no.25)	
	Male	Female	Male	Female
7–17	2 (2.66%)	3 (4%)	0	2 (8%)
18–28	7 (9.3%)	11 (14.6%)	3 (12 %)	3 (12 %)
29–39	4 (5.3%)	12 (16%)	3 (12%)	2 (8%)
40–50	11 (14.6%)	5 (6.6%)	1 (4%)	4 (16%)
51–61	3 (4%)	3 (4%)	2 (8%)	1 (4%)
62–74	11 (14.6%)	3 (4%)	2 (8%)	2 (8%)
mean ± SD	46.62 ± 19.323	33.07 ± 14.691	38.86 ± 14.607	38.67 ± 16.014
Std. Error of Mean	3.71880	2.72822	3.90387	3.77470
Maximum	74.00	72.00	62.00	60.00
Minimum	7.00	12.00	21.00	12.00
p-value	0.004**		0.9 N. S	

Significant

Table 2. Distribution of patients according to the type of tuberculosis for both males and females.

		Gender		Total	Chi-square
		Male	Female		
Type.of.Tb	pulmonary	22	22	44	0.6
	Extra Pulmonary	14	17	31	
Total		36	39	75	

Table 3. Levels of IL-1β in the tuberculosis patients and control group.

Group	Parameter
	IL-1 Beta Pg/ml (mean + SD)
Patient	B 18.841 ± 2.722
Control	A 20.638 ± 1.972
P-Value	**0.021**
Sign.	**Sign.**

Table 4. The levels of IL-1 β in pulmonary and extrapulmonary tuberculosis patients.

Group	Parameter
	IL-1 Beta Pg/ml (mean + SD)
Pulmonary	A 19.103 ± 3.077
Extra pulmonary	A 18.33 ± 2.304
P-Value	**0.253**
Sign.	**Non-Sign.**

fever, hyperlipidemia, neutrophilia, hypotension, and hypoferremia, in addition to others [14].

IL-1β causes human and murine macrophages to directly destroy the Mtb while also increasing the recruitment of antibacterial effector molecules. Caspases-3 are activated by increased expression of TNF-α and tumor necrosis factor receptor 1 (TNFR1) on cell surfaces. The IL1B gene is directly activated by the 25-dihydroxy form of vitamin D1 (1,25D), and this gene is critical for the macrophage's response to *M. tuberculosis* infection. Host resistance to Mtb infection depends on IL-1β [15]. The fact that IL-1β−/− or IL1R−/− animals considerably outlived healthy mice following infection serves as proof. Infants who have been infected have been the subject of studies that have revealed lower levels of IL-1 and the impact on its production capability, demonstrating immunological sensitivity to TB in this population. Several investigations have exhibited a correlation between host resistance and variations in the IL-1 or IL-1 receptor genes, corroborating the notion that IL-1 plays a role in tuberculosis immunity in humans [15].

The cytokines TNF-α and IL-1β increase the expression of endothelial adhesion molecules throughout the inflammatory phase, which makes it easier for more inflammatory cells to aggregate with the active endothelium. Additionally, they stimulate macrophages and/or DC, aiding in the regulation of mycobacterial replication and the direct inhibition of *M. tuberculosis* intracellular growth [16].

The level of IL-1β in this study population of tuberculosis patients was less than the healthy group and this was indicated by a P value (0.02). This result was not consistent with [17,18] who found high levels of IL-1β in tuberculosis patients, while consistent with Prashant Mishra et al. [20].

Low levels of 25-dihydroxy vitamin D (1,25D), a form of vitamin D that directly activates the IL1B gene and is essential for macrophage defense against *M. tuberculosis* infection, maybe the root cause of these individuals' low levels of this interleukin. The NOD-like receptor 3 (NLRP3) inflammasome is necessary for pro-IL-1β maturation. The second stage needed to produce IL-1b is

the conversion of pro-IL-1b into active IL-1b. The normal processing of pro-IL-1b in bone marrow-derived macrophages infected with *M. tuberculosis* in vitro was related to the inflammasome NLRP3 and its apoptosis-associated speck-like proteins, which include a CARD (ASC) and CASP1 component [23,24] and dendritic cells produced from bone marrow [23,25]. It has also been demonstrated that M. tuberculosis-infected macrophages and monocytes absent in melanoma 2 (AIM2) inflammasome contribute to the in vitro generation of IL-1b [25–27]. In contrast, the situation in vivo is less clear. Mice lacking AIM2 or ASC, however, displayed a higher vulnerability to *M. tuberculosis* infection [26], When comparing the sensitivity and production of IL-1β in mice lacking CASP1 or NLRP3 to those of wild-type mice, no differences were observed [28]. According to our unpublished results in the same patient population, this may be associated with variation in the aforementioned genes. Mycobacterial proliferation in the macrophage is controlled by IL-1β and 1,25D in combination. Patients who have active TB often have 1,25D deficiency. In general, this vitamin enhances cytokine/chemokine responses that are triggered by infections and expands its function in human innate immune control. Vitamin D insufficiency and TB susceptibility are related, as has been recognized by researchers [19]. A functional issue in monocytes following activation with TLR ligands has been shown by other research that shows the production of cytokines defect, which was demonstrated by reduced IL-1β frequency in the cases compared to the controls, remained constant throughout several TLR stimulations [21].

This study shows no significant differences between pulmonary and extra-pulmonary tuberculosis in the level of IL-1β, this result was consistent with Nathella Pavan Kumar et al. [22]. These results may be due to that the number of extra-pulmonary tuberculosis was less than pulmonary tuberculosis patients.

4.1. Conclusion

This study concludes that individuals with low levels of IL-1β may be more susceptible to tuberculosis infection, while the level of this interleukin hasn't a role if the infection is pulmonary or extends to extrapulmonary tuberculosis.

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