Comparison of Interleukin 17A and Interleukin-18 Cytokines During Active and Latent TB Infection in Iraqi Patients

Sarah Kassab Shandaway Al-Zamali[1], Jawad Kadhim Tarrad AL-Khafaji[2], Ahmed Asmar Mankhi[3]

[1] College of Nursing, Department of Medical Microbiology, Telafer University, Telafer, Iraq.
[2] College of Medicine, Department of Microbiology, Babylon University, Babylon, Iraq.
[3] National Tuberculosis Institute (NTI) / National Reference Laboratory (NRL) for Tuberculosis in Baghdad, Ministry of Public Health, Baghdad, Iraq.
*Corresponding author: sarah.alzamali85@gmail.com
E-mail: dr.jawad66@yahoo.com

Abstract

Introduction: Despite international control programs, tuberculosis remains a public health issue. People with latent TB infection (LTBI) significantly increase the number of active tuberculosis (TB) cases and carry a lifelong risk of developing the disease. Therefore, the present study aims to determine the changes in cytokine production at two phases during the development of active pulmonary and latent tuberculosis infection and to evaluate their role as predictive markers in active and latent infections.Method: Blood specimens were collected from 60 patients with active pulmonary TB, 60 cases with latent TB infection and 40 healthy controls to obtain serum. ELISA kit for IL-17A and IL18 was used to determine the concentrations of IL-17A and IL18 according to the manufacturer's instructions (Elabscience / China).Result: The results of the current study found that the mean serum concentration of interleukin-18 was significantly higher in cases with Active pulmonary tuberculosis compared to cases with latent TB infection and healthy control, respectively (P<0.001), also the mean serum concentration of IL-18 was significantly higher in subjects with latent TB infection as compared to healthy control (P<0.001). Also, the present study found that the mean serum concentration of IL-17A showed an insignificant variation in cases with Active pulmonary TB compared to healthy control (P< 0.069). In contrast, the mean serum concentration of IL-17A was significantly higher in subjects with latent TB infection as compared to healthy control(P<0.002) and Active pulmonary TB (P<0.001)Conclusion: A comparison of latent and active tuberculosis cases may provide some insight into factors that shield them from disease development, as well as new insights into the roles of interleukin -17A and interleukin -18 at two critical stages of the M. tuberculosis infection. These findings suggest that IL-17A and IL18 play distinct roles in two phases of tuberculosis infection and can potentially be used to develop novel diagnostics. The IL-18 ELISA results revealed a highly significant difference between the three groups. All of this information allows to distinguish TB patients and LTBI from healthy controls. Furthermore, the current findings indicated that IL-17A could be alternative biomarker for LTBI diagnosis.

Keyword: Interleukin 17A, Interleukin 18, ELISA, Active TB, Latent TB

1. Introduction

Despite international control programs, tuberculosis remains a public health issue. According to the World Health Organization, approximately 10 million cases occur worldwide, with about 2-3 million deaths yearly. In 2020, it was estimated that nearly one-quarter of the world's population was infected with Mycobacterium tuberculosis (Mtb), but only 5-15 % of those people with latent TB infection would develop the disease in the near or distant future (a process known as "TB reactivation") [1] Latent tuberculosis infection (LTBI) is a condition in which the host immune system continues to respond to Mtb antigen stimulation in the absence of clinically manifested active TB. TST or interferon-release assays (IGRA) can diagnose latent tuberculosis (LTBI). In contrast, clinically suspected TB disease is evaluated using a chest radiograph, diagnostic microbiology for acid-fast bacilli, culture, and the GeneXpert assay [2]

In the case of tuberculosis infection, the cellular immune response is essential. Th17 cells are critically important in the fight against Mycobacterium tuberculosis (Berringer et al., 2016). IL-17 is a key cytokine involved in acquired immunity, and IL17A is primarily secreted by TH17 cells. It can increase the levels of chemokines such as CXCL9, 10, and 11, which attract interferon-producing cells to the site of inflammation [3], the interleukin 17 cytokine is a proinflammatory cytokine that primarily acts as a mediator of resistance to extracellular bacteria and fungi. However, these cytokines have also been linked to host immunity to tuberculosis and the production of autoimmune and inflammatory disorders. IL-17A plays a role in the formation and maintenance of granulomas by stimulating the production of chemokines, which in turn assists in the

doi.org/10.31838/hiv23.02.3 Received: 16.10.22, Revised: 21.11.22, Accepted: 12.12.22

recruitment of inflammatory cells that are migrating to Mtb-infected sites

Interleukin-18 has been shown to play a significant part in M.tb infection. It stimulates Natural killer cell cytotoxicity and the development of Th1 cell responses. This mechanism is connected with the production of interferon (IFN)-γ, which is an essential component in protecting against mycobacteria Interleukin-18 was first identified as a factor capable of inducing robust production of interferon-γ from Th1 cells, particularly in the presence of IL-12. [4] found that when naive T cells stimulate with antigen and IL-12, they develop into Th1 cells that express the IL-18 receptor (IL-18R). These cells increase their production of IFN-γ in response to IL-18 stimulation. The present study aims to determine the changes in cytokine production at two phases during the development of active and latent tuberculosis infection and to evaluate their role as predictive markers in active and latent infections.

2. Materials and Methods

2.1. Ethical approval and consent

All subjects involved in this work were informed, and the agreement was obtained verbally from each one before collecting samples. The committee approved this study of publication ethics at the college of medicine, Babylon university, Iraq.

2.2. Study design and settings

The current study was designed as a case-control study and this study was performed from February 2021 – to September 2021 at the National Tuberculosis Institute (NTI) / National Reference Laboratory (NRL) for Tuberculosis in Baghdad. A total of 160 subjects were enrolled in this study; All participants were classified into three groups: 60 patients with active TB (ATB) were included (39 males and 21 females) with ages ranging from 16 -68 years and diagnosed by the specialist doctors who were based on clinical symptoms and signs, chest radiography (CXR) suggestive of tubercle bacilli (TB) and a positive results from at least one form of microbiological evidence; AFB smear microscopy, MTB culture or from Xpert MTB/RIF. At the time of recruitment, 60 subjects who tested positive for QFT-Plus were considered to be latent tuberculosis consisting of (37 males and 23 females) with ages ranging from 18 – 66 years. The diagnosis of latent tuberculosis was made based on a positive QFT-Plus test and the absence of any microbiological, clinical, and radiological characteristics that would indicate active tuberculosis. A 40 healthy control individual was asymptomatic with a normal chest x-ray, and a QFT- Plus negative result was included in this study. Healthy controls (HC) showed no evidence of TB exposure (individuals with no TB infection or disease), including 22 males and 18 females with ages ranging from 15 to 70 years.

2.3. Inclusion& Exclusion criteria

The inclusion criteria in the present study included all active pulmonary tuberculosis cases with no record of prior anti-TB treatment (ATT) and newly diagnosed patients. The exclusion criteria included patients with autoimmune diseases, diabetes mellitus, nephropathy, Follow up patients and extrapulmonary TB. Subjects had close contact for TB with an indeterminate IGRA result or with IGRA negative. Also, the subjects who refused to participate in this study.

2.4. Serum specimens' collection

A 3 ml of blood was drawn from each subject via vein puncture, placed in gel tubes and allowed to clot at room temperature for 30 minutes, then centrifuged at 3000g for ten minutes to collect the serum. The serum specimens were kept at -20 C° until the ELISA assay was performed.

IL17A and IL18 Cytokines measurement

Assessment of Human IL-17A and IL18 serum levels by ELISA assay. This was done by the method recommended by the manufacturer company (Elabscience /China).

2.5. Statistical analysis

The statistical package for social sciences (SPSS) version 23 was used to analyze and present the data. After performing the Kolmogorov-Smirnov normality test and making decisions about normally and non-normally distributed variables, numerical data were presented as mean and standard deviation. The one-way ANOVA test was used to investigate the difference in mean between more than two groups when the variable was normally distributed. A P-value of less than 0.05 was used to determine significance.

3. Results and Discussion

Measurement of Serum IL-18 level in studied groups by ELISA

The present study enrolled 60 cases with active pulmonary TB (ATB) and latent TB infection (LTBI) subjects, 40 healthy controls (HC). Table 1 shows the demographic characteristics of the studied groups.

Table (1): Demographic characteristics of TB patients, LTBI cases, and healthy controls in this study.

	Number Gender			Age (years)	
Group	Total	Male (%)	Female (%)	Mean ± SD	Range
HC	40	22(55.0%)	18 (45.0 %)	38.46 ±14.59	15- 70
ATB	60	39 (65.0%)	21 (35.0%)	36.16 ±14.16	16 - 68
LTBI	60	37 (61.7 %)	23(38.3 %)	42.45±13.62	18 – 66

Abbreviations: HC, healthy controls; LTBI, latent TB infection; ATB, Active pulmonary TB.

The present study found that the mean serum concentration of IL-18 was highly significantly higher in cases with Active pulmonary TB 412.74 ± 74.54 pg /ml compared to cases with latent tuberculosis and healthy control, respectively, 346.39 ± 70.55 pg/ml and 269.02± 55.14 pg /ml (p<0.001) as shown in the figure (1) and the table (2), table (4), also the mean serum concentration of IL-18 were significantly higher than in cases with latent TB infection 346.39 ± 70.55 pg/ml as compared to healthy control 269.02± 55.14 pg/ml, (p<0.001) as shown in the figure (1) and table (5).

Table (2): Comparison of Serum levels of *Interleukin-18* in Active TB patients and healthy controls.			
Cases control comparison			P- value
IL-18 pg/ml	Active pulmonary TB N= 60	Healthy controls N=40	
Mean	412.74±74.54	269.02±55.14	< 0.001 † HS
SD	74.54	55.14	
Range	302.55- 710.75	191.0–358.39	
N: number of cases; SD: standard deviation; †: one way anova; ; HS: highly significant at P≤ 0.001.			

Table (3): Comparison of Serum levels of *Interleukin-18* in LTBI cases and healthy controls.			
Cases control comparison			P value
IL-18 pg/ml	Latent TB infection N= 60	Healthy controls N=40	
Mean	346.39±70.55	269.02±55.14	< 0.001 † HS
SD	70.55	55.14	
Range	206.04 – 482.9	191.0–358.39	
N: number of cases; SD: standard deviation; †: one-way anova; HS: highly significant at P≤ 0.001.			

Table (4): Comparison of Serum levels of Interleukin-18 in Active TB patient and latent TB infection cases .			
Cases control comparison			*P value*
IL-18 *pg/ml*	*Active pulmonary TB* N= 60	*Latent TB infection* N= 60	
Mean	412.74±74.54	346.39±70.55	< 0.001 † HS
SD	74.54	70.55	
Range	302.55- 710.75	206.04 – 482.9	
N: number of cases; SD: standard deviation; †: one way anova; HS: highly significant at P≤ 0.001.			

Measurement of Serum IL-17A level in studied groups by ELISA

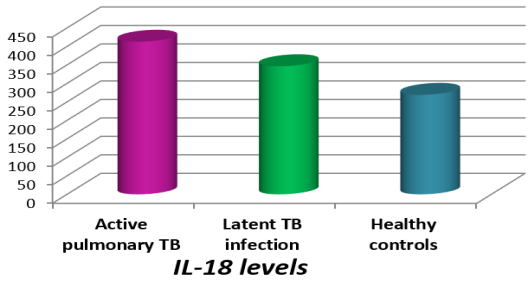

Figure (1): Distribution of Active pulmonary TB, Latent TB infection cases and healthy controls groups according to the mean level of serum IL-18.

The present study found that the mean serum concentration of IL-17A showed an insignificant variation in cases with Active pulmonary TB 107.95±41.18 pg/ml as compared to healthy control 122.14 ±39.80pg/ml, (p=0.069) as shown in the table (5) , while the mean serum concentration of IL-17A was significantly higher in cases with latent TB infection 147.14±43.99 pg/ml as compared to healthy control 122.14 ±39.80 pg/ml, (p=0.002) shown in the table (6). Also, the mean concentration level of IL-17A was significantly higher with latent TB infection 147.14±43.99 pg/ml, (p<0.001) as compared to Active pulmonary TB 107.95±41.18 pg/ml as shown in the table (7) and figure (2).

Table (5): Comparison of Serum levels of Interleukin-17A in Active TB Patient and healthy controls.			
Cases control comparison			P value
IL-17A *pg/ml*	*Active pulmonary TB N=60*	Healthy controlN=40	
Mean	107.95	122.14	0.069† NS
SD	41.18	39.80	
Range	51.76- 202.46	60.30-215.64	
SD: standard deviation; †: one-way ANOVA; HS: highly significant at P ≤ 0.001, NS: not significant at P ≥ 0.05.			

Table (6): Comparison of Serum levels of Interleukin-17A in LTBI cases and healthy controls			
Cases control comparison			P value
IL-17A pg/ml	Latent TB infection N= 60	Healthy controls N=40	
Mean	147.14	122.14	0.002† S
SD	43.99	39.80	
Range	90.59-300.38	60.30-215.64	
SD: standard deviation; †: one-way ANOVA; HS: highly significant at P ≤ 0.001 , NS: not significant at P ≥ 0.05.			

Table (7): Comparison of Serum levels of Interleukin-17A in Active pulmonary TB and LTBI cases			
Cases control comparison			*P value*
IL-17A *pg/ml*	*Latent TB infection* N= 60	*Active pulmonary TB* N= 60	
Mean	147.14	107.95	< 0.001 † HS
SD	43.99	41.18	
Range	90.59-300.38	51.76- 202.46	
N: number of cases; SD: standard deviation; †: one-way anova; HS: highly significant at P≤ 0.001.			

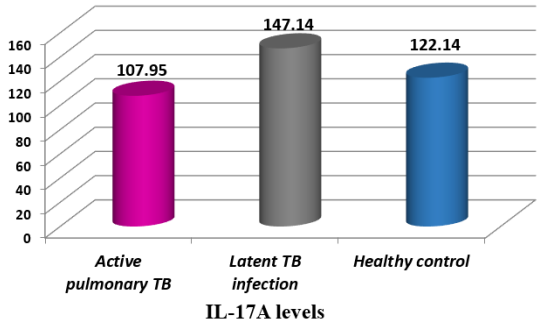

Figure (2): Distribution of Active pulmonary TB, Latent TB infection cases and healthy controls groups according to the mean level of Serum IL-17A.

4. Discussion

The present study found that the mean serum concentration of IL-17A showed an insignificant variation in cases with Active pulmonary TB compared to healthy control. This result is consistent with a local study by 5 who showed an insignificant variation between PTB patients and controls in the serum level of IL17A.

The present findings showed that IL-17A levels were increased in latent tuberculosis cases compared to healthy controls and active pulmonary tuberculosis. This was compatible with 6. when comparing LTBI groups vs. Active TB., The results of the current study were consistent with a study in 4 and Iran6 Coulter et al. (2017) reported that the main source of interleukin-17 was CD26+ Th17 cells in latent and active tuberculosis patients, but the levels of interleukin-17 were significantly lower than in latent tuberculosis. In Argentina, 7 documented that CD4 + T cells from active TB produce less IL-17 in response to Mycobacterium antigens than CD4 + T cells from healthy tuberculin reactors. 8 displayed that the frequency of Th17 cells in the blood and pleural fluid of active tuberculosis patient was lower than in cases with latent tuberculosis.

Interestingly, 6 reported that Th1 and Th17 were at significantly lower levels in active TB pretreatment compared to latent TB infection; the results of the current study are supported by the results of previous studies, which showed that IL-17 is increased during early Mtb infection before disease progression. In contrast with the present results, a local study by 9 and other studies by 10 They reported that IL-17A was significantly increased in active TB in comparison healthy group. There were some discrepancies between the results of this study and previous reports may be due to several factors, including Variable immunity of experimental subjects, subjects may be sampled before, after, or during treatment, and the choice of practical methods can also affect results 9.

Subjects with latent TB infection and active TB react differently to mycobacterium Individuals with latent tuberculosis infection (LTB) and active tuberculosis (TB) have CD4 T cells that produce IL-17, which recognize different antigens at different stages of the disease. This is one of the possible explanations for this matter 4 It has also been demonstrated that the depletion of regulatory T cells in LTB individuals results in an increase in the production of IL-17, which suggests that regulatory T cells might be involved in the differences in IL-17 that are observed between active and LTB subjects. Therefore, the stage of the disease, the number of regulatory T cells, and the production of CTLA-4 and FOXp3 may all contribute to the variations in the levels of IL-17 found in different studies involving Mtb 6 Differences in Mtb environmental exposure in Iraq should also be considered.

IL-17A is a cytokine that protects the host from getting an infection by mycobacteria; suppressing IL-17A production will increase TB susceptibility 11 reported that there is a possibility that a genetic variant causes the down-regulated of IL-17A expression and represents an increased risk of M.tb infection.

The findings of the current study suggest that interleukin-17A (IL-17A) could be an immunological marker that has the potential to help identify subjects who are latently infected with tuberculosis (TB), which is consistent with Teklu et al. (2018). IL-17A has been shown to play a protective role during the early stages of Mtb infection, according to several reports. This role includes contributing to the recruitment of neutrophils and IFN- secreting cells to the site of infection, which is necessary to establish an efficient memory response 12 These findings imply that IL-17A plays distinct roles at two different stages of the TB infection spectrum. These roles can be utilized in the development of novel diagnostics and treatments.

IL-18 is one of the IL-1 family members and one of the cytokines that have been implicated in both protective and pathological processes associated with M.tb infection , which is produced by a variety of immune cells including monocytes, macrophages, dendritic cells, epithelial cells, keratinocytes, synovial fibroblasts, and T and B lymphocytes 13 IL-18 is regarded as a member of the family of Th1 cytokines because of its capacity to stimulate IFN- in T cells and natural killer cells 14

Interleukin -18 is one of the IL-1 family members and

produced by a variety of immune cells, including monocytes, macrophages, dendritic cells, epithelial cells, keratinocytes, synovial fibroblasts, and T and B lymphocytes. It has been linked to both protective and pathological processes associated with mycobacterium infection 7. Because it can induce IFN-γ in T cells and natural killer cells, IL-18 is regarded as a member of the Th1 cytokine family 8

The present study found that the mean serum concentration of IL-18 was significantly higher in cases with Active pulmonary TB compared to cases with latent TB infection and healthy control, respectively (P<0.001). Also, the mean serum concentration of IL-18 was significantly higher in subjects with latent TB infection compared to healthy control (P<0.001). In line with the present findings, A recent study in China by 6 reported that IL-18 was remarkably upregulated in TB patients compared to healthy controls. In addition, IL-18 levels in LTBI were first measured, and it was discovered that these were significantly higher in the LTBI group than in the control group, while these levels were lower than those in the TB group. In Poland, a study by Wawrocki et al. (2019) found that ATB patients' serum levels of IL-18 were significantly higher than those of LTBI or healthy controls. This finding suggests that there has been a significant loss of balance in the range of the IL-18 signalling complex in ATB. The current study's findings were partially consistent with those of 5 They found that LTB subjects had a significantly higher relative level of IL-18 mRNA expression than healthy controls without M.tb infection. The difference in values between the ATB and Control groups was not statistically significant.

Interleukin-18 is a proinflammatory cytokine that is essential in the inflammatory cascade. The production of IL-18 in response to mycobacterial antigens (Ags) is strongly linked to the production of IFN- and mycobacterial defensive immunity (Lee et al.,2011). However, IL-18 levels were elevated in patients with active pulmonary TB (ATB), suggesting that IL-18 may play a role in both protective immunity and human TB pathological responses 10 The role of IL-18 in the pathogenesis of many diseases, including cancer and digestive disorders, has been extensively researched 15

M. tuberculosis may use the IL-18 signalling complex to expand the clinical manifestation of pulmonary TB, according to 12 As a result, direct analysis of serum components of the IL-18/IL-37 signalling complex could help develop novel rapid screening tests for pulmonary tuberculosis.

16 confirmed the role of IL-18 in the development of the immune response against mycobacteria by observing an increased level of IL-18 in a group of patients with active pulmonary TB. Furthermore, IL-18 is an important mediator of macrophage activation in the control of M.tb. Under certain conditions, IL-18 is required for the production of IFN-γ from T cells, and its absence can result in increased susceptibility to Mtb. Indeed, 14 discovered a significant positive correlation between serum levels of IL-18 and IFN-γ. Interleukin -18 exhibits a similar tendency to IFN-γ, which has been recognized for a long time as one of the markers that can be used to evaluate the state of tuberculosis infection. In this content, in tuberculosis pleurisy, parallel concentrations of IL-18 and IFN-γ were detected.

5. Conclusion

A comparison of latent and active tuberculosis cases may provide some insight into factors that shield them from disease development, as well as new insights into the roles of interleukin -17A and interleukin -18 at two critical stages of the M. tuberculosis infection. These findings suggest that IL-17A and IL18 play distinct roles in two phases of tuberculosis infection and can potentially be used to develop novel diagnostics. The IL-18 ELISA results revealed a highly significant difference between the three groups. All of this information allows to distinguish TB patients and LTBI from healthy controls. Furthermore, the current findings indicated that IL-17A could be alternative biomarker for LTBI diagnosis.

References

Akitsu, A., & Iwakura, Y. Interleukin-17-producing γδ T (γδ17) cells in inflammatory diseases. Immunology,2018. 155(4), 418-426.

Shen, H., & Chen, Z. W. The crucial roles of Th17-related cytokines/signal pathways in M. tuberculosis infection. Cellular & molecular immunology,2018. 15(3), 216-225.

Matsuzaki, G., & Umemura, M. Interleukin-17 family cytokines in protective immunity against infections: role of hematopoietic cell-derived and non-hematopoietic cell-derived interleukin-17s. Microbiology and immunology,2018. 62(1), 1-13.

Jolink, H., de Boer, R., Hombrink, P., Jonkers, R. E., van Dissel, J. T., Falkenburg, J. F., & Heemskerk, M. H. Pulmonary immune responses against Aspergillus fumigatus are characterized by high frequencies of IL-17 producing T-cells. Journal of Infection,2017. 74(1), 81-88.

Beringer, A., Noack, M., & Miossec, P. (2016). IL-17 in chronic inflammation: from discovery to targeting. Trends in molecular medicine, 22(3), 230-241.

Coulter, F., Parrish, A., Manning, D., Kampmann, B., Mendy, J., Garand, M., ... & Sutherland, J. S. IL-17 production from T helper 17, mucosal-associated invariant T, and γδ cells in tuberculosis infection and disease. Frontiers in immunology, 2017.8, 1252.https://doi.org/10.3389/fimmu.2017.01252

Vecchié, A., Bonaventura, A., Toldo, S., Dagna, L., Dinarello, C. A., & Abbate, A.. IL-18 and infections: Is there a role for targeted therapies? Journal of Cellular Physiology,2021. 236(3), 1638-1657.

Wawrocki, S., Druszczynska, M., Kowalewicz-Kulbat,

M., & Rudnicka, W.Interleukin 18 (IL-18) as a target for immune intervention. Acta Biochimica Polonica,2016. 63(1), 59-63.

World Health Organization. Global tuberculosis report 2020. Geneva: World Health Organization;2020.

Kumar, N. P., Hissar, S., Thiruvengadam, K., Banurekha, V. V., Suresh, N., Shankar, J., ... & Babu, S. Discovery and Validation of a Three-Cytokine Plasma Signature as a Biomarker for Diagnosis of Pediatric Tuberculosis. Frontiers in immunology,2021 12, 1143. DOI=10.3389/fimmu.2021.653898

Akgun, M., Saglam, L., Kaynar, H., Yildirim, A. K., Mirici, A., Gorguner, M., ... & Ozden, K. . Serum IL-18 levels in tuberculosis: comparison with pneumonia, lung cancer and healthy controls. Respirology,2005. 10(3), 295-29

Akitsu, A., & Iwakura, Y. Interleukin-17-producing γδ T (γδ17) cells in inflammatory diseases. Immunology, 2018.155(4), 418-426.

Elarab, A. & Garrad, H. (2012). Serum level of interferon gamma (inf-ɣ), il-12, and il-18 in active pulmonary tuberculosis. AAMJ; 10 (3): 21-33.

Etna, M. P., Giacomini, E., Severa, M., & Coccia, E. M. Pro-and anti-inflammatory cytokines in tuberculosis: a two-edged sword in TB pathogenesis. In Seminars in immunology ,2014. 26(6), 543-551.

Hafid, E. S., & Ismael, M. K. Detection of Serum Levels of IL-17 and CCL-5 in a Sample of Iraqi Pulmonary Tuberculosis Patients. Iraqi Journal of Science,2021. 2887-2893.

He, J., Fan, Y., Shen, D., Yu, M., Shi, L., Ding, S., & Li, L. Characterization of cytokine profile to distinguish latent tuberculosis from active tuberculosis and healthy controls. Cytokine,2020. 135, 155218. https://doi.org/10.1016/j.cyto.2020.155218.