Journal of Animal Environment

(E-ISSN 2717-1396, ISSN 2717-1388)

Original Research Paper

Evaluation the role of CD 4, CD8, CD56 and interleukin - 2 in pulmonary TB patients before and after treatment in waist province

Abbas Mohammed Jubair[1] , Haethem Qassim Mohammed[2]

Std2024201.AbbasmjAlmyahi@uowasit.edu.iq[1]
hmohammed@uowasit.edu.iq2

[1,2] Wasit University, College of Medicine Department of Medical Microbiology

Abstract

Background: Tuberculosis (TB) remains one of the leading infectious diseases worldwide despite the availability of effective chemotherapy. Host immune responses, particularly cell-mediated immunity, play a critical role in controlling *Mycobacterium tuberculosis* infection. Cellular immune biomarkers such as $CD4^+$ T lymphocytes, $CD8^+$ cytotoxic T cells, $CD56^+$ natural killer (NK) cells, and the cytokine interleukin-2 (IL-2) have attracted considerable attention because of their potential value in monitoring disease progression and treatment response.

Objective: This study aimed to evaluate the immunological changes in $CD4^+$, $CD8^+$, $CD56^+$ lymphocyte subsets and serum IL-2 concentrations among patients with pulmonary tuberculosis before and after anti-tuberculosis treatment in Wasit Province, Iraq.

Methods: A comparative case-control study was conducted involving pulmonary tuberculosis patients and healthy controls. Peripheral blood samples were collected before treatment and during anti-tuberculosis therapy. Flow cytometry was used to quantify $CD4^+$, $CD8^+$, and $CD56^+$ lymphocyte populations, whereas serum IL-2 concentrations were determined using enzyme-linked immunosorbent assay (ELISA). Statistical analyses were performed to compare immunological parameters among the study groups, and correlations between immune markers and treatment status were evaluated.

Results: Patients with newly diagnosed pulmonary tuberculosis demonstrated significant alterations in cellular immune markers compared with healthy controls. $CD4^+$ T-cell counts and serum IL-2 concentrations were markedly reduced before treatment, whereas gradual recovery was observed following anti-tuberculosis therapy. $CD8^+$ T-cell and $CD56^+$ NK-cell profiles also exhibited significant changes during treatment, suggesting restoration of cellular immune function. These findings indicate that successful chemotherapy is associated with progressive normalization of host immune responses.

Conclusion: $CD4^+$, $CD8^+$, $CD56^+$ lymphocyte subsets and IL-2 represent valuable immunological biomarkers for evaluating immune status and monitoring treatment response in pulmonary tuberculosis patients. Their combined assessment may improve clinical follow-up and provide additional prognostic information beyond conventional microbiological investigations.

Keywords: Pulmonary tuberculosis; CD4; CD8; CD56; Interleukin-2; Flow cytometry; ELISA; Cellular immunity; Biomarkers; Iraq.

INTRODUCTION

Tuberculosis (TB) remains one of the leading causes of morbidity and mortality due to infectious diseases worldwide. Despite substantial advances in diagnosis, vaccination, and chemotherapy, TB continues to pose a major public health challenge, particularly in low- and middle-income countries (WHO, 2024).

Pulmonary tuberculosis, caused by *Mycobacterium tuberculosis* (Mtb), accounts for approximately 80–85% of all TB cases and represents the primary source of disease transmission Ferlita (Ferlita *et al*, 2019).

Among adaptive immune cells, $CD4^+$ T-helper lymphocytes play a pivotal role in orchestrating protective immunity against TB. After activation by antigen-presenting cells, $CD4^+$ T cells differentiate into functional subsets, particularly Th1 cells, which secrete interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and interleukin-2 (IL-2). These cytokines activate infected macrophages, enhance intracellular killing of *M. tuberculosis*, and contribute to the maintenance of granuloma architecture (Fang *et al*, 2022).

***Corresponding Author:**

Received: 25th May, 2026; Revised: 6th June, 2026; Accepted: 8th June, 2026; Available Online: 04rd August, 2026

(DOI): 10.70102/AEJ.2026.18.2s.55

In addition to helper T cells, $CD8^+$ cytotoxic T lymphocytes are increasingly recognized as critical contributors to anti-tuberculosis immunity. These cells recognize infected macrophages through major histocompatibility complex class I (MHC-I) molecules and eliminate infected cells via perforin- and granzyme-mediated cytotoxicity (Flores-Gonzalez *et al*, 2024).

Natural killer (NK) cells, identified by the surface marker CD56, constitute an essential component of the innate immune response against intracellular pathogens, including *Mycobacterium tuberculosis* (Quatrini *et al*, 2021).

Several investigations have demonstrated that NK-cell populations undergo substantial alterations during tuberculosis infection. Patients with active pulmonary TB frequently exhibit reduced frequencies of circulating $CD56^+$ NK cells compared with healthy individuals or subjects with latent tuberculosis infection (Han *et al*, 2025).

Interleukin-2 (IL-2) is a multifunctional cytokine predominantly secreted by activated $CD4^+$ T-helper lymphocytes following antigen recognition. IL-2 acts as an autocrine and paracrine growth factor that promotes proliferation, differentiation (Clifford *et al*, 2019).

Aim of the Study

The present study aimed to evaluate the immunological changes in $CD4^+$, $CD8^+$, and $CD56^+$ lymphocyte subsets together with serum interleukin-2 concentrations among patients with pulmonary tuberculosis before treatment, after two weeks of anti-tuberculosis therapy, and after two months of treatment in Wasit Province, Iraq. In addition, the study sought to determine whether these immune markers could serve as potential biomarkers for monitoring treatment response and immune recovery.

MATERIALS AND METHODS

Study Design and Participants

This comparative case-control study was conducted between November 2025 and May 2026 in Wasit Province, Iraq. The study was designed to investigate alterations in selected cellular immune markers among patients with pulmonary tuberculosis before and during anti-tuberculosis treatment.

A total of 160 participants was enrolled and classified into four equal groups (n = 40 per group):

Group I: Healthy control subjects without any clinical or laboratory evidence of tuberculosis.

Group II: Newly diagnosed pulmonary tuberculosis patients before initiation of anti-tuberculosis therapy.

Group III: Pulmonary tuberculosis patients after two weeks of standard anti-tuberculosis treatment.

Group IV: Pulmonary tuberculosis patients after two months of treatment.

The diagnosis of pulmonary tuberculosis was established by chest physicians based on clinical manifestations together with positive sputum smear microscopy for acid-fast bacilli (AFB) and confirmation using the GeneXpert MTB/RIF assay. Participants were recruited from the Chest and Respiratory Diseases Consultation Center in Wasit Province.

The age of participants ranged from 18 to 53 years, and both males and females were included in the study.

Inclusion and Exclusion Criteria

Patients with newly confirmed pulmonary tuberculosis who agreed to participate were included in the study.

Individuals were excluded if they had one or more of the following conditions: Human immunodeficiency virus (HIV) infection, Diabetes mellitus, Autoimmune disorders, Malignancy, Chronic inflammatory diseases, Pregnancy, Extrapulmonary tuberculosis, Current smoking, Obesity, Immunosuppressive therapy

Ethical Approval

The study protocol was approved by the Ethical Committee of the Ministry of Health and the Ministry of Higher Education and Scientific Research, Iraq. Written informed consent was obtained from all participants before enrollment, and all procedures were performed in accordance with the ethical principles outlined in the Declaration of Helsinki.

Blood Sample Collection

Approximately 5 mL of peripheral venous blood was collected aseptically from each participant.

Each blood sample was divided into two portions:

2 mL was transferred into EDTA tubes for immunophenotyping by flow cytometry.

3 mL was collected into gel tubes, allowed to clot, centrifuged, and serum was separated for IL-2 analysis using ELISA.

Separated serum samples were aliquoted into sterile microtubes and stored at −20°C until laboratory analysis.

Immunophenotyping by Flow Cytometry

Determination of Serum Interleukin-2

Serum concentrations of interleukin-2 (IL-2) were measured using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, China).

RESULTE

Demographic Characteristics

A total of 160 participants were enrolled in the present study and equally distributed into four groups: healthy controls (n = 40), newly diagnosed pulmonary tuberculosis patients before treatment (n = 40), patients after two weeks of anti-tuberculosis therapy (n = 40), and patients after two months of treatment (n = 40). No statistically significant differences were observed regarding age or sex distribution among the study groups, indicating that the enrolled populations were comparable for subsequent immunological analyses (Tables 1,2).

Changes in Cellular Immune Markers During Anti-Tuberculosis Treatment

Marked alterations in cellular immune markers were observed throughout the different stages of tuberculosis treatment (Table 3). Overall, statistically significant differences were detected among the four study groups for CD4, CD8, CD56, and IL-2 (all $P < 0.001$).

CD4 T Lymphocytes

$CD4^+$ T-cell percentages increased progressively during treatment. Healthy controls exhibited the lowest mean CD4 level (42.92 ± 10.67%), whereas newly diagnosed patients showed higher values (52.29 ± 10.52%). Following initiation of anti-tuberculosis therapy, CD4 levels continued to rise to 55.93 ± 10.10% after two weeks and reached the highest level after two months (62.00 ± 12.55%). This gradual increase indicates progressive restoration of helper T-cell immunity during successful chemotherapy (Table 3).

CD8 T Lymphocytes

Unlike CD4 cells, $CD8^+$ cytotoxic T lymphocytes demonstrated a biphasic response during treatment. Mean CD8 expression increased markedly from 47.76 ± 12.38% before treatment to 58.33 ± 13.44% after two weeks. However, a moderate decline was observed after two months (51.62 ± 12.83%), although values remained higher than those recorded in healthy controls (33.23 ± 15.02%). These findings suggest an early activation of cytotoxic immunity followed by gradual normalization during treatment (Table 3).

CD56 Natural Killer Cells

Natural killer (NK) cells expressing CD56 also exhibited dynamic changes throughout treatment. Baseline CD56 values increased substantially during the first two weeks of therapy (50.50 ± 14.32%), followed by a significant reduction after two months (38.47 ± 12.68%). Despite this decline, CD56 expression remained above control values, suggesting partial recovery of innate immune activity after bacterial clearance (Table 3).

Serum Interleukin-2

Serum IL-2 concentrations changed significantly during treatment. Patients before treatment exhibited a mean IL-2 concentration of 208.18 ± 20.56 pg/mL, which increased markedly after two weeks of therapy (236.48 ± 21.61 pg/mL). Following two months of treatment, IL-2 levels declined significantly to 196.97 ± 25.67 pg/mL, approaching values observed in healthy controls (198.03 ± 21.90 pg/mL). These findings indicate that IL-2 production is strongly associated with early immune activation during anti-tuberculosis therapy (Table 3).

After 2 months	21 (52.5%)	19 (47.5%)	40 (100.0%)	
Total	**84 (52.5%)**	**76 (47.5%)**	**160 (100.0%)**	

Table 2: Frequencies and percentages of sample distribution across study groups by age category (N = 160)

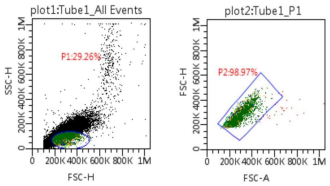
Group	≤35 years Count (%)	> 35 Count (%)	Total Count (%)	p-value	Mean ±SD	p-value

Table 1: Frequencies and percentages of sample distribution across study groups by sex (N = 160)

Group	Male Count (%)	Female Count (%)	Total Count (%)	p-value
Control	20 (50.0%)	20 (50.0%)	40 (100.0%)	0.978
Before	21 (52.5%)	19 (47.5%)	40 (100.0%)	
After 2 weeks	22 (55.0%)	18 (45.0%)	40 (100.0%)	

Markers	Control(n=40)	Before(n=40)	After 2 weeks(n=40)	After 2 months(n=40)	p-value
CD4	42.92 ±10.67	52.29 ±10.52	55.93 ±10.10	62.00 ±12.55	**<0.001**
CD8	33.23 ±15.02	47.76 ±12.38	58.33 ±13.44	51.62 ±12.83	**<0.001**
CD56	18.50 ±14.43	35.95 ±14.38	50.50 ±14.32	38.47 ±12.68	**<0.001**
IL-2	198.03 ±21.90	208.18 ±20.56	236.48 ± 21.61	196.97 ± 25.67	**<0.001**

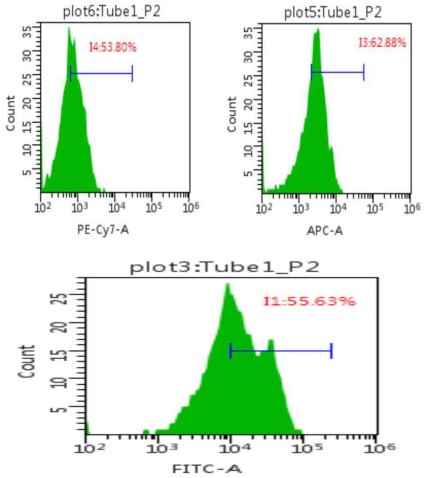
Graph AREA:



Area:Data

Control	32 (80.0%)	8 (20.0%)	40 (100.0%)	0.078	30.27 ±8.20	0.388
Before	26 (65.0%)	14 (35.0%)	40 (100.0%)		31.40 ±10.16	
After 2 weeks	25 (62.5%)	15 (37.5%)	40 (100.0%)		31.73 ±9.67	
After 2 months	21 (52.5%)	19 (47.5%)	40 (100.0%)		34.15 ±10.13	
Total	**104 (65.0%)**	**56 (35.0%)**	**160 (100.0%)**		**31.89 ±9.59**	

Table 3: Comparative analysis of immune markers among distinct control and time-point groups (N=160)



DISCUSSION

The present study demonstrated significant alterations in the cellular immune markers $CD4^+$, $CD8^+$, $CD56^+$, and serum IL-2 concentrations among pulmonary tuberculosis (PTB) patients before treatment and during anti-tuberculosis therapy. These findings highlight the dynamic nature of the host immune response during the course of infection and its gradual restoration following successful chemotherapy.

A significant progressive increase in $CD4^+$ T lymphocytes was observed throughout the treatment period, with the highest values recorded after two months of therapy. Similar observations were reported by Fang *et al* (2022) who demonstrated recovery of $CD4^+$ T-cell populations after successful treatment of active tuberculosis. Also, Paul *et al* (2023) suggested that effective chemotherapy promotes normalization of helper T-cell function, resulting in enhanced macrophage activation and improved immune control of intracellular bacilli.

The subsequent decline in $CD8^+$ cells after two months may indicate gradual resolution of inflammatory responses as bacterial load decreases. Nevertheless, the persistence of higher $CD8^+$ values compared with controls suggests that immune activation continues even

after clinical improvement. Similar findings were described by Chávez-Galán *et al* (2019) who reported that $CD8^+$ T-cell activation is greatest during the early phase of treatment and gradually decreases with bacterial clearance. Furthermore, Flores-Gonzalez *et al* (2024) observed that $CD8^+$ lymphocyte dynamics closely reflect disease activity and may therefore represent a useful biomarker for monitoring therapeutic response.

The reduction observed after two months may represent normalization of innate immune activation after effective bacterial clearance. Comparable observations were reported by Quatrini *et al* (2021) who demonstrated restoration of NK-cell function during successful tuberculosis treatment. Likewise, Esaulova *et al* (2021) reported that circulating NK-cell subsets increase during early treatment before gradually returning toward normal levels. Recent investigations by Zhou *et al* (2022) further confirmed that $CD56^+$ NK-cell recovery is associated with favorable clinical outcomes and may serve as an indicator of immune restoration.

The subsequent decline in IL-2 after two months suggests attenuation of inflammatory responses following reduction of antigenic stimulation. These findings support the concept that IL-2 may serve as a dynamic biomarker reflecting changes in immune activity during treatment rather than simply indicating the presence or absence of infection. Similar patterns were described by Wang *et al* (2012), who demonstrated increased IL-2 production during the early phase of anti-tuberculosis therapy followed by normalization after successful treatment. Moreover, Jafrasteh *et al* (2021) concluded that combined evaluation of IL-2 with T-cell subsets improves assessment of treatment response compared with measurement of cytokines alone.

REFERENCES

Chávez-Galán, L., Illescas-Eugenio, J., Illescas-Eugenio, J., Baez-Saldaña, R. and Baez-Saldaña, R. (2019). Tuberculosis patients display a high proportion of CD8+ T cells with a high cytotoxic potential. Microbiology and Immunology. 2019;63 :316–327.

Clifford, V., Tebruegge, M., Zufferey, C., Germano, S., Forbes, B. and Cosentino, L. (2019). Cytokine biomarkers for the diagnosis of tuberculosis infection and disease in adults in a low prevalence setting. Tuberc (Edinburgh, Scotland). 114: 91-102.

Esaulova, E., Das, S., Singh, D.K., Choreno-Parra, J.A., Swain, A., Arthur, L., Rangel-Moreno, J., Ahmed, M., Singh, B. and Gupta, A. (2021). The immune landscape in tuberculosis reveals populations linked to disease and latency. Cell Host Microbe, 29, 165–178.e168.

Fang, H., Sha, Y., Yang, L., Jiang, J., Yin, L. and Li, J. (2022). Macrophage targeted hydroxychloroquine nanotherapeutics for rheumatoid arthritis therapy. ACS Appl Mater Interfaces. 14: 8824-37.

Ferlita, S., Yegiazaryan, A., Noori, N., Lal, G., Nguyen, T. andTo, K. (2019). Type 2 diabetes mellitus and altered immune system leading to susceptibility to pathogens, especially Mycobacterium tuberculosis. J Clin Med. 8: 2219.

Flores-Gonzalez, J., Ramón-Luing, L. A., Falfán-Valencia, R., Batista, C. V. F., Soto-Alvarez, S., Huerta-Nuñez, L., et al. (2024). The presence of cytotoxic CD4 and exhausted-like $CD8^+$ T-cells is a signature of active tuberculosis. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1870(6), 167219. https://doi.org/10.1016/j.bbadis.2024.167219

Han, Z., Liguo, L., Jie, H., Xiaolin, W., Jianhua, Z., Henan, X., Jiang, D., Jiarong, Y., Zizheng, L., Zhuoran, W., Lei, G., Rongmei, L., Haidan, S., Xiaobing, Z. and Qi J. (2025). Transcriptomic and proteomic signatures of host NK cells delineate distinct immune states across tuberculosis infection statuses. doi: 10.3389/fimmu.2025.1607770.

Jafrasteh, A., Karimi, A., Hoseinialfatemi, S.M., Azimi, L., Tabarsi, P., Nasehi, M. (2021). Evaluation of interleukin-2 to detect active and latent tuberculosis among household contacts of pulmonary tuberculosis cases. Arch Pediatr Infect Dis; 9: e109398.

Paul, S., Shivani, P., Andrew, C., Shafi, M., Uzma, N., Violet Q., Mira, B. and Vishwanat, V. (2023). Role of B Cells in Mycobacterium Tuberculosis Infection. https://doi.org/10.3390/vaccines11050955.

Quatrini, L., Della Chiesa, M., Sivori, S., Mingari, M.C., Pende, D. and Moretta, L. (2021). Human NK cells, their receptors and function. Eur. J. Immunol. 51, 1566–1579.

Wang, S., Diao, N., Lu, C., Wu, J., Gao, Y., Chen, J., Zhou, Z., Huang, H., Shao, L., Jin, J., Weng, X., Zhang, Y. and Zhang, W. (2012). Evaluation of the diagnostic potential of IP-10 and IL-2 as biomarkers for the diagnosis of active and latent tuberculosis in a BCG-vaccinated population. PLoS One.7(12): e51338.

World Health Organization. (2024). Global Tubercu2021losis Report 2024. Geneva: World Health Organization.

Zhou, Y., Lan, H., Shi, H., Wu, P. and Zhou, Y. (2022). Evaluating the diversity of circulating natural killer cells between

active tuberculosis and latent tuberculosis infection. Tuberculosis. 135, 102221.