# Evaluation of Interleukin- 12 and Tumor necrotic factor- *alpha* Concentration in Serum of Iraqi Tuberculosis Patients

**Ekhlas Saddam Falih *[1]and Suhad Hassan obaid [2]**

[1,2] Collage of Health and Medical Technology/Baghdad -Iraq

E-mail(*): khlassaddam@yahoo.com

**Abstract**

A total of (100) patients attending the Center of Tuberculosis and Chest Disease in Baghdad were included in this study. Their ages ranged between (17-68) years. They all complained of a reproductive cough. The study was accomplished during the period extending from November 2019 till May 2020. Twenty-five were found to have ***M. tuberculosis*** by using the Ziehl Neelsen stain and culture technique (15 males, 10 females), their ages ranging between 17 and 68 years. Twenty-five age and sex-matched normal healthy-looking individuals were also chosen as control group for comparison. In this work, the concentrations of the cytokines, interleukine-12(IL-12) and tumor necrotic factor alpha (TNF-α) were evaluated in the sera of Tuberculoid patients by using Enzyme Linked Immunosorbent Assay (ELISA). Results of this work revealed that the mean concentrations of (IL-12 and TNF-α) were significantly higher in the sera of patients with ***M. tuberculosis*** infection when compared with their concentration in the sera of the control group ($P<0.001$). No significant differences were found in the mean concentration of serum (IL-12 and TNF-α) between males and females with ***M. tuberculosis*** infection ($P>0.05$).

***Keywords:*** *M. tuberculosis, IL-12 and TNF-α.*

# تقييم الانترلوكين -12 وتركيز عامل التنخر الورمي- الفا في مصل المرضى العراقيين المصابين بالسل الرئوي

**م.د.اخلاص صدام فالح و م.د. سهاد حسن اعبيد**

**الخلاصة**

تضمنت الدراسة الحالية مجموعه 100 مريض يعانون من السل الرئوي وأمراض الصدر الاخرى اجريت الدراسة في مركز الامراض الصدرية والتنفسيه في بغداد. تراوحت أعمار المرضى بين (17-68) عامًا. حيث كانو يشتكون من سعال. تم إجراء الدراسة خلال الفترة الممتدة من نوفمبر 2018 إلى مايو 2019. تم تشخيص على خمسة وعشرون مصابًا بمرض السل عن طريق استخدام صبغة Ziehl Neelsen , والزرع الجرثومي (15 ذكور ، 10 إناث). كما تم اختيار خمسة وعشرين من الأفراد الذين يتمتعون بصحة جيدة ومتناسبين مع الجنس ، كمجموعة ضابطة . في هذا الدراسة ، تم تقييم تركيزات السيتوكينات ، الانترلوكين -12 (IL-12) والعامل النخري الورمي ألفا (TNF-α) في مصل مرضى للسل

الرئوي باستخدام استخدام إنزيم مقايسة مناعية موصلة (ELISA). كشفت نتائج هذا العمل أن متوسط تركيزات (IL-12 و TNF-α) كانت أعلى بكثير في الأمصال من المرضى الذين يعانون من عدوى السل بالمقارنة مع تركيزها في الأمصال من السيطرة على المجموعة (P <0.001). لم يتم العثور على اختلافات كبيرة في متوسط تركيز المصل (IL-12 و TNF-α) بين الذكور والإناث مع ضمن مرضى السل الرئوي (P> 0.05) .

***الكلمات المفتاحية :*** *السل الرئوي .IL-12 and TNF-α*

## Introduction

Tuberculosis (TB) is the chronic infection caused by *M. tuberculosis*. The characteristic pathology of *M. tuberculosis* infection is the formation of granulomas via cell mediated immunity [1]. Although TB can affect a wide number of organ systems, pulmonary tuberculosis remains the most important [2]. About 90% to 95% of immuno competent individual control the initial infection via cellular immune response involving macrophage, both in the lung and lymph node [3]. TB today is the number one cause of death by infectious disease worldwide with 3 million deaths per year [4], and more than 30 million people are expected to become sick with TB [5], cell mediate immunity is the only immunity operative in tuberculosis. Humoral immunity has no influence on the course of disease [6].in non-immune host bacillus is able to multiply inside phagocyte and destroy the cell. In immune host activated T-lymphocytes release lymphokines which make the macrophages bactericidal [7]. Recognition of *M. tuberculosis* by phagocytic cells leads to cell activation and production of cytokines [8], which in itself induce further activation and cytokines production in a complex process of regulation and cross-regulation. This cytokines network plays a crucial role in the inflammatory response and the outcome of mycobacterium infections [9]. This study was conducted with estimation the level of TNF-α and IL-12 concentration in mycobacteria infection.

## Materials and methods

### Subject selection

One hundred patients attending the center of tuberculosis and chest disease in Baghdad were included in this study. The study was accomplished during the period extending from November 2018 till May 2019.Thier ages ranged between (17-68) years. Twenty-five were found to have *M. tuberculosis* using the Ziehl Neelsen stain and culture technique (15 males,10 female). Twenty-five age and sex-matched (15 males,10 females) individuals who were healthy looking were chosen as controls.

**Sample collection**

**Sputum:** Two sputum samples was collected from each patient. Ziehl neelsen stain and culture on a special medium (Lowenstein-Jensen) was done for each sputum sample, for detection of *M.. tuberculosis*

**Blood**: Five milliliter of Blood was collected from all members of the study groups. The concentrations of the serum IL-12 and TNF-α in patients and healthy controls were determined by using ELISA Kit, according to the manufacturer's guidance (MyBiosource .USA).

**Statistical analysis**

Statistical analyses were conducted using the SPSS statistical package for Social Sciences (version 20.0 for Windows, SPSS, Chicago, IL, USA). Data are displayed as mean ± SD for quantitative variables.While number and percentage for qualitative variables. Quantitative data were tested using ANOVA and Kruskal-Wallis test for differences between groups, Pearson's correlation for the relation between groups; while qualitative relations were evaluated using the Chi-square test. P-value of <0.05 was considered statistically significant.

**Results**

**Mean concentration of IL-12 (pg/ml) according to gender**

There was no significant difference in mean conc. of serum IL-12 in the males (89.98 pg/ml) when compared with concentration in the sera of females (96.99 pg/ml) p>0.05 as shown in table (1).

**Table (1):** Mean concentration of IL-12 (pg/ml) in patients sera with *M. tuberculosis* according to gender

| P. value | Range | Median | SD ± | Mean Conc. of Serum IL-12 (pg/ml) | Number | Gender |
|---|---|---|---|---|---|---|
| P> 0.05 | 72.79-112.89 | 97.90 | 2.89 | 89.98 | 15 | Male |
| | 68.88-127.8 | 3.01 | 3.02 | 96.99 | 10 | Female |

(*) NS: Non -Significant. at P>0.05.

**Mean concentration of IL-12 according to studied groups**

Table (2) showed that there was significant increase in mean conc. of IL-12 in patients sera (77.16 pg/ml), when compared with its conc. in the control group (0.0476 pg/ml) p<0.001.

**Table (2):** Mean concentration of IL-12 (pg/ml) in the sera of patients with *M. tuberculosis* in comparison to the control group

| P. value | SD ± | Mean Conc. of Serum IL-12 (pg/ml) | Number | Groups |
|---|---|---|---|---|
| P< 0.001 | 2.29 | 77.16 | 25 | Patients |
| | 0.00224 | 0.0476 | 25 | Controls |

**Mean concentration of TNF-α (pg/ml) according to gender**

Data obtained from table (3) indicates that there was no significant difference(p>0.05) in the mean conc. TNF-α in males (0.0441 ρg/ml) when compared with its conc. in sera of females (0.0088 ρg/ml) .

**Table (3):** Mean concentration of TNF-α (pg/ml) in the patient's sera with *M. tuberculosis* according to gender

| P. value | Range | Mediam | SD ± | Mean Conc. of TNF-α Serum (ρg/ml) | Number | Gender |
|---|---|---|---|---|---|---|
| P> 0.05 | 0.0089-0.2110 | 0.0554 | 0.0103 | 0.0441 | 15 | Male |
| | 0.0100-0.1620 | 0.0004 | 0.0066 | 0.0068 | 10 | Female |

(*) NS: Non -Significant. at P>0.05.

**Mean concentration of TNF- α according to studied groups**

Data in table (4) demonstrate that the mean conc. of TNF- α in patient's. Sera was significantly higher (0.0204) when compared with its conc.in the sera of the control groups (0.00521) p<0,001.

**Table (4):** Mean concentration of TNF-α (pg/ml) in the sera of patients with *M. tuberculosis* in comparison to the control groups

| P. value | SD ± | Mean Conc. of Serum TNF-α (pg/ml) | Number | Groups |
|---|---|---|---|---|
| P< 0.001 | 0.0085 | 0.0204 | 25 | Patients |
| | 0.00521 | 0.00521 | 25 | Controls |

Highly Sig. P=0.000 among patients groups and healthy control

**Discussion**

*Mycobacterium tuberculosis* (*M. tuberculosis)*, was the causative agent for tuberculosis (TB), and responsible for approximately 1.5 million deaths and 9 million new cases of TB in 2010 [1]. According to the above data this result may be explained in accordance to the fact that TB infection is a complex disease influenced by various etiological factor [11]. Therefore, cytokine production is affected by the disease itself not by gender differences. The above result assumed to be in accordance with that reported by investigators whom found a significant increase in the mean conc. of IL-12 in mycobacterial infection [12]. The protective role of IL-12 can be inferred from the observation that IL-12 KO mice are highly susceptible to mycobacterial infections [13, 14]. Previous study showed that in tuberculosis, IL-12 has been detected in pleurisy, in lung infiltrates [15,16], and lymphadenitis [17].

The expression of IL-12 receptors is also increased at the site of disease [18]. Another studies showed that IL-12 is a regulatory cytokine which connects the innate and adaptive host response to mycobacteria, and which exert its protective effects mainly through the induction of INF-γ [19, 20, 21]. Protective immunity against pulmonary tuberculosis is characterized by the formation of granulomas at the site of infection [22]. TNF- α plays a key role in granuloma formation, induces macrophages activation, and has immuno regulatory properties [23, 24]. This study agreed with result obtained from the studies which observed elevation in the conc. of TNF- α especially at the site of disease [25, 26, 27]. Several studies in mice have shown that TNF- α plays a central role in the successful host response to tuberculosis [28- 30].Various investigators have shown that TNF- α clearly plays an important potentially complex in the host response to *M. tuberculosis* [31,32], not only synergizing with IFN- γ in activation of macrophages [33], but also by playing a role in the modulation of macrophage apoptosis [34] and granuloma formation [35].

## References


1. W.H.O. (2010). W.H.O. global TB control report, 2010 WHO / CDC/ TB; 287, Geneva.

2. Dolin, P., Dye, C. and Raviglione, A. (2009). Global tuberculosis incidence, prevalence and mortality during. Bull. W.H.O. 72: 213-220.

3. Bleed, D., Dye, C. and Gullibly, I. M. (2007). Dynamic and control of global tuberculosis epidemic. USA. Am. J. Public Health; 6: 74-79.

4. W.H.O. (2011b). W.H.O. Report: Global tuberculosis control, Geneva.

5. Bean, A., Roach, D. R., Brisco, H., France, M. P., Korner, H., Sedgwick, J. D. and Britton, W. J. (2009). Structural deficiencies in granuloma formation in TNF-gene-targeted mice underlie heightened susceptibility to aerosol *Mycobacterium tuberculosis* infection, which is not compensated for by Lymphotoxin . J. Immunol. 162: 3504-3511.

6. Chretein, J. (2000). Tuberculosis: The illustrated history of a disease propose, France, HAUTS; 1: 7-14.

7. Iraqi TB and Chest Disease Institute (2004). Tuberculosis Report Baghdad.

8. Keane, J., Remold, H. G. and Kornfeld, H. (2015). Virulent *Mycobacterium tuberculosis* strains evade apoptosis of infected alveolar macrophages. J. Immunol. 164: 2016.

9. Al-Damluji, S. T. (1976). Tuberculosis: for medical students and practitioners in Iraq. London William Heimann Medical Brook limited.

10. Snedecor, W. G. and Cochran, G. W. (2007). Statistical methods. Iwa state university. Press. Iowa, USA.

11. Harrison, E. (2002). Tuberculosis. In: Textbook Harrison's principles of Internal Medicine, International edition. Vol. 1, 15th ed. McGraw-Hill Medical Publishing Division, p. 160-163.

12. Trinchieri, G. (2015). Interleukin-12: a pro-inflammatory cytokine with immunoregulation functions that bridge innate resistance and antigen-specific adaptive immunity. Annu. Rev. Immunol. 13: 251-276.

13. Fulton, S. A., Johnsen, M. J., Wolf, F., Sieburth, D. S. and Boom, W. H. (2011). Interleukin-12 production by human monocytes infected with *Mycobacterium tuberculosis*: role of phagocytosis. Infect. Immun. 64: 2523-2531.

14. Ladel, C. H. and Kaufmann, S. H. (1997). Interleukin-12 secretion by *Mycobacterium tuberculosis*- infected macrophages. Infect. Immun. 65: 1936-1938.

15. Casarini, M., Ameglio, F., Alemanno, L., Zangrilli, P., Mattia, P., Paone, G., Bisetti, A. and Giosue, S. (2000). Cytokine levels correlate with a radiologic score in active pulmonary tuberculosis. Am. J. Respir. Crit. Care. Med. 159: 143-148.

16. Taha, R. A., Kotsimbos, T. C., Song, Y. L., Menzies, D. and Hamid, Q. (1997). IFN-γ and IL-12 are increased in active compared with inactive tuberculosis. Am. J. Respir. Crit. Care Med. 155: 1135-1139

17. Zhang, M., Gately, M. K., Wang, E., Gong. J., Wolf, S. F., Lu, S., Modlin, R. L. and Barnes, P. F. (2000). Interleukin-12 at the site of disease in tuberculosis. J. Clin. Investig. 93: 1733-1739.

18. Lin, Y., Zhang, M. F., Hofman, J., Gong, J. and Barnes, P. F. (1996). Absence of a prominent Th2 cytokine response in human tuberculosis. Infect. Immun. 64: 1351-1356.

19. O'Neil, L. and Green, C. (2011). Signal transduction pathways activated by the IL-1 receptor family: ancient signaling machinery in mammals, insects, and plant. J. Leukoc. Biol. 63: 650-657.

20. Sieling, P. A., Wang, M. K., Gately, L. J., Oliverose, J. L., Me Hugh, T., Wolf, S. F. and Yogi, Y. (2015). IL-12 regulates T-helper type 1 cytokine responses in human infectious disease. J. Immunol. 153: 36369-3647.

21. Cooper, A. M., Magram, J., Ferrante, A. and Orme, I. M. (1997). Interleukin-12 is crucial to the development of protective immunity in mice intravenously infected with *Mycobacterium tuberculosis*. J. Exp. Med. 186: 39-45.

22. Rojas, M., Garcia, L. F., Nigou, J., Puzo, G. and Oliver, M. (2000). Mannosylated Lipoarabinomannan antagonizes *Mycobacterium tuberculosis* induced macrophage apoptosis by altering $Ca^{+2}$ dependent cell signaling. J. Infect. Dis. 182: 240-251.

23. Kennedy, M. K. and Park, L. S. (2009). Characterization of interleukin-15 and the IL-15 receptor complex. J. Clin. Immunol. 161: 134-143.

24. Jonis, E. M. and Parodll, M. D. (2010). Activation of gamma/ delta T-cells in the primary immune response to *Mycobacterium tuberculosis*. Science. 244: 713-716.

25. Condos, R. W., Rom, Y. M. and Liu, Y. M. (2011). Local immune responses correlate with presentation and outcome in tuberculosis. Am. J. Respir. Crit. Care. Med. 157: 729-735.

26. Barnes, P. F., Abrams, J. S., Lu, S., Sieling, P. A., Rea, T. H. and Modlin, R.L. (2011). Cytokine production at the site of disease in human *Mycobacterium tuberculosis*. Infect. Immun. 61: 3482-3489.

27. Law, K., Weiden, T., Harkin, K. Techou wong, K., Chi, C. and Rom, W. N. (2000). Increased release of interleukine-1 beta, interleukin-6, and Tumor Necrosis Factor-Alpha by bronchoalveolar cells lavaged from involved sites in pulmonary tuberculosis. Am. J. Respir. Crit. Care Med. 153: 799-804.

28. Vancerval, R. and Netea, M. G. (2000). Disease specific ex vivo stimulation of whole blood for cytokine production: applications in the study of tuberculosis. J. Immunol. Methods. 222: 145-153.

29. Fulton, S. A., Cross, J. V., Tossi, Z. T. and Boom, W. H. (2014). Regulation of interleukin-12 by IL-10, TGF-β, TNF, and IFN-γ in human monocytes infected with *Mycobacterium tuberculosis* H37Ra. J. Infect. Immun. 64: 2523-2531.

30. Sporn, M. B., Reberts, A. B., Wakefield, L. M. and Assoian, R. K. (2000). TNF-beta: biological function and chemical structure. Science. 233: 532-534.

31. Fenhalls, G., Stevens, L., Bezuidenhout, J., Amphlett, G. E., Duncan, K., Bardin, P. and Lukey, P. (2000). Distribution of IFN-γ, IL-4 and TNF-α protein and CD8 T-cells producing IL-12 p20 mRNA in human lung tuberculosis granulomas. Immunol. 105: 325.

32. Hickman, S. P., Chan, J. and Salgame, P. (2009). *Mycobacterium tuberculosis* induces differential cytokine production from dendritic cells and macrophages with divergent effects on naive T cell polarization. J. Immunol. 168: 4636.

33. Saha, B., Das, G., Vohra, H., Ganguly, N. K. and Mishra, S. C. (2004). Macrophage T-cell interaction in experimental mycobacterial infection. Selective regulation of co-stimulatory molecules on Mycobacterium infected macrophages and its implication in the suppression of cell-mediated immune response. Eur. J. Immunol. 24: 2618-2624.

34. Hirsch, C.S., Tossi, S. Z., Johnson, J. L., Luzze , H., Ntambi, L., Peters, P., McHugh, M., Kwera, A., Joloba, M., Terebuh, P. and Ellner, J. J. (2001). Augmentation of apoptosis and interferon – gamma production at the sites of active *Mycobacterium tuberculosis* infection in human tuberculosis. J. Infect. Dis. 183: 779-788.

35. Boussiotis, V. A., Tsai, E. Y., Yunis, E. J., Thim, S., Delgado, J. D., Dascher, C. C., Rousset, D., Reynes, J. M. and Goldfeld, A. E. (2010). IL-10 producing T-Cells suppress immune responses in anergic tuberculosis patients. J. Clin. Investig. 105:1317-1325.