



---

---

**THE POSSIBLE RELATIONSHIP BETWEEN SERUM TUMOR NECROSIS FACTOR  
ALPHA LEVEL AND THE RECOVERY OF PATIENTS WITH PULMONARY  
TUBERCULOSIS**

**AUDA JG<sup>1\*</sup>, AUDA IG<sup>2</sup>, SALIH WH<sup>1</sup>, ISMAIL H AND ISMAIL A<sup>1</sup>**

**1:** Al-kindy college of Medicine, University of Baghdad, Iraq

**2:** College of Science, Al-Mustansryia University, Iraq

**\*Corresponding Author: E Mail: [jamelauda@yahoo.com](mailto:jamelauda@yahoo.com); Mob.: 009647705870707**

**ABSTRACT**

*Mycobacterium tuberculosis* is the cause of the major world health issue, tuberculosis (TB). The cytokine, tumor necrosis factor alpha (TNF- $\alpha$ ) has been implicated in protection against TB in the early stages of the disease. TNF- $\alpha$  is an effective cytokine in the killing of intracellular *M. tuberculosis*. This study inducted to investigate whether there is any relationship between levels of TNF- $\alpha$  in sera of TB patients and their recovery, and is there any difference in the level of this cytokine in sera of female and male TB patients. This study included 29 patients with pulmonary TB (18 female and 11 male), their ages ranging from 37 to 59 years. All of them received first line TB therapy. They were consulted at Pasture Center during September 2012, at Baghdad city, Iraq. TNF- $\alpha$  level in sera was estimated by ELISA. Data were analyzed using Two sample T test for measuring the differences between the groups. , no significant difference ( $p=0.198$ ) was found in serum TNF- $\alpha$  concentration between TB patients (mean= 8.09 pg/ml) and in control group (mean=5.62 pg/ml). On the other hand no significant difference was found between serum TNF- $\alpha$  concentration in male TB patients (mean=8.42pg/ml,  $p=0.71$ ) and female TB patients (mean=7.89pg/ml). As a conclusion, TNF- $\alpha$  level in TB patients may associate with recover of the patient after treatment. On the other hand no relationship was found between the levels of TNF- $\alpha$  in TB patients' sera and their gender.

**Keywords: Tuberculosis, *Mycobacterium tuberculosis*, Cytokines, Tumor Necrosis Factor  
Alpha**

## INTRODUCTION

Tuberculosis is the world's second commonest cause of death from infectious disease [1]. Cellular immunity plays a major role in the control of the infection [2]. The cytokine, tumor necrosis factor alpha (TNF- $\alpha$ ) has been implicated in protection against TB in the early stages of the disease [3]. No cytokines were as effective as TNF- $\alpha$  in the killing of intracellular *M. tuberculosis*. Blocking TNF- $\alpha$  has been shown to allow the disease to emerge from latency [4]. TNF or TNF receptor-knockout (KO) mice exhibit significantly increased susceptibility to *M. tuberculosis* infection [5] and have poorly formed granulomas, mass regions of necrosis, and infiltration of the neutrophilic alveoli. Defective TNF-TNF receptor signaling culminates in massive inflammation and necrosis as a result of an uncontrolled T-helper type 1 immune response to the overproduction of IFN- $\alpha$ , and IL-12 [6]. The levels of tumour necrosis factor TNF- $\alpha$  produced by infected high responder macrophages was significantly higher than low responders [7].

Systemic spillover of TNF $\alpha$ - may account for unwanted inflammatory effects like fever and wasting [8]. Macrophages produce a distinct pattern of cytokines including; Interleukin-1 (IL-1), IL-2, IL-10, IL-12, tumor necrosis

factor-alpha (TNF- $\alpha$ ) and interferon-gamma (IFN- $\gamma$ ) [9, 10]. TNF- $\alpha$  is required for induction of apoptosis in response to infection with *M. tuberculosis* [11]. This study inducted to investigate whether there is any relationship between levels of TNF- $\alpha$  in sera of TB patients and their recovery, and is there any difference in the level of this cytokine in sera of female and male TB patients.

## MATERIALS AND METHODS

### Patients

This study included 29 patients with pulmonary TB (18 female and 11 male), their ages ranging from 37 to 59 years. All of them received first line TB therapy. The treatment was with isoniazid, rifampicin, and pyrazinamide for two months, followed by isoniazid and rifampicin for four months. They were defined and diagnosed as TB cases by the specialist physicians according to the clinical picture, chest x-rays, direct sputum smear examination by Ziehl-Neelsen's staining technique and by culture on Löwenstein- Jensen media [12]. They were consulted at Pasture Center during September 2012, at Baghdad city, Iraq. For comparison, 12 healthy persons also included.

### Methods

Serum TNF- $\alpha$  level: Blood samples (5 ml) were drawn and sera were separated and

stored at -20 C° until analyzed. Estimation of TNF- $\alpha$  concentration in serum was done by ELISA method using US Biological kit, USA. It also called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to TNF- $\alpha$ . Standards and samples are added to plate wells with a biotin-conjugated polyclonal antibody preparation specific for TNF- $\alpha$ . TNF- $\alpha$  if present will bind and become immobilized and then be “sandwiched” by biotin conjugate. In order to quantization determine the amount of TNF- $\alpha$ , Avidin conjugated to Horseradish Peroxidase is added. Only those wells that containing TNF- $\alpha$  biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color, and the color is measured spectrophotometrically at wavelength 450nm. Serum TNF- $\alpha$  was measured in TB patients (both female and male) and in control groups.

-Statistical analysis: Data were analyzed statistically using descriptive statistics

Infrequencies tables, median and slandered deviation. Two sample T test are used for measuring the differences between the groups. These were done using Mini Tab Statistical Software Program 13.20. A p-value < 0.05 was considered significant.

## RESULTS

### Level of TNF- $\alpha$ in Patients and Control Groups

ELISA result reveals that, no significant difference (p=0.198) was found in serum TNF- $\alpha$  concentration between TB patients (mean= 8.09 pg/ml) and in control group (mean=5.62 pg/ml). **Table 1** show these result.

### Level of TNF- $\alpha$ in Male and Female TB Patients

No significant difference was found between serum TNF- $\alpha$  concentration in male TB patients (mean=8.42pg/ml, p=0.71) and female TB patients (mean=7.89pg/ml), **Table 2**.

**Table 1: Level of TNF- $\alpha$  in Serum of TB Patients and Control Groups**

Subjects	Number	Mean (pg/ml)	Median (pg/ml)	Standard Deviation	SE Mean	T-value
Patients	29	8.09	6.80	3.62	0.67	1.35*
Control	12	5.62	4.27	5.90	1.70	

NOTE: Two-Sample T-Test: p=0.198

**Table 2: Level of TNF- $\alpha$  in Serum of Male and Female TB Patients**

Gender	Number	Mean (pg/ml)	Median (pg/ml)	Standard Deviation	SE Mean	T-value
Male	11	8.42	6.70	3.53	1.07	0.38*
Female	18	7.89	6.83	3.76	0.88	

NOTE: Two-Sample T-Test: p=0.71

## DISCUSSION

No significant difference was found between TNF- $\alpha$  concentration in sera of TB patients and in control group, they were previously found that increased levels of expression of TNF- $\alpha$  after 2 months of intensive anti-TB therapy were significantly associated with poorer outcomes on chest radiography [13]. This result may indicate that there is an association between increases of TNF- $\alpha$  level and TB clinical deterioration early in treatment. Other researchers found that, quick recovery is associated with a rapid decrease of TNF- $\alpha$  in plasma [8]. To limit the deleterious effects of TNF- $\alpha$ , and soluble TNF- $\alpha$  receptors which block TNF- $\alpha$  activity are increased [14]. IL-10 antagonizes the proinflammatory cytokine response by downregulation of production of IFN $\gamma$ , TNF- $\alpha$ , and IL-12 [15]. However, further studies are warranted before implementation of a cytokine-based predictive scale for the clinical setting. On the other hand, the plasma TNF- $\alpha$  level of female did not showed any significant difference when compare with that of male in this study. No studies were investigating the presence of such difference. Male and female are equal in the production of TNF- $\alpha$  when they get infection with TB. As a conclusion, TNF- $\alpha$  level in TB patients may associate with recover of the patient after

treatment. On the other hand no relationship was found between the levels of TNF- $\alpha$  in TB patients' sera and their gender.

## REFERENCES

- [1] Frieden TR, Sterling TR, Munsiff SS, Watt CJ and Dye C, Tuberculosis, *Lancet*, 362 (9387), 2003, 887-899.
- [2] Azzurri A, Sow OY, Amedei A, Bah B, Diallo S, Peri G, Benagiano M, D'Elios MM, Mantovani A and Del Prete G, IFN-gamma-inducible protein 10 and pentraxin 3 plasma levels are tools for monitoring inflammation and disease activity in Mycobacterium tuberculosis infection, *Microbes Infect.*, 7, 2005, 1-8.
- [3] Olobo JO, Geletu M, Demissie A, *et al.*, Circulating TNF-alpha, TGF-beta, and IL-10 in tuberculosis patients and healthy contacts, *Scand. J. Immunol.*, 2001, 53, 85-91.
- [4] Gomez-Reino JJ, Carmona L, Valverde VR, *et al.*, Treatment of rheumatoid arthritis with tumor necrosis factor inhibitors may predispose to significant increase in tuberculosis risk: a multicenter active-surveillance report, *Arthritis Rheum*, 48, 2003, 2122-7.

- [5] Roach DR, Bean AG, Demangel C, France MP, Briscoe H and Britton WJ, TNF regulates chemokine induction essential for cell recruitment, granuloma formation, and clearance of mycobacterial infection, *J. Immunol.*, 168, 2002, 4620-4627.
- [6] Zganiacz A, Santosuosso M, Wang J, Yang T, Chen L, Anzulovic M, Alexander S, Gicquel B, Wan Y, Bramson J, Inman M and Xing Z, TNF-alpha is a critical negative regulator of type 1 immune activation during intracellular bacterial infection, *J. Clin. Invest.*, 113, 2004, 401-413.
- [7] Gaikwad AN and Sinha S, Determinants of natural immunity against tuberculosis in an endemic setting: factors operating at the level of macrophage-*Mycobacterium tuberculosis* interaction, *Clin. Exp. Immunol.*, 151 (3), 2008, 414-422.
- [8] Hsieh SM, Hung CC, Chen MY, Sheng WH and Chang SC, Dynamics of plasma cytokine levels in patients with advanced HIV infection and active tuberculosis: implications for early recognition of patients with poor response to anti-tuberculosis treatment, *AIDS*, 13, 1999, 935-941.
- [9] Mattos AMM, Almeida CS, Franken KLM et al., Increased IgG1, IFN- $\gamma$ , TNF- $\alpha$  and IL-6 responses to *Mycobacterium tuberculosis* antigens in patients with Tuberculosis are lower after chemotherapy, *Int. Immunol.*, 2010, 22 (9), 775-782.
- [10] Masood IK, Martin E Rottenberg, Salahuddin ME, Irfan N, Rao M, Islam NBCM, Hussain R and Hasan Z, Expression of *M. tuberculosis*-induced suppressor of cytokine signaling (SOCS) 1, SOCS3, FoxP3 and secretion of IL-6 associates with differing clinical severity of tuberculosis, *BMC Infect. Dis.*, 13, 2013, 13.
- [11] Crevel R, Ottenhoff THM and Van Der Meer JWM, Innate Immunity to *Mycobacterium tuberculosis*, *Clin. Microbiol. Review*, 15 (2), 2002, 294-309.
- [12] Baron EJ, Peterson ILR and Fingol SM, Baily and Scott's Diagnostic Microbiology, Mosby, New York, USA, 1999.
- [13] Su W, Perng W, Huang C, Yang C, Wu C and Chen J, Association of Reduced Tumor Necrosis Factor

---

Alpha, Gamma Interferon, and Interleukin-1 $\beta$  (IL-1 $\beta$ ) but Increased IL-10 Expression with Improved Chest Radiography in Patients with Pulmonary Tuberculosis, Clin. Vaccine Immunol., 17 (2), 2010, 223-231.

[14] Roach DR, Briscoe H, Saunders B, France MP, Riminton S and Britton WJ, Secreted lymphotoxin-alpha is essential for the control of an intracellular bacterial infection, J. Exp. Med., 193, 2001, 239-246.

[15] Vázquez-Armendáriz AI, Rosas-Taraco AG, Salinas-Carmona MC and Arce-Mendoza AY, *Mycobacterium tuberculosis* lipids induce an immunosuppressive environment in diabetic patients, Next Document Medicina Universitaria, 14, 2012, 80-5.