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## **Evaluation of some immunological markers related to immune anergy in tuberculosis patients**

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**Abstract**---This study was applied on 90 tuberculosis patients admitted in the Babylon Center of Tuberculosis and Chest Diseases in Hilla, Iraq. They were divided into four groups; 50 patients with Pulmonary, 40 patients with Extra pulmonary, 20 people as healthy contact group and 20 people as healthy control group. Blood samples were collected from patients and control group to estimate Anti Ascaris lumbricoides-IgG, also Zinc and copper level were also spectrophotometrically estimated. The ages range of all TB cases were ranged from 11 to more than 71 years; TB patients consisted of 51 males and 39 females. The mean of Anti- Ascaris lumbricoides IgG in PTB patient's sera was  $0.43 \pm 0.17$ , while in control subjects was  $0.20 \pm 0.19$ . The study significantly shows difference between PTB patients and controls, while The mean of anti Ascaris lumbricoides IgG in EPTB patients sera was  $0.23 \pm 0.02$ , while in control subjects was  $0.20 \pm 0.19$ . The study significantly shows no difference between EPTB patients and controls. The zinc level in serum was significantly decreased in the TB patients ( $28.3 \mu\text{g/dl}$ ,  $27.2 \mu\text{g/dl}$  for EPTB patients compared with control ( $89.16 \mu\text{g/dl}$  and copper level was significantly increased in the TB patients ( $203.6 \mu\text{g/dl}$ ,  $182.7 \mu\text{g/dl}$  for EPTB patients compared with control ( $128.9 \mu\text{g/dl}$ ).

**Keywords**---pulmonary tuberculosis, extra pulmonary tuberculosis, anti ascaris lumbricoides-IgG, zinc, copper, anergy.

## Introduction

Tuberculosis (TB) is considered one of the most important infectious diseases in the world and its incidence is on rise [1]. Tuberculosis is a leading global cause of morbidity and death [2]. TB infection is acquired by the inhalation of droplets containing *Mycobacterium Tuberculosis* bacilli (MTB). Tuberculosis is an immunological disease and the clinical manifestations of tuberculosis are the functions of the immune status of the host [3] The world health organization (WHO) considered Iraq within a high burden of TB among seven countries of the Eastern Mediterranean Region include 20 thousand represent of new and relapse TB cases (represent 54% of all cases), of these four thousand death recorded annually (4).

Immune anergy as a mechanism of peripheral tolerance may increase the severity of TB (5). In anergic TB patients , sustained stimulation by *M. tuberculosis* , which results in IL 10 but not IFN -  $\gamma$  production , mediates the generation of anergic *M. tuberculosis* - specific T - cells with immunosuppressive properties (6) .Many predisposing factors increase the frequency of immune anergy including viral infections and metabolic disorders especially renal failure and diabetes mellitus (7). Helminth infections such as ascariasis elicit a type 2 immune response resembling that involved in allergic inflammation, but differing to allergy, they are also accompanied with strong immunomodulation (8) Most hypotheses suggest that *A. lumbricoides* activate the Th2 inflammatory response to type 2 immunity (Th2-mediated allergic inflammation) that play a protective role against asthma and other allergic diseases (9). The pulmonary migrations of *Ascaris* larvae would create a highly polarized Th2 immune environment in the lung mucosa, which might enhance Th2 cytokine production to non parasite antigens (e.g., aeroallergens or pathogens such as *Mycobacterium tuberculosis*) at the same site, enhancing allergic reactivity to environmental allergens and thus contributing to the enhanced allergic pulmonary reactivity that has been associated with geo helminth infections (10).Also ,Malnutrition affects both the innate and adaptive immunity of an individual rendering them susceptible to a variety of infections. Phagocytosis and complement cascade are two main mechanisms involved with elimination of pathogenic organisms from the body (11,12). Zinc is a potent mediator of host resistance to infection because it can influence the innate and adaptive immune response in many ways (13). It can increase the release of IFN- $\gamma$  and other cytokines by PBMC although at high concentrations, and induce the proliferation of CD8+ T-cells in combination with an exposure to IL-2. In such studies, the addition of zinc can also affect the proliferation of different cell types in response to various mitogenic stimuli although an excessive supplementation by zinc could also have a deleterious effect on immune functions (14) . During zinc deficiency, the production of Th1 cytokines in particular IFN- $\gamma$ , IL2, and tumor necrosis factor (TNF) is reduced where as the levels of the Th2 cytokines IL4, IL6, IL10 were not affected in cell culture models and ( 15). Copper (Cu) is a trace element essential for the development of almost all aspects of mammalian physiology, thus defects in Cu

homeostasis almost certainly impact immune responses to microbial infections. Dietary Cu deficiency in farm animals is linked to a higher incidence of bacterial infections (16). In Mycobacterium tuberculosis, the most prominent Cu binding enzymes include cytochrome c oxidase and the Cu/Cu superoxide dismutase11, which contributes to resistance to oxidative stress(17).Copper overload is one of the mechanisms used by macrophages to destroy MTB within their phagosomes through iron-sulfur cluster degradation [Fe-S] and metal cofactor replacement (18,19)

### **Aim of this study**

The aim of this study is the estimation of immunological markers related to immune anergy involving nutritional factors( Zn and Cu) as well as Ascaris lumbricoides infections in some of Iraqi patients that have Pulmonary and Extra pulmonary TB and comparing these levels with that of the healthy subjects.

### **Materials and Methods**

A total of 90 TB patients consisting of 51 males and 39 females were involved in this study. Their age ranged from 11to more than70 years. They were admitted to Babylon Center of Tuberculosis and Chest Diseases during the period January to October 2021. The age range the TB patients (11 to more than 70) years. 3 ml of blood were collected by vein puncture, 2 ml of them were left for 2 to 4 h, then, the serum was collected in clean test tube and stored at -20°C until use for serological tests.

### **Results and Discussion**

#### **Anergic factors**

#### **Anti Ascrais lumbricoides-IgG**

The mean of Anti- Ascrais lumbricoides IgG in PTB patients sera was  $0.43\pm 0.17$ , while in control subjects was $0.20\pm 0.19$ . The study significantly shows difference between PTB patients and controls ( $p < 0.01$ ), tables (1). The study shows Anti-Ascrais lumbricoides IgG was significantly higher in TB patients than in controls.

Table (1): concentration anti Ascrais lumbricoides-IgG among pulmonary tuberculosis patients and healthy control

Parameters	Study groups	Number	M±SD	P- Value
Anti Ascrais lumbricoides- IgG	PTB Patients	50	$0.43\pm 0.17$	0.000**
	Healthy control	20	$0.20\pm 0.19$	

\*\*( $P < 0.01$ ), SD: standard deviation.

The mean of anti *Ascaris lumbricoides* IgG in EPTB patients sera was  $0.23 \pm 0.02$ , while in control subjects was  $0.20 \pm 0.19$ . The study significantly shows no difference between EPTB patients and controls ( $p < 0.357$ ), tables (2).

Table (2): concentration anti *Ascaris lumbricoides*-IgG among Extra pulmonary patients and healthy control

Parameters	Study groups	number	M±SD	P- Value
Anti <i>Ascaris lumbricoides</i> - IgG	Extra pulmonary Patients	40	$0.23 \pm 0.02$	0.357
	Healthy control	20	$0.20 \pm 0.19$	

( $P > 0.05$ ), SD: standard deviation.

The study shows Anti- *Ascaris lumbricoides* IgG was significantly higher in TB patients than in controls. But no significantly difference between EPTB patients and controls ( $p < 0.357$ ). In study by (20) who found that helminth infection is associated with increased regulatory T-cell and Th2-type responses. Considering the fact that TB is a chronic disease, it is difficult to determine whether or not helminth infection preceded the development of active TB. The presence of intestinal worms may shift the immune background towards a Th2 profile as well as increased IL10- producing Tregs (21) which may favour the establishment of mycobacterial infection and the development of active diseases. A study determining worm load is needed to evaluate whether individuals with high worm burden have an increased risk of developing TB to further support the hypothesis that worms are one of the risk factors besides HIV infection, for developing active TB in worm-endemic countries. However, we evaluated the association between the number of helminth species a person carried and active TB and showed that the odds of being an active TB patient increased progressively with the number of species of helminth parasites the person carried. This strongly supports the hypothesis that helminth infection may be an important risk factor for the development of active TB (22, 23,24). Previous studies have confirmed a link between chronic helminth infection during TB infection and an increased Th2-type immune response with a concomitant impaired immune control of Mtb. helminth antigen exposure models using human-naïve PBMCs as the source of monocytes for investigating early host control of virulent Mtb growth in monocytes/macrophages. Our main result is that human phagocytes had increased ability to control virulent Mtb during early stages of helminth exposure. Additionally, *Ascaris lumbricoides* or *Schistosoma mansoni* antigens showed differential effects on M1/M2 polarization markers of monocyte macrophages and cytokine expression, which indicated a gradual transition into a Th2-type immune response (25).

### Zinc Concentration

The mean level of serum zinc was  $28.3 \mu\text{g} / \text{dl}$  for PTB patients,  $27.2 \mu\text{g} / \text{dl}$  for EPTB patients and  $89.16 \mu\text{g} / \text{dl}$  for control subjects. The study shows significantly difference between TB patients and control subjects ( $p < 0.01$ ) figure (1). The study shows that the zinc level higher in controls than TB patients.

It showed that zinc level was lower in TB patients than controls. This finding was matched with (26) who mentioned that serum zinc levels were significantly reduced in patients with pulmonary tuberculosis (PTB) compared to healthy controls. This finding also matched with (27) who showed that zinc level in serum of TB patients is significantly decreased when it is compared to control group. The reason for low serum zinc levels in TB could be multifactorial. Firstly a change in distribution of zinc in the body tissue is known to occur in chronic infections, with a net flow of zinc to the liver for the synthesis of acute phase reactants including metallo enzymes. Secondly, zinc may be utilized by *Mycobacterium tuberculosis* for the growth and multiplication (28). Other study found that there was a significant decrease in serum zinc levels of TB patients when it was compared to control group. The low plasma zinc levels can be observed in our study among children with active pulmonary TB is similar to the finding of other studies(29). Other studies showed that Anti-tuberculosis therapy results were significantly increased in serum zinc levels during treatment (30). (31) showed that zinc supplementation improves the effect of tuberculosis medication after 2 months of anti-tuberculosis treatment and the results in earlier sputum smear conversion. Zinc deficiency was reported in patients with pulmonary tuberculosis in India and China, and thus deficiency may severely affect human health. (32)

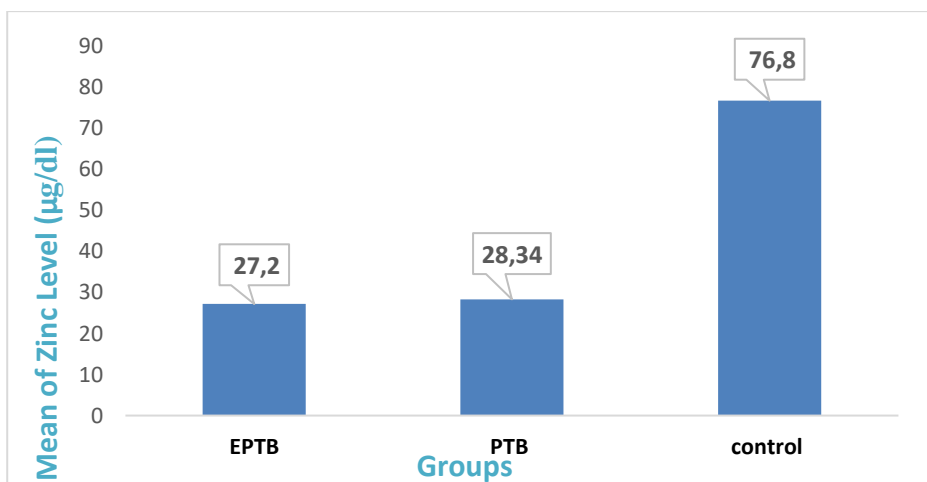


Figure (1): Zinc level (µg/dl) for TB Patients and Controls

### Copper Concentration

The mean level of serum copper was 203.6 µg /dl for PTB patients, 182.7 µg /dl for EPTB patients and 128.9 µg /dl for control subjects. The study shows significantly difference between TB patients and control subjects ( $p < 0.01$ ) figure (2). The study shows that the copper level higher in TB patients than controls. This finding was matched with (33) who mentioned the level of copper was higher in the biological samples of pulmonary Tuberculosis patients as compared to control ( $p < .001$ ). This finding also matched with (34) who mentioned that there was a significant increase in serum copper levels of TB patients. While (29) found increased copper level in serum of children with pulmonary tuberculosis. (35) studied the serum levels of copper, zinc in patients suffering from pulmonary TB.

Their study showed that TB patients had higher serum copper level and Cu/Zn ratio as compared to normal individuals, while serum zinc level was lower as compared to control group. The serum level of copper in the patients under anti TB treatment was significantly decreased (36).

The most reason for increased of copper level in serum of TB patients that the most important aspect of copper homeostasis is considered to be copper exporters because of their ability to pump excess Cu outside the cell before it can damage intracellular components. Based on our results, (30) began to sketch the components utilized by *M. tuberculosis* to survive the fluctuation in Cu level inside the phagosome.

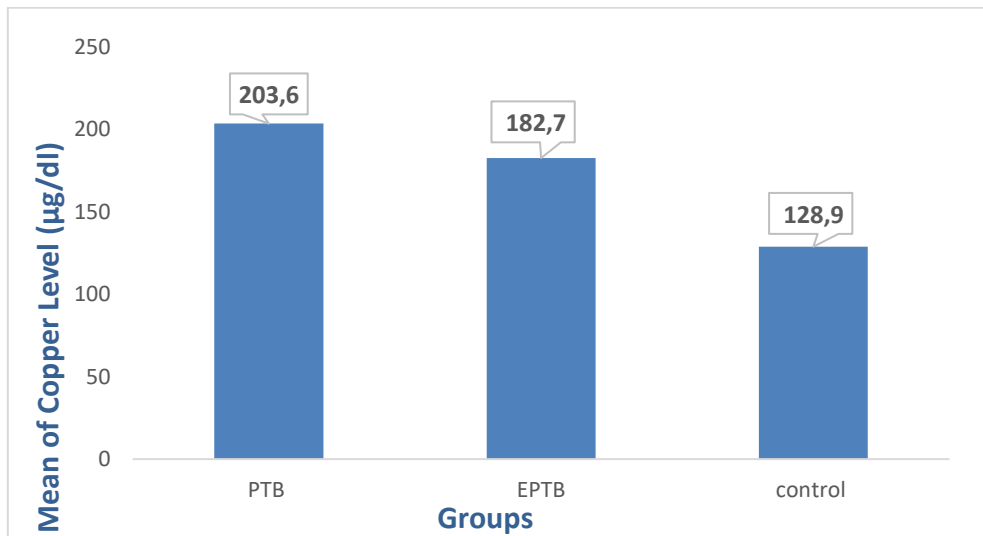


Figure (2) : Copper level (µg/dl) for TB Patients and Controls

## Conclusion

From the current study, it has been shown that levels of Anti- *Ascaris lumbricoides* IgG highest in patients suffering of tuberculosis compared to healthy control group and this correlates with many other studies as these Anti- *Ascaris lumbricoides* IgG play an important role as immune mediators in tuberculosis disease. However, we think that the immune modulation induced by helminth infection may affect immunity to TB. Copper and zinc are important elements for the human body, copper and zinc are imbalanced in TB patients.

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