

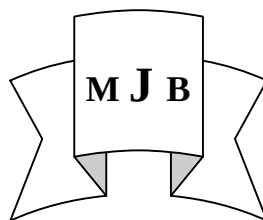
Studying The Effect of Zinc and copper on Cellular Immunity in Tuberculosis Patients

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Abstract

This study aims at studying the effect of zinc and copper level on the cell mediated immunity against tuberculosis. This work was applied on 60 TB patients admitted to the Babylon Center of Tuberculosis and Chest Diseases from October 2009 to January 2010.

TB patients consisted of 38 males and 22 females with age range 9-60 years. This study involved also 40 apparently health subjects as controls, which consisted of 25 males and 15 females with age range 10-60 years. Blood samples were collected from patients and control to estimate phagocytic index, and interferon gamma (IFN- γ). Zinc and copper level were also spectrophotometrically estimated. The cell mediated parameters showing a significantly decreased ($p < 0.05$) in phagocytosis activity in TB patients (7.3%) compared to control group (11.6%) and no significantly increased ($p > 0.05$) of IFN- γ level in the TB patients (0.123) IU/ml compared to control group (0.071) IU/ml. The zinc level in serum was significantly decreased ($p < 0.05$) in the TB patients (58.27) $\mu\text{g/dl}$ compared with control (79.92) $\mu\text{g/dl}$ and copper level was significantly increased ($p < 0.05$) in the TB patients (148.36) $\mu\text{g/dl}$ compared with control (89.16) $\mu\text{g/dl}$. The results provide a good correlation between the levels of zinc and copper and the cellular immunity against tuberculosis.

الخلاصة

تهدف هذه الدراسة الى دراسة بعض معايير المناعة الخلوية المتضمنة نشاط الالتهام وتركيز IFN- γ لمصنوع مرضى التدرن ودراسة تأثير مستوى الزنك والنحاس على المناعة الخلوية لمرضى التدرن.

تضمنت الدراسة ٦٠ مريضاً (٣٨ ذكراً ، ٢٢ انثى) تراوحت اعمارهم (٩-١٠) عاماً المراجعين لمركز بابل للأمراض الصدرية والتدرن وللفترة من تشرين الاول ٢٠٠٩ لغاية كانون الثاني ٢٠١٠ و ٤٠ شخصاً سويّاً (٢٥ ذكراً ، ١٥ انثى) تراوحت اعمارهم (١٠-٦٠) عاماً .

جمعت عينات الدم من كلا المجموعتين لتقدير نشاط الالتهام وتركيز IFN- γ بطريقة الأمتزاز المناعي المرتبط بالأنزيم باستخدام و قياس مستوى الزنك والنحاس باستخدام مقياس الضوء الطيفي

أظهرت النتائج لمعايير المناعة الخلوية زيادة في نشاط الالتهام للخلايا العدة بشكل معنوي ($p < 0.05$) حيث كان نسبة نشاط الالتهام للخلايا العدة (٧,٣%) مقارنة بالاصحاء (١١,٦%). وتركيز IFN- γ لم يظهر زيادة معنوية لدى مرضى التدرن ($p > 0.05$) حيث كانت IU/ml (٠,١٢٣) مقارنة بالاصحاء (٠,٠٧١) .

كان مستوى الزنك أظهر نقصان معنوي حيث كان لمرضى التدرن $\mu\text{g/dl}$ (٥٨,٢٧) مقارنة بالاصحاء $\mu\text{g/dl}$ (٧٩,٩٢) ومستوى النحاس أظهر زيادة معنوية ($p < 0.05$) حيث كان لمرضى التدرن $\mu\text{g/dl}$ (١٤٨,٣٦) مقارنة بالاصحاء $\mu\text{g/dl}$ (٨٩,١٦)

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]. Zinc deficiency is known to cause impaired cell mediate immunity this can increase susceptibility to TB because the cell mediated immunity plays a major role in the disease [4]. Several studies have demonstrated that the serum level of zinc decreased significantly during active tuberculosis and increase following recover after institution of antituberculosis therapy and improvement of nutritional status [5]. The copper is one from the trace elements Fe, Zn, Cu, and Se work in synergy to support the protective activities of immune cells[6]. Ray [7] studied the changes in the serum level of copper and zinc in the patients that are under antiTB therapy. After anti TB therapy, there is a significant rise in the serum zinc level, while serum copper level decreases with anti TB treatment [1]. This study aimed to illustrate the effect zinc and copper on the cell-mediated immunity against tuberculosis.

Materials and Methods

Patients

A total of 60 TB patients consisting of 38 males and 22 females were involved in this study. Their age ranged from (9– 60) years. Case information was taken for each patient include name, sex, age, residency, duration of infection. All TB cases were clinically diagnosed by a specialist clinician. Those patients were admitted to Babylon Center of Tuberculosis and Chest Diseases

during the period October 2009 – January 2010.

Control

A total of 40 apparently healthy subjects (25 males and 15 females) were involved as controls group. The age range of controls was matched to the patients (10 – 60) years.

Blood samples

Three ml of blood were collected by vein puncture into two sterile test tubes , in one of them 2 ml of blood were put and left for (2 – 4) hours , then the upper layer (serum) was collected in clean test tube and stored at – 20 C° until using it in serological tests and determination of zinc and copper. The second sample of blood (1 ml) put in another test tube containing anticoagulant EDTA and used for assessment of phagocytic activity [8].

Methods

1-Estimation of phagocytic index for neutrophils by using[9] method .

2- Estimation of Serum Interferon Gamma (IFN- γ) by using EASIA methods (Biosource)

4-Determination of Serum Zinc and copper by sperctrophotometer (LTA – Italy).

Results and Discussions

Zinc Concentration

The mean level of serum zinc was 58.27 $\mu\text{g/dl}$ for TB patients and 79.92 $\mu\text{g/dl}$ for control subjects. This study reveals a significant difference between the serum zinc level for patients and control subjects ($p < 0.05$), figure (1). It showed that zinc level was lower in TB patients than controls. This finding was matched with [10]who mentioned that serum zinc levels were significantly reduced in patients with pulmonary tuberculosis (PTB) compared to healthy controls. This finding also matched with [11] 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 metalloenzymes. Secondly, zinc may be utilized by *Mycobacterium tuberculosis* for the growth and multiplication [4].

There was a significant decrease in serum zinc levels of TB patients when it was compared to control group[12].

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. Other studies showed that Anti-tuberculosis therapy results were significantly increased in serum zinc levels during treatment [1]. While Karyadi *et al*[13] 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.

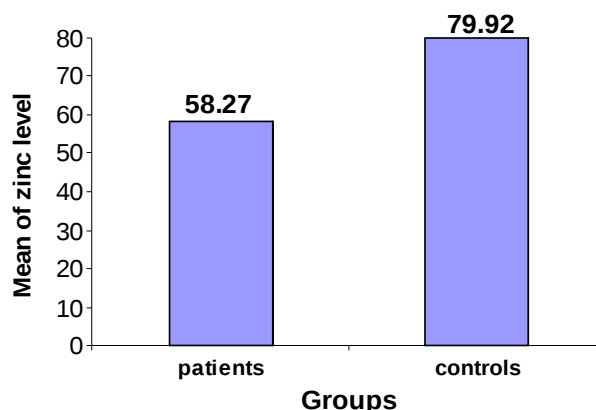


Figure 1 Zinc level (µg/dl) for TB Patients and Controls

Copper Concentration

The mean level of serum zinc was 148.36 µg /dl for TB patients and 89.16 µg /dl for control subjects. The study shows significantly difference between TB patients and control subjects ($p < 0.05$), figure (2). The study shows that the copper level higher in TB patients than controls. This finding was matched with [12] who mentioned that there was a significant increased in serum copper levels of TB patients. While [1] found increased copper level in serum of children with pulmonary tuberculosis.

Koyonagi *et al*. [14] 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 [1,7].

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 [15].

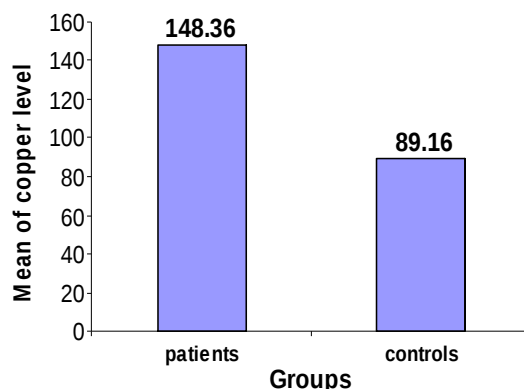


Figure 2 Copper level (µg/dl) for TB Patients and Controls

Effect of Zinc Level on Cell Mediated Parameters

Effect of Zinc Level on Phagocytic Index

In this study, the level of zinc and the mean of phagocytic index were decreased in patients (58.27 µg/dl) and (7.3%) respectively, when was compared to controls, table (1-A). the results explained the decreasing of zinc level in serum of TB patients caused decreased phagocytosis activity of TB patients. This finding was matched with [16] who mentioned that zinc deficiency causes impaired functions of innate immune cells by impaired phagocytosis, oxidative burst of neutrophil granulocytes while high zinc concentrations induce chemotaxis

of polymorphonuclear cells. And this finding was matched with [4] who mentioned that zinc deficiency is known to cause impaired cell mediated immunity and compromise neutrophil function this can increase susceptibility to TB because the cell mediated immunity plays a major role in the disease. While [17] found that the production of IL-2 are decreased and found imbalance between Th1 and Th2 functions in zinc deficiency. Our studies show that even a mild deficiency of zinc in humans may be accompanied by decreased serum thymulin activity, decrease percentage of T cytolytic cells, and decreased NK cell lytic activity.

Table (1-A) Zinc Level and Phagocytic Index

Subject	Zinc Mean(µg/dl)±SD	phagocytic index Mean(%) ±SD(%)
patients (n=60)	58.27±24.47	7.3% ±2.1%
Controls (n=40)	79.92 ± 18.10	11.6%±1.9%

The effect of zinc level on phagocytosis activity was clearly found, in table (1-B) , when was taken samples of TB patients have zinc level

less than the normal value (79.92 $\mu\text{g/dl}$) for these patients showed more decreased in phagocytic index (6.73%).

Table (1-B) Zinc level and Phagocytic Index for Patients have more than the normal value of Zinc Level

Subject	Zinc Mean($\mu\text{g/dl}$) $\pm$ SD	phagocytic index Mean(%) $\pm$ SD(%)	Significance between A,B
A-patients (n=37)	50.01 $\pm$ 16.33	6.73% $\pm$ 1.61%	Significant (P<0.05)
B-Controls (n=40)	79.92 $\pm$ 18.10	11.6% $\pm$ 1.9%	

Effect of Zinc Level on Concentration of IFN- γ

In this study, the level of zinc was decreased (58.27 $\mu\text{g/dl}$) and concentration of IFN- γ was (0.123 IU/ml) no significantly difference between patients and controls, table (2-A). This finding was matched with [18] who mentioned that the effect of zinc deficiency on tuberculosis could result from the impairment of several important effector mechanisms of the

immune system, including cellular immunity. The IFN- γ is an important regulator of the cellular immune response; it directly activates macrophages and enables then to kill ingested pathogens, including *M. tuberculosis*. The zinc deficiency caused an imbalance between Th1 and Th2 functions and the production of interferon- γ and IL2 were decreased[17].

Table (2-A) Zinc Level and Concentration of IFN- γ

Subject	Zinc Mean ($\mu\text{g/dl}$) $\pm$ SD	IFN- γ Mean(IU/ml) $\pm$ SD
patients (n=60)	58.27 $\pm$ 24.47	0.123 $\pm$ 0.071
Controls (n=40)	79.92 $\pm$ 18.10	0.071 $\pm$ 0.070

Through the comparison of zinc level for patients show less than the normal value (79.92 $\mu\text{g/dl}$) with concentration of IFN- γ for these patients shows zinc level more decreased (50.01 $\mu\text{g/dl}$) and concentration of IFN- γ also was

(0.197 IU/ml) no significantly difference between patients and controls , table (2-B). This finding was matched with [6] who mentioned that zinc deficiency lead to decreased cell mediated cytotoxicity, helper T-cell and NK cell activities.

Table (2-B) Zinc Level and Concentration of IFN- γ for Patients have more than the normal value of Zinc Level

Subject	Zinc Mean ($\mu\text{g/dl}$) $\pm$ SD	IFN- γ Mean(IU/ml) $\pm$ SD	Significance between A,B
A-patients(n=37)	50.01 $\pm$ 16.33	0.197 $\pm$ 0.412	No significant (P>0.05)
B-Controls(n=40)	79.92 $\pm$ 18.10	0.071 $\pm$ 0.070	

Effect of Copper Level on Cell Mediated Parameters**Effect of Copper Level on Phagocytic Index**

The results of this study, show that copper level was increased (148.36 $\mu\text{g/dl}$), whereas the mean of phagocytic index was decreased (7.3%) for patients when compared to controls, table (3-A). The result indicates the decreased phagocytosis activity for TB patients during

increased copper level .Whereas other studies show that the knowledge about the effects of excess copper on human health is clearly insufficient [19]. While [6]mentioned that deficiency and oversupply of copper modulate the immune response. The decreased in phagocytic activity may be regared to the effect of decreased zinc level in TB patients, this leads to decreased phagocytic activity.

Table (3-A) Copper Level and Phagocytic Index

Subject	Copper Mean ($\mu\text{g/dl}$) $\pm$ SD	Phagocytic index Mean % $\pm$ SD%
patients (n=60)	148.36 $\pm$ 46.47	7.3 % $\pm$ 2.1%
Controls (n=40)	89.16 $\pm$ 5.65	11.6% $\pm$ 1.9%

The effect of copper level on phagocytic activity was clearly found, when we take the samples of the patients we find that the copper level is more than the normal value

(89.16 $\mu\text{g/dl}$), the level of copper is more increased (158.04 $\mu\text{g/dl}$) , whereas the mean of phagocytic index is more decreased (6.7%), in these patients, table (3-B).

Tables (3-B) Copper Level and Phagocytic Index for Patients have more than the normal value of Copper Level

Subject	Copper Mean ($\mu\text{g/dl}$) $\pm$ SD	Phagocytic index Mean % $\pm$ SD%	Significance between A,B
A-patients (n=37)	158.04 $\pm$ 36.18	6.7 % $\pm$ 1.6%	Significant (P<0.05)
B-Controls (n=40)	89.16 $\pm$ 5.65	11.6% $\pm$ 1.9%	

Effect of Copper Level on Concentration of IFN- γ

The level of copper is increased in the patients (148.36 $\mu\text{g/dl}$) and found no significantly difference in concentration of IFN- γ for the patients when compared to controls , table (4-A). The results indicate the presence of reduced cell mediate immunity in TB patients. Maggini *et al.*[6] mentioned that copper has a role in supporting Th1 response. Other studies on mice found that the excess

of Cu reduced the NK values and the main subpopulation of the innate immunity NK cell was affected substantially[20].

Significantly suppress the proliferation of cytotoxic NK cells and the progenitors of NK T cells (CD4+ CD8+) in mice. Others studies showed that decreased copper intake reduced lymphocyte proliferation by decreasing the production of interleukin-2 [21].

Table (4-A) Copper Level and Concentration of IFN- γ

Subject	Copper Mean($\mu\text{g/dl}$) $\pm$ SD	IFN- γ Mean(IU/ml) $\pm$ SD
patients (n=60)	148.36 $\pm$ 46.47	0.123 $\pm$ 0.071
Controls (n=40)	89.16 $\pm$ 5.65	0.071 $\pm$ 0.070

The effect of copper level on concentration of IFN- γ was clearly found when we take the samples of the patients we find that the copper level is more than the normal value (89.16

$\mu\text{g/dl}$) for these patients , the level of copper is more increased (158.04 $\mu\text{g/dl}$) and no significantly difference in concentration of IFN- γ (0.184 IU/ml) in these patients, table (4-B).

Tables (4-B) Copper Level and Concentration of IFN- γ for Patients have more than the normal value of Copper Level

Subject	Copper Mean ($\mu\text{g/dl}$) $\pm$ SD	IFN- γ Mean(IU/ml) $\pm$ SD	Significance between A,B
A-patients (n=37)	158.04 $\pm$ 36.184	0.184 $\pm$ 0.394	No significant (P>0.05)
B-Controls (n=40)	89.16 $\pm$ 5.65	0.071 $\pm$ 0.070	

Thus, Cell mediated parameters: Phagocytosis activity of neutrophil was significantly lower in TB patients whereas IFN- γ concentration was not significantly increase in TB patients in comparison with controls. Level of zinc was significantly lower in TB patients whereas level of copper

was significantly higher in TB patients in comparison with controls. Therefore, the CMI parameters should be evaluated periodically to monitor the TB infections and Checking for TB infections for persons with zinc level below the normal.

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