

# Trace Elements Levels In Iraqi Immunocompetent Patients With Toxoplasmosis

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## ABSTRACT

Toxoplasmosis is a widespread infection usually caused by *Toxoplasma gondii* (*T. gondii*) parasite. It occurs in humans and other warm blooded animals, causing severe problems. It was found that there is an alteration in the trace elements concentrations levels associated with some human diseases.

This study aimed to investigate the changes in the concentrations of some trace elements (Mg, Fe, Zn, and Cu) in the sera of 60 immunocompetent patients with chronic toxoplasmosis and 82 healthy individuals as a control group.

Measuring the serum level of seropositivity rate of anti-*T. gondii* antibodies was done by Enzyme Linked Immunosorbent Assay (ELISA) Kit, while the concentrations of trace elements were measured by absorption spectrophotometry.

The copper element showed significant difference between patients and controls with lower average of concentration in seropositive patients than the control. Non-significantly difference was found for this element between females and males of both control and patient groups ( $p > 0.05$ ).

Non-significantly difference was found in Mg, Zn and Fe levels between patients and control groups.

Such results indicate the significance of additional knowledge of the mineral homeostasis and the regulatory processes during toxoplasmosis infection.

**Keywords:** Toxoplasmosis, trace elements, Iraq, immunocompetent, serum

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## INTRODUCTION

In *T. gondii* infection, similar to other intracellular pathogens, innate immune response is the first line defense that is important in restricting parasite dissemination while adaptive immune function is crucial for the suppression of parasite growth and long-term defense against infection (Khan *et al*, 2019; Sasai *et al*, 2018). In immune-competent patients infected with toxoplasmosis, it is mostly has no signs or mild symptoms and provides non-specific symptoms such as enlarged lymph nodes, stiff neck, painful swallowing, fever, myalgia, and abdominal pain (Waree, 2008).

Trace elements are micronutrients which are needed in low concentration and play important functions for human body. Several trace elements (as zinc, copper, magnesium and iron) modulate the function of the immune system and effect on host susceptibility to infection (Rivera *et al*, 2003).

Magnesium used for many biochemical functions in the body such as synthesis of proteins, lipids and antioxidant glutathione, regulation of blood pressure and control of blood glucose level, although Mg deficiency is not common, this can occur because of the low dietary intake, alcohol abuse, or diuretic drugs using (Al-Fartusie & Mohssan, 2017).

Zinc is act as important cofactor for over than three thousand metalloenzymes and proteins such as copper/zinc -superoxide dismutase (SOD) and metallothionin (Weiss & Carver, 2018). There is increasing evidence of pathogen-specific protective effects of zinc (Astiazarán García *et al*, 2015).

Iron is important component of many proteins such as hemoglobin and myoglobin which have a role in transport of oxygen, act as a cofactor for enzymes like ribonucleotide reductase that is function in synthesis of DNA. Iron deficiency is very common public health

problem, its deficiency exceed 50% in developing countries and can also be seen in developed world (Watson *et al*, 2010).

Copper is another trace elements required for many important metabolic processes, it binds to many Cu-dependent enzymes like cytochrome c oxidase and tyrosinase. Similar to zinc, copper is important for maintaining and improving of immune system response (Salman, 2014; Stafford *et al*, 2013).

Decreased concentration of these elements may affect the activities of innate and adaptive immune system cells, through decreased cell number and/or impaired their function. Such effect may trigger raised morbidity and mortality to microbial, viral as well as parasitic infections (Stafford *et al*, 2013).

This research aims to compare the levels of certain trace elements in immunocompetent patients with chronic toxoplasmosis and healthy individuals.

## MATERIALS AND METHODS

**subjects:** Sixty of both sexes Iraqi immunocompetent patients who were seropositive in toxoplasmosis test in addition to 82 healthy individuals for control (who have no toxoplasmosis), were participated in this study. These cases were collected during the period from September 2019 to January 2020. The patients and healthy individuals (control) have been of different geographic residencies and different age groups, ranged between 23 -70 years old. Each participant was provided with informed consent. Cases have IgM positive, and cases used currently trace elements supplementation were excluded. Five milliliters of blood samples were obtained from all participations in sterile conditions. They were processed in  $-20^{\circ}\text{C}$  after having collected serum by centrifugation blood at 3,000 rpm. In time of assay, sera was carried in room temperature. Toxoplasmosis was

diagnosed through serological examinations by ELISA method (ACON Laboratories, USA), IgG positive sample have a ratio absorbance/cut-off > 1.1 according to the manufacturer instructions.

It can be predictable to have a chance of chronic toxoplasmosis by increasing the level of IgG while IgM antibodies mean primary, or acute toxoplasmosis (Yuliawati & Nasronudin, 2015). Both IgM and IgG antibodies in the same serum may be overcome by testing for toxoplasmosis for both antibodies to remove residual IgM if the result is negative for IgG antibodies.

**Assay for serum metals:** Calorimetrically using commercial diagnostic kits (Labkit, Spain for Mg and LTA, Italy for Zn, Fe and Cu), 4 trace elements were analysed for their concentrations in the sera of all cases by absorption spectrophotometry.

#### STATISTICAL ANALYSIS

The statistical analysis was performed in this study using the SPSS program, version 16.0. Variables have been described as mean  $\pm$  standard deviation (SD). Data was analyzed using an independent sample of the Student's t test. Significance was applied to p values < 0.05 with 95% confidence interval.

#### RESULTS AND DISCUSSION

Toxoplasmosis is classified into 5 classes; toxoplasmosis in patients' immunocompetent, toxoplasmosis in immune-compromised patients, ocular toxoplasmosis, congenital toxoplasmosis and toxoplasmosis in pregnancy (Montoya, 2002).

Current study found that the mean value of IgG-*Toxoplasma* antibodies for immunocompetent patients with toxoplasmosis was  $5.02 \pm 0.41$  according to the manufacturer instructions of the kit, which have a ratio absorbance/cut-off > 1.1. For optimal functioning, the immune system needs important micronutrients and trace elements, including zinc, copper, magnesium and iron (Stafford *et al*, 2013). Deficiency in micronutrients and infectious diseases frequently occur together and has complicated interactions. This combination is more observed in people living in developing countries, especially in rural regions (Salman, 2014).

There were no significant variations in the means of 3 studied elements (Mg, Zn and Fe) concentrations. The fourth element (Cu) exhibited significant depression in its concentration in patients groups compared to the control (table 1).

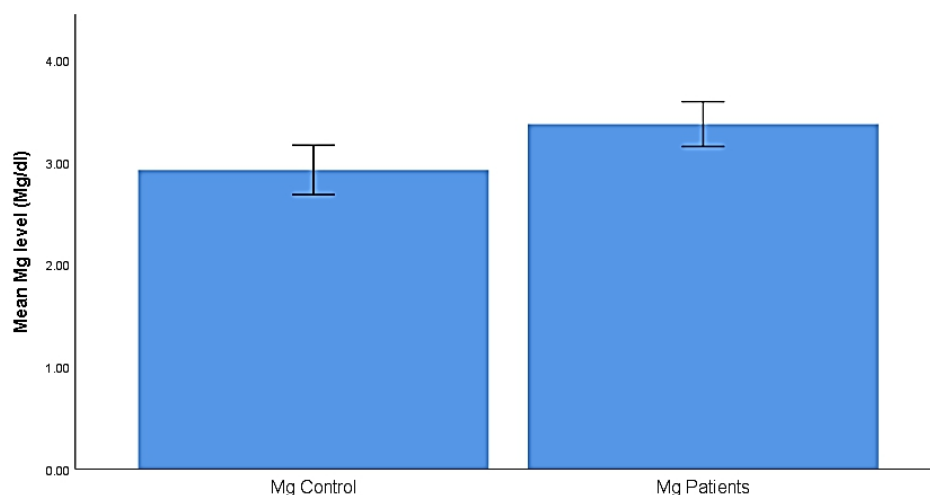
**Table (1):** Serum levels of Magnesium, Zinc, Iron and Copper in chronic toxoplasmosis patients and controls.

Trace element	Patients n=60 Mean $\pm$ SD	Control n=82 Mean $\pm$ SD	p- value
Magnesium(mg/dl)	3.37 $\pm$ 0.21	2.92 $\pm$ 0.24	> 0.05
Zinc ( $\mu$ g/dl)	41.84 $\pm$ 2.57	43.17 $\pm$ 3.71	> 0.05
Iron ( $\mu$ g/dl)	121.5 $\pm$ 10.53	126.13 $\pm$ 11.49	> 0.05
Copper ( $\mu$ g/dl)	81.81 $\pm$ 6.54	115.71 $\pm$ 12.1	< 0.05

Current results were consistent with the study on Iraqi women with abortions infected with toxoplasmosis (Salman, 2014).

In patients with toxoplasmosis (Figure 1), a raise in serum Mg level was noticed in comparison with the control group. This alteration has not been statistically significant (p> 0.05) between control and patients, and

between females and males of both groups. That result is different to the study made by Yazar *et al*. who documented reduced levels of Mg in chronic toxoplasmosis patients. That's in other words, according to our results of Mg-linked enzyme systems, like alkaline phosphatase and ATPase, do not appear to be influenced by *T.gondii* in humans (Yazar *et al*, 2003).



**Figure-1:** Serum Mg concentrations (mg/dl) in the control and the Toxoplasmosis patients.

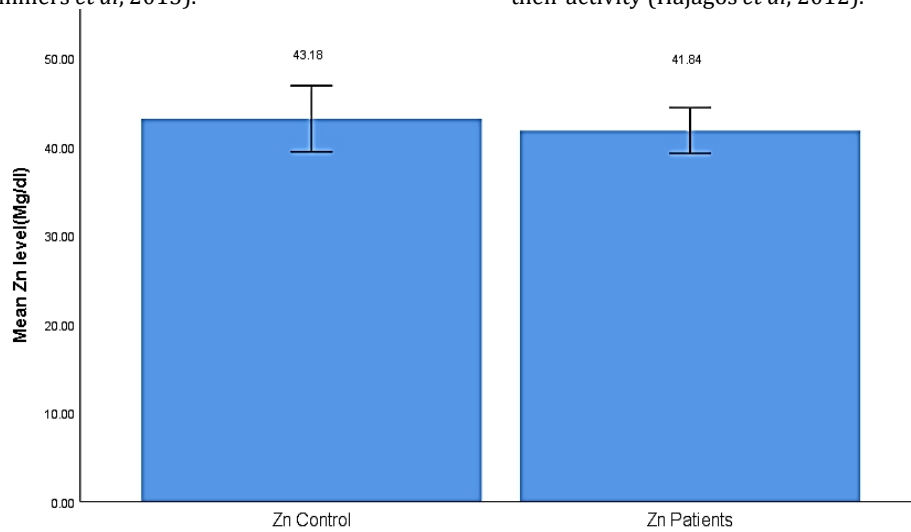
The average Zn concentration in seropositive patients was lower than in controls (figure 2). However, non-significantly difference was found between patient and control groups, and between females and males of both groups (p>0.05). The serum levels are stated to be between 70-115  $\mu$ g/ dl in humans according to the kit used. However, according to the results in table 1, even

the control group showed mean concentrations below the normal value.

Zinc deficiency is a rising public health concern. Zinc deficiency was reported to be due to malabsorption and insufficient intake, and was associated with growth, cognitive impairment function, abnormal functions of immune systems and death (Fattahi Bafghi *et al*, 2015). Zinc deficiency is more common in developing countries which lead to impair the function of phagocytes, depletion of lymphocytes, reduced production of

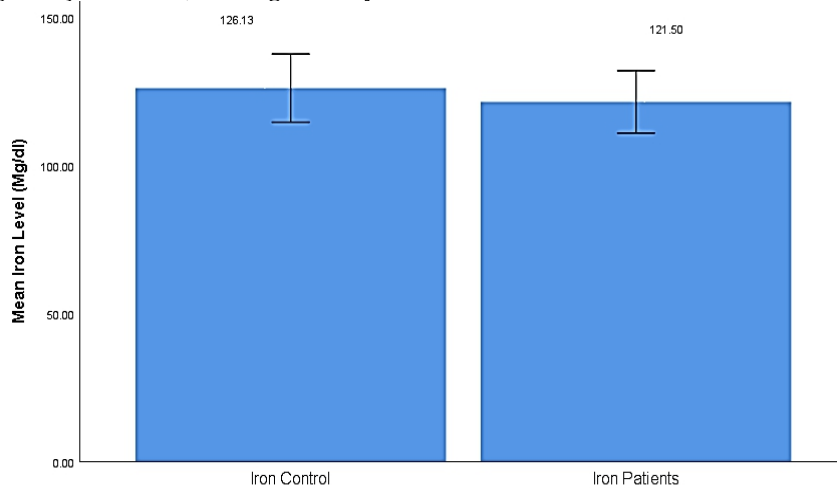
antibodies, lowering of CD4+/ CD8+ ratio and reduction in production of interleukin-2. In addition to low dietary intake deficiency of zinc also can occur due to physiological stress and in patient with diarrhea (Weiss & Carver, 2018; Stammers *et al*, 2015).

It was known that Zn is an important element for immune function that plays a significant role in immune response to parasites, there are many metalloproteases in *T. gondii* that are supposed to need Zn<sup>2+</sup> as a cofactor for their activity (Hajagos *et al*, 2012).



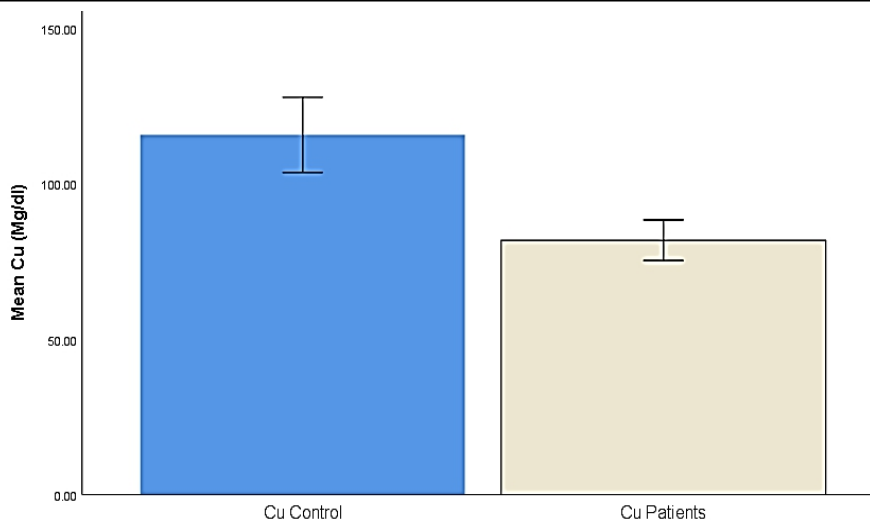
**Figure-2:** Serum Zn concentrations (µg/dl) in the control and the Toxoplasmosis patients. The mean value of the Fe in blood of patients was lower than in controls (figure 3). However, non-significantly

difference was found between patient and control groups, but there was a significant difference between females and males of control group ( $p < 0.05$ ).



**Figure-3:** Serum Fe concentrations (µg/dl) in the control and the Toxoplasmosis patients. Toxoplasmosis somehow led to decreased Fe levels in sera (Al-Fartusie, 2012). Iron deficiency make individual more susceptible to infection by affecting T- cells of innate immunity and antibody response of adaptive

immunity (Ward *et al*, 2011). Regarding the copper element, the average of concentration in seropositive patients was lower significantly than the control (figure 4) at the level 0.05. Non-significantly difference was found between females and males of both patient and control groups ( $p > 0.05$ ).



**Figure-4:** Serum Cu concentrations ( $\mu\text{g}/\text{dl}$ ) in the control and the Toxoplasmosis patients. Copper is another important trace element needed for growth. Infections change the metabolism of Cu and Zn, and even copper deficiency occurs seldom, defects of Cu and/or Zn can increase the risk of infection (Astiazaran-Garca *et al*, 2015; Watson *et al*, 2010; Oz- Helieh *et al*, 2017).

Copper and zinc are necessary part of the group of superoxide dismutase (SOD) enzymes, past researches recorded the reduction of liver SOD, due to parasitic infections (inar *et al*, 2018; Nabi *et al*, 2017). The depletion in these activities has been reported significantly in the second and the third weeks after infection (Fahmy *et al*, 2019).

Hypo-cupriemia has been reported among women with toxoplasmosis (Musstafa, 2000), meanwhile hypercupremia was recorded in some viral and bacteria infections. (Kadi *et al*, 1995).

Gene expression on control and infected cells explained infection-specific up regulation of five genes belonging to the metallothionin gene family, encoding heavy metal-binding proteins that have a significant role in transition metal homeostasis (e.g. Cu, Fe and Zn) (Klaassen *et al*, 2009; Al-Sandaqchi *et al*, 2018).

The copper complexes are useful candidate drugs, may have possible effects on *T. gondii* and can in the future be used as pharmaceutical products or acts as prototypes for the production of new toxoplasmosis treatment (Portes *et al*, 2017).

Variations in the findings of previous studies can be due to discrepancies in the number of specimens, type of procedure, source and quality of toxoplasma kits.

In conclusion, toxoplasmosis-infected immunocompetent patients exhibited significantly reduced in serum Cu levels and non-significantly increased in Mg concentrations. Zn and Fe concentrations showed non significantly decreased in serum. This can affect numerous of the enzyme systems. To the best of the author's knowledge, the first observation of immunocompetent patients infected with chronic toxoplasmosis is a decreased Cu levels significantly reported in this study. Further investigations of mineral homeostasis and the regulatory processes during infection with other categories of toxoplasmosis, are suggested.

#### Conflict of Interest

There are no declared conflicts of interest.

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