Study of Some Trace Elements and Antioxidant Vitamins in Sera of Iraqi Women with Toxoplasmosis

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ABSTRACT

Toxoplasmosis is a common infection that occurs in humans and other warm-blooded animals usually caused by the parasite Toxoplasma gondii (T. gondii). It causes serious problems to the pregnant women that lead to incidence an abortion. This study was aimed to investigate the alterations in the levels of vitamins (A, B6, C, D and E) and some trace elements Zn, Cu, Fe, and Mg in the sera of women with toxoplasmosis with a history of congenitally infected new born, and compare them with the results of age matched healthy volunteers as control group. Thirty five patients with positive anti-T gondii (IgG) antibodies and 15 healthy individuals were included in this study. The levels of serum vitamins were measured using high performance liquid chromatography technique, while serum Zn, Cu, Fe and Mg concentrations were measured using atomic absorption spectrophotometry. Although, there was no significant variations in serum Zn, Cu and Mg levels, serum Fe exhibited significant depression in its concentration in the patients group compared to the control. However, serum vitamins (A, B6, C, D and E) were observed to be decreased significantly in patients when compared with those of control group. In conclusion, the results indicated that the levels of antioxidant vitamins and Fe may have an important role to increase possibility of exposure to toxoplasmosis in women.

INTRODUCTION

Toxoplasmosis is an infection caused by the protozoan Toxoplasma gondii (T. gondii) and is contracted either congenitally or by eating food contaminated with cat faeces containing infectious oocysts or tissue cysts. It is a cosmopolitan disease, where it is a common disease...
affecting women in different countries of the Arab world. For example, in Iraq, it has been reported an infection rate with toxoplasmosis of 34.7% among pregnant women in Baghdad [1]. In fact, *T. gondii* is an obligate intracellular parasite with two phases life cycle, the first phase called the intestinal phase which occurs in cats only (wild and domesticated cats) and mainly lead to produce oocysts. While the second phase called extra intestinal phase, which occurs in all infected animals (including cats) and eventually lead to produces zoitocysts [2]. The disease toxoplasmosis can be transmitted to humans via ingestion of food or water contaminated with oocysts shed by cats, by ingesting tissue cysts in undercooked, uncooked or raw meat, and via contact with cats faeces (directly or indirectly through the soil) [3]. Also, it can be transmitted transplacentally when a woman is infected with toxoplasmosis while she is pregnant. It is important to note here that, if the woman is infected before she is pregnant, transplacental transmission will not occur [4, 5].

It has been found that a strong humoral and cellular immune response can be induced against *T. gondii* in an infected host. Therefore, cell-mediated immunity is considered as a main factor responsible for resistance against this parasite [6].

In toxoplasmosis, like others human diseases, free radicals, such as superoxide radicals, hydrogen peroxide and hydroxide radicals, and other reactive oxygen species (ROS) are constantly formed in the human body. The major defence system against superoxide involves a family of metalloenzymes, called superoxide dismutases, by which superoxide is converted to oxygen and $\text{H}_2\text{O}_2$ [7]. So the degradation of superoxide produces hydrogen peroxide, but while hydrogen peroxide is not as potent an oxidising agent as superoxide, it is still dangerous to the cell. Hydrogen peroxide is therefore removed by two peroxidatic types of enzyme, catalase and peroxidase [8]. Catalases decompose hydrogen peroxide into water and oxygen, whereas peroxidases reduce hydrogen peroxide to water.

Vitamins, minerals and trace elements play an important role in the prevention of many age-associated diseases [9] and in maintaining normal immune and cognitive functions [10]. Aerobic organisms are protected against free radicals by enzymatic and non-enzymatic antioxidant defenses [11]. Non-enzymatic antioxidants include vitamin A, vitamin C, vitamin E and elements such as zinc (Zn), copper (Cu) and magnesium (Mg).

Vitamin A is a fat soluble vitamin and essential for the immune system, cellular differentiation, and the maintenance of the respiratory epithelium [12]. Moreover, it has anti-infective, anti-inflammatory and anti-oxidant activities. Vitamin C, also known as ascorbic acid, it is a
water soluble vitamin that is known as one of the most powerful non-enzymatic antioxidant in human body. This vitamin acts as an electron donor, reducing agent, in several non-enzymatic reactions [13]. Vitamin E is also classified as a fat soluble vitamin; it also has antioxidant properties for protecting cells against the accumulation of the dangerously reactive peroxide compounds [14].

Trace elements have important functions in the human body. They are required in low concentrations and essentially they serve as very important cofactors for many antioxidant enzymatic reactions, e.g. Cu and Zn as cofactors for cytoplasmic Cu/Zn-superoxide dismutase (SOD) and Fe or Mn superoxide dismutase types (which bind either iron or manganese) [15]. Trace elements have also an important role in production of T lymphocytes (T-cell), which have a central role in cell-mediated immunity [16]. In fact, all activities of human tissues and organs depend on minute mineral and element concentrations that act as an enzyme biocatalyst. It has found that the trace element Zn is important for immune function and participates in many important immune processes. Magnesium (Mg) is one of the important elements that contribute to the synthesis of proteins, nucleotides, lipids, and carbohydrates. Trace element Fe plays a key role in supplying oxygen for tissues where it is involved in the heme structure of hemoglobin [17]. The present study was conducted to obtain information and to clarify the relationship between antioxidant vitamins as well as trace elements with toxoplasmosis. This was achieved by investigating the alteration in serum levels of vitamins (A, B₆, C, D and E) and trace elements zinc (Zn), copper (Cu), iron (Fe), and magnesium (Mg) in Iraqi women with Toxoplasmosis.

**MATERIALS AND METHODS**

The current study was conducted in the Medical Research Unit/ College of Medicine/ Al-Nahrain University in Baghdad, Iraq between October, 2010 and July, 2011. The randomly selected study group comprised of 35 female patients with toxoplasmosis with mean ages of 45.5 ± 12.6 years. The control group comprised of 15 healthy female volunteers with mean ages of 40.4 ± 16.8 years. Blood samples were collected from Al-Khadimiya Hospital in Baghdad, Iraq. All sera were collected in the morning. The healthy volunteers were free from acute or chronic pathologies, clinically evident at the moment of examination. Sera were collected from patients before drug administration. Patients with toxoplasmosis were diagnosed through clinical and serological examinations.
Blood samples were taken from all subjects in accordance with standard procedure; 5 mL of blood was collected from the vein in evacuated tubes without adding any anticoagulants. Collected blood samples were allowed to clot. The blood samples were centrifuged at 3000 rpm for 10 min; sera were transferred and stored in plastic vials at – 4 °C, until further analysis.

TRACE ELEMENTS ANALYSIS:
Sera were diluted 10 folds with de-ionized water and then analyzed, by completely controlled atomic absorption spectrophotometer (AA-6200; Shimadzu, Kyoto, Japan), to determine the levels of Cu, Zn, Fe and Mg elements in sera of the studied groups.

VITAMINS ANALYSIS
In order to measure the vitamins levels, the samples were prepared by adding 50 µl of 15% 5-sulphosalicylic to 400 µl of serum, then mixed and centrifuged at 3000 rpm for 10 min. The supernatant was taken and diluted 10 folds with distilled water, then filtered using millipore filter paper. All samples and standard solutions of vitamins were chromatographically analyzed by High Performance Liquid Chromatography (HPLC), Shimadzu (Kyoto, Japan) which consisted of a system controller model SCL-10 AVP, a degasser model DGU-12A, two liquid delivery pumps model LC-8AVP, UV-Visible detector model SPD-10AVP, and injector model SIL-10A, equipped with 20 µl sample loop. The HPLC system has been interfaced with computer via a Shimadzu class-VP5 chromatography data system program supplied by the manufacturer; Epson LQ-300 printer model P852A (Japan).

STATISTICAL ANALYSIS
Statistical analysis was performed using SPSS program, version 16.0. Variables were expressed as mean ± standard deviation (SD). Data were analyzed using independent sample Student’s t test. Significance was assigned for p values <0.05 with 95% Confident Interval.

RESULTS AND DISCUSSION
The serum trace elements concentrations for zinc, copper, iron and magnesium were found to be (Zn: 1.119 ± 0.048 mg/L), (Cu: 1.091 ± 0.192 mg/l), (Fe: 0.027 ± 0.006 mg/l), and (Mg: 19.4 ± 1.018 mg/l)in patients with toxoplasmosis, these levels remained without significant variations compared with those of healthy individuals controls (Zn: 1.119 ± 0.059 mg/l), (Cu: 1.096 ± 0.238 mg/l), (Fe: 0.035 ± 0.013 mg/l), and (Mg: 20.29 ± 3.393 mg/L) at (p<0.05), Figure 1. But, at p<0.1, serum Fe concentration (Fe: 0.027 ± 0.006 mg/l) was significantly
lower in women with toxoplasmosis than those with healthy individuals control (Fe: 0.035 ± 0.013 mg/l).
The results obtained for serum vitamins A, B_6, C, D and E concentrations were (A: 38.233 ± 4.521 mg/L), (B_6: 4.038 ± 1.212 mg/l), (C: 1.058 ± 0.221 mg/l), (D: 18.556 ± 2.188mg/l) and (E: 4.183 ± 0.75 mg/l) respectively in patients with toxoplasmosis, Figure 2. These results were lower than those obtained of controls (A: 43.727 ± 2.718 mg/l), (B_6: 13.187 ± 3.135 mg/l), (C: 1.615 ± 0.093 mg/l), (D: 28.085 ± 2.188mg/l) and (E: 13.644 ± 1.036 mg/L) in healthy individuals, respectively. Serum vitamins (A, B_6, C, D and E) were observed to be decreased significantly (p<0.05) in patients with toxoplasmosis when compared with those of the control group.

Figure-1: Serum Cu, Zn, Fe and Mg concentrations (mg/L) in the control (n = 15) and the Toxoplasmosis patients (n = 35) shown as mean ±SD (p < 0.05).

Figure-2: Serum vitamins (A, B_6, C, D and E ( mg/L) in the control (n=15) and the Toxoplasmosis patient (n=35) shown as mean ± SD (p<0.05).
The trace elements and antioxidant vitamins play important roles in human health rather than their intoxication. It has been confirmed that the presence of various trace elements is essential for human body, where they are responsible for many biochemical, immunological, and physiological activities. Furthermore, it was found that there is an alteration in the trace elements concentrations levels, such as Zn, Cu, Mg and Fe, associated with some human diseases [18]. However, our results show that toxoplasmosis somehow led to decreased Fe levels in sera. This is the first study that gives a clear indication of the relationship between toxoplasmosis and deficiency in iron among all trace elements. It has been reported that iron deficiency affects 20-50 % of the world's population. Therefore, it represents the most common trace element deficiency worldwide [19]. It has been observed that this deficiency in iron usually associated with impairments in cell-mediated immunity and reductions in neutrophil action, and also associated with decrease in peroxidase activity, particularly myeloperoxidase activity. In general, it leads to lower the ability of body to resistance disease [20]. Although, the results obtained show a decreased in serum iron level of toxoplasmosis patients relative to that of control value, the trace elements Zn, Cu and Mg levels did not show variation in their concentrations level. In other words, toxoplasmosis did not affect the Zn, Cu, Mg-linked enzyme systems, such as SOD, catalase, carbonic dehydrogenase, alcohol dehydrogenase, alkaline phosphatase and ATPase [2].

Unlike trace elements, the results obtained show that vitamins (A, B₆, C, D and E) levels were significantly decreased in patients with toxoplasmosis when compared to controls. Because of free radical formation can induce oxidative stress, the decrease in the levels of vitamins A, C and E (non-enzymatic antioxidants) in patients with toxoplasmosis may be due to the increased turnover for preventing oxidative damage in these patients, suggesting an increased in defense system against oxidant damage in toxoplasmosis disease. It has been reported that vitamin A is required for adaptive immunity and plays a role in the evolution of T both-helper cells (Th-cells) and B-cells [21]. Also, it was reported that vitamin B₆ is involved in immune function, where it promotes lymphocyte and interleukin-2 production. There is a positive association between B₆ and interleukin-2 [22, 23]. Therefore, the results obtained in this study suggest that the significant lowering determined for vitamins A and B₆ may be the reason for the decrease of the immunity to toxoplasmosis. Finally, vitamin D also exhibited decreasing in toxoplasmosis patients compared to controls. No one has referred to the relationship between toxoplasmosis and vitamin D, but the decrease in vitamin D level here
may be regarded to an imbalance in calcium metabolism. However, the reason(s) for vitamin D deficiency in patient with toxoplasmosis should be further investigated.

This is the first study indicating that the levels of some vitamins significantly decrease in patients with toxoplasmosis; this may possibly affect some specific enzyme systems, which can, consequently, exhibit serious pathology, including hepatitis, pneumonia, blindness, and severe neurological disorders.

REFERENCES:
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