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Evaluation of Age, Education, Trace Elements and Vitamins in Men with *Toxoplasma Gondii* in Maysan, Iraq

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Abstract

Background & Objectives: Toxoplasmosis is a disease caused by the intracellular protozoan *Toxoplasma gondii (T. gondii)*. The parasite's presence within cells affects various cellular activities and mechanisms. While evidence suggests that certain vitamins and trace elements can alleviate the disease and its complications, the impact of toxoplasmosis on these factors remains unclear. This study aims to evaluate the relationship between age, education, trace elements and vitamins and toxoplasmosis in men with toxoplasmosis from Maysan city, Iraq.

Materials & Methods: This study involved 200 participants, comprising 150 men infected with toxoplasmosis and 50 healthy men as a control group. Various measurements were conducted, including serum levels of trace elements (Mg, Zn, Fe) and vital vitamins (C, D, E) in both the toxoplasmosis-infected and healthy groups.

Results: The findings revealed that toxoplasmosis prevalence was highest among men aged 31-40 years with low educational attainment (up to diploma level). Furthermore, toxoplasmosis was associated with significant decrease in mean serum levels of trace elements (Mg, Zn, Fe) and vitamins (C, D, and E) ($P<0.05$).

Conclusion: Our results suggest that higher education levels may reduce the incidence of toxoplasmosis. Additionally, the complications caused by *T. gondii* may be related to the reduction in the aforementioned trace elements and vitamins. Evaluation of these factors could prove beneficial in the diagnosis and treatment of this disease.

Keywords: Life stage, Academic Background, *Toxoplasma gondii*, Microneurients, Vitamines

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Introduction

Toxoplasmosis is a zoonotic disease caused by the protozoan parasite *Toxoplasma gondii* (*T. gondii*). The primary mode of transmission to humans occurs through the consumption of food contaminated with *T. gondii* sporulated oocysts or by ingesting food contaminated with infected feline feces. The infection is usually asymptomatic or associated with self-limited symptoms such as fever, malaise, and cervical lymphadenopathy (1). Humans may remain infected but asymptomatic unless immunosuppression occurs (2). In immunocompetent hosts, acute infection is typically followed by asymptomatic latent infection, during which the parasite encysts

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in various organs, particularly the cardiac and skeletal muscles, brain parenchyma, and retina. Latent infection can reactivate locally in the retinas of immunocompetent individuals, potentially leading to significant loss of visual acuity and economic productivity (3). Evidence from human and animal studies has demonstrated that certain vitamins and trace elements can ameliorate complications caused by toxoplasmosis. For instance, zinc and copper are trace minerals that influence the development of both acute and chronic toxoplasmosis. In chronic experiments, early supplementation with biogenic zinc prophylactically reduced the number of brain cysts or completely prevented their development (4). ZnO nanoparticles have demonstrated antiparasitic activity against *T. gondii*, exhibiting minimal toxic effects and high efficiency in increasing the survival of infected mice (5). Additionally, *in vitro* and *in vivo* studies have shown the antiprotozoal activity of magnesium oxide (MgO) nanoparticles (6). Research has shown that iron is a vital nutrient for the survival of *T. gondii*. Iron deficiency affects various parasitic metabolic processes, inducing the differentiation of tachyzoites into bradyzoites; however, excessive amounts can lead to toxicity (7). Furthermore, some studies have indicated that vitamins C and E, along with selenium, are effective in reducing parasite burden (8). One study demonstrated that administration of vitamin C, E, or both significantly enhanced the killing of invasive parasites through elevated NO production by activated macrophages, suggesting potential complementary therapeutic effects (9).

It is believed that vitamins C, D, and E act against *T. gondii* tachyzoites, potentially by activating the NO-mediated mechanism to enhance parasite killing. However, vitamin E appears to be detrimental to the development of chronic toxoplasmosis (8-10). The prevalence of toxoplasmosis is high in many societies, including developed nations such as the United States. Studies have shown that it is more common among the elderly and those with lower levels of education (11). Given the various complications associated with this disease and considering the aforementioned information, several questions arise. What is the frequency of toxoplasmosis among men in Maysan city, and how does it correlate with age and education? Furthermore, do changes occur in the serum levels of trace elements such as Mg, Zn, and Fe, as well as vitamins C, D, and E in patients with *T. gondii*? This study was designed to address these questions.

Material and Methods

Sample Collection and Identification

To collect data for this research, a questionnaire was administered to 150 patients and 50 healthy men, gathering information on age and education level. Additionally, blood samples were obtained from all participants for biochemical tests.

Serological Tests

Anti-Toxoplasma IgM and IgG antibodies were detected in serum samples using enzyme linked immunosorbent assay (ELISA) kits manufactured by Pishtaz Tab Iran Company. According to the manufacturer's protocol, an IgG anti-Toxoplasma level <15 IU/ml was reported as negative, while a level >15 IU/ml was considered positive. For IgM, levels lower than 1 IU/ml were reported as negative, whereas levels equal to or higher than 1 IU/ml were deemed positive.

Measurement of Serum Metals

Three trace elements were analyzed for their concentrations in the sera of all subjects using colorimetric methods with commercial diagnostic kits (Lab kit, Spain for Mg and LTA, Italy for Zn and Fe). The analysis was performed using absorption spectrophotometry.

Measurement of Serum Vitamins

The concentrations of 25-hydroxy Vitamin D, Vitamin C, and Vitamin E were determined

in heparinized plasma samples using ELISA kits and an ELISA reader from Sun Red Biological Technology (China), following the manufacturers' instructions. 25-hydroxy Vitamin D concentration was expressed in ng/ml, while Vitamin C and Vitamin E concentrations were expressed in µmol/L.

Data Analysis

Data were analyzed using SPSS software. Results were expressed as mean ± standard deviation (SD). Comparisons of mean values between the two groups were performed using independent sample t-tests. P-values less than 0.05 were considered statistically significant.

Results

The results of this study are presented in two parts: 1) descriptive and demographic statistics, and 2) inferential statistics. Descriptive and demographic statistics were collected through questionnaires completed by both control and patient groups, including information on age and education level. In the inferential statistics section, we examine the results of t-tests comparing the control and patient groups.

1. Descriptive and Demographic Statistics

a. Age distribution of men in the control and patient groups

Table 1 shows the age distribution and corresponding percentages for men in the control and patient groups. Analysis of the data in Table 1 reveals that the highest percentage of patients with toxoplasmosis (52%) falls within the 31-40 age range, with the incidence of the disease decreasing in older age groups.

b. Data on the education level of men in the research community

Table 2 shows data on the education level of men in the research community, indicating that the highest incidence rate of the disease is related to patients with a diploma level of education and below (48%), and that with an increase in education, the incidence of toxoplasmosis decreases.

Table 1. Data on the men's ages and their percentage in the control group and patient group

Age of patients group	Number	%
20-30	39	26
31-40	78	52
>40	33	22
Total	150	100
Age of control group		
20-30	20	40
31-40	19	38
>40	11	22
Total	50	100

Table 2. Data on the education level of men in the research community

Groups	Education level	Number	%
Patients' group	Diploma and below	72	48
	Associate degree	21	14
	Bachelor's degree	38	25.3
	Master's degree and higher	19	12.6
Total		150	100
Control group	Diploma and below	18	36
	Associate degree	9	18
	Bachelor's degree	12	24
	Master's degree and higher	11	22
Total		50	100

Table 3. Mean values and standard deviation of the research variables for patients and control groups

Variables	Control group	Patient group	P value
Magnesium (mg/dL)	4.1±0.24	3.5±0.14	0.001
Zinc (µg/dL)	59±3.1	52±2.8	0.001
Iron (µg/dL)	140±11.6	134±9.8	0.05
Vitamin D (ng /ml)	190±31	151±44	0.05
Vitamin E (µg/ml)	10.3±2.4	6.3±0.4	0.01
Vitamin C (mg/L)	2.3±0.4	1.3±0.3	0.001

2. Inferential Statistics

The results obtained separately from the control group and the patient group for men are given in the table below. Table 3 presents the mean and standard deviation for the two groups of patients and the control group for each of the research variables. These values can be used to investigate and evaluate the relationship between toxoplasmosis and each of the variables.

Magnesium: The results of the t-test analysis in Table 3 show a significant difference in magnesium levels between the control group and patients (P<0.001), indicating that toxoplasmosis causes a significant decrease in serum magnesium.

Zinc: According to Table 3, which shows the average amount of zinc measured in both the control and patient groups, the average amount of zinc in the patient group is 52 mg/dl, while in the control group, it is 59 mg/dl. The t-test analysis for men shows a significant difference in zinc levels between the control group and patients (P<0.001), indicating that toxoplasmosis significantly decreases serum zinc.

Iron: Iron is another trace metal in the body, and this research has investigated the relationship between toxoplasmosis and iron levels in men's bodies. The results of the t-test analysis for men show an inverse relationship between toxoplasmosis and iron levels. At a P<0.05 level, there is a significant difference between the patient group and the control group, with the iron level in the patients being lower than that of the control group.

Vitamin D: The average amount of vitamin D among 50 people in the control group who do not have the disease is 190 ng/ml, whereas the average amount of vitamin D measured in the patient group is 150 ng/ml. The t-test analysis shows that the level of vitamin D has decreased in male patients (P<0.05). Consequently, we can conclude that there is an inverse relationship between toxoplasmosis and vitamin D (P<0.05).

Vitamin E: A statistical comparison of serum vitamin E levels between patients and healthy people shows a significant decrease in people with toxoplasmosis (P<0.01).

Vitamin C: A comparison of vitamin C levels between patients and healthy people shows a significant decrease in people with toxoplasmosis (P<0.001), indicating that toxoplasmosis decreases the level of serum vitamin C.

Discussion

Toxoplasmosis is a disease caused by the intracellular protozoan *T. gondii*. The presence of parasites within cells affects various cellular activities and mechanisms (1, 2). Research has revealed that the global spread of toxoplasmosis is alarmingly high and challenging. More than 40 million individuals in the United States carry the parasite, which affects approximately one-third of the human population (11). There is evidence that some vitamins and trace elements alleviate the disease and complications of toxoplasmosis, but the effect of this disease on these factors remains unclear (4-6). Furthermore, no study has been conducted on the epidemiology of this disease across different age groups and educational levels in the city of Maysan, Iraq.

Our study in the Iraqi city of Maysan revealed that the highest rate of toxoplasmosis occurs in

men between the ages of 31 and 40 with low education levels (up to a diploma). Additionally, the results demonstrated that the levels of Mg, Zn, Fe, and vitamins D, E, and C were significantly lower in patients compared to the control group.

An epidemiological study in America has shown that parasitic and infectious diseases, including toxoplasmosis, are more prevalent among older adults, foreign-born individuals, those with lower educational attainment, and individuals working in soil-related occupations (11). As observed in our results, the rate of toxoplasmosis among men in Maysan city appears to be similar to that of America, particularly in older adults between 31 to 40 years old with less education (up to diploma), thus corroborating our findings.

Vitamins and minerals (macro and microelements) play a significant role in the physiological activities of dairy animals, including appropriate growth, production, and reproduction, as well as immunity, oxidative metabolism, nutrition, and energy metabolism (12).

Regarding the reduced levels of trace elements such as magnesium, zinc, and iron observed in male patients with toxoplasmosis in this study, several pieces of evidence support our findings. For example, Yazar et al. (2003) demonstrated that the mean concentration of Mg in blood was significantly lower in seropositive patients than in their controls, for both females and males. In contrast, no change was observed in Zn levels (13). Additionally, Al-Masoudi et al. (2020) reported that serum zinc levels significantly decreased in women aged 34-43 years compared to the control group (14). It has also been shown that the average Zn concentration in serum from seropositive sheep was significantly ($P<0.05$) lower than in their controls. However, the mean concentration of Mg in serum did not change significantly ($P>0.05$) in sero-positive sheep compared to their controls (15). Most of these studies corroborate our results.

Trace elements such as Mg, Zn, and Fe are essential for normal physiological processes in the body. Magnesium (Mg) is required by many enzymes such as alkaline phosphatase and ATPase (16). Zinc is a necessary cofactor for many enzymes and plays an important role in synaptic transmission and immune function, particularly in the immune response against parasites (17). Iron performs several vital functions in the body. Iron deficiency can cause low birth weight, reduced immune-competence, poor cognitive development, behavioral complications, reduced work capacity, and maternal mortality (18). Some researchers have identified iron's role in ocular toxoplasmosis (OT), a form of toxoplasmosis that causes blindness. They observed reduced iron concentrations in the vitreous humor of human patients and iron accumulation in the retinas of mice. Treatment of mice with an iron-chelating compound successfully reduced their symptoms. These findings highlight the critical role of iron in the disease and suggest that controlling iron levels may lead to successful treatment (19). This information aligns with our study's results, which demonstrated decreased serum iron levels.

Regarding the relationship between vitamins and toxoplasmosis, some evidence suggests that acute toxoplasmosis (characterized by high levels of IgM) accompanied by vitamin D deficiency is more prevalent among women aged 15 to 25 years, which may play a role in women's abortions and congenital abnormalities. This indicates a significant relationship between toxoplasmosis and vitamin D deficiency (20). Furthermore, studies have shown a direct correlation between vitamin D deficiency and the severity of toxoplasmosis in Saudi women. Consequently, it is hypothesized that vitamin D supplementation may offer protection against toxoplasma infection (21). However, it remains unclear whether individuals with vitamin D deficiency are more susceptible to toxoplasmosis or if toxoplasmosis itself causes vitamin D deficiency.

In the case of vitamin E, it has been observed that school-aged children with *T. gondii* who

experience vitamin E deficiency also exhibit memory impairment, suggesting a direct relationship between vitamin E levels and memory function (22). Thus, the memory loss associated with toxoplasmosis may be attributed to vitamin E deficiency. With regard to vitamin C, while there is evidence of its therapeutic effect in toxoplasmosis (9), few studies have investigated its serum levels in patients with *T. gondii*. Interestingly, the aforementioned factors either play an antioxidant role or support antioxidants, and their reduction can evidently disrupt cellular metabolism and function.

In cardiovascular disease, magnesium supplementation has demonstrated antioxidant and anti-inflammatory properties in patients both with and without magnesium deficiency (23). Zinc, as an antioxidant, competes with iron (Fe) and copper (Cu) ions for binding to cell membranes and proteins, displacing these redox-active metals that catalyze the production of OH from H2O2. Zinc enhances the activation of antioxidant proteins, molecules, and enzymes such as glutathione (GSH), catalase, and SOD, while also reducing the activities of oxidant-promoting enzymes and inhibiting the generation of lipid peroxidation products (24).

Iron and oxidative stress play essential roles in cell biology and physiology but carry potential harmful effects, especially in overload states. There is a close connection and interplay between iron and oxidative stress, influencing metabolic liver disease and liver fibrosis (25). Both iron deficiency and iron overload may have detrimental consequences for health.

Vitamins C, E, D, and carotenoids are well-known antioxidants (26). Research has shown that humans possess a complex endogenous defense system designed to protect tissues from ROS/RNOS-induced cell injury. This system comprises special enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (including their cofactors selenium, zinc, manganese, and iron), sulfhydryl group donors (e.g., glutathione), and vitamins (e.g., vitamins E, C, and β-carotene), forming a network of functionally overlapping defense mechanisms (26).

ElSayed et al. (2024) demonstrated a beneficial effect of antioxidant vitamins (A, D, E) and trace elements (Cu, Mn, Se, Zn) on metabolic, antioxidant, and immunological markers in dromedary camels throughout their transition period (27). Given that researchers have recently attributed the pathophysiology of toxoplasmosis to increased oxidative stress (28), it appears that the reduction of antioxidant factors may be the primary cause of this increase. This finding could potentially inform and strengthen treatment approaches.

Conclusions

Our results suggest that higher levels of education may reduce the incidence of toxoplasmosis. Considering the antioxidant role of the aforementioned trace elements and vitamins, it appears that their reduced levels in serum increase the vulnerability of body cells to oxidative stress reactions. These findings not only could prove useful in the diagnosis and treatment of this disease but also warrant further investigation by researchers and clinicians in developing therapeutic approaches.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Ethical Considerations

This study was approved by the Human Research Ethics Committee of Shahid Chamran University of Ahvaz, Iran. Written informed consent was obtained from all participants prior to their involvement in the study.

Code of Ethics

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Authors' Contributions

Mahnaz Kasmati was responsible for guiding the main idea and supervising this study. Zahraa Hatem collected the data and prepared the initial manuscript with the supervisor's assistance. Haitham Mohammed aided in sampling and conducting biochemical tests. Mohammad Shafiei contributed to revising the manuscript and analyzing the interpretation of the data.

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