Original Article

Association of *Toxoplasma gondii* Infection with Serum Copper, Iron, and Vitamin C Levels in Women with Recurrent Spontaneous Abortion in Babylon Province, Iraq

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Abstract

Background: One of the factors contributing to poor pregnancy outcomes, such as recurrent spontaneous abortion (RSA), is *Toxoplasma gondii* infection. Micronutrient and antioxidant disorders levels of a particular nutrient or a physiologic molecule's capacity to withstand oxidation, such as in copper (Cu), iron (Fe), and vitamin C may increase the risk of pregnancy loss because of oxidative stress, inflammation brought on by infection, and placental dysfunction. **Objectives:** This study has investigated the serum Cu, Fe, and vitamin C levels of women with RSA and serological evidence of *T. gondii* infection in Babylon Province in Iraq and their comparison with healthy people. **Materials and Methods:** A case-control study was done in Babylon Maternity and Children's Hospital during the period of October 2025 to January 2026. A total of 100 women were recruited; 50 cases (RSA with *T. gondii* seropositivity) and 50 controls (no history of abortion and *T. gondii* seronegativity) were included. *Anti-T. gondii* IgG and IgM antibodies were determined by means of indirect immunodiffusion and enzyme immunoassay. Serum Cu and Fe levels were measured by atomic absorption spectrophotometry, and vitamin C was measured using the 2,4-dinitrophenyl hydrazine (DNPH) colorimetric method. Data analysis was performed using an independent-samples t-test and Pearson correlation. $p<0.05$ was considered significant. **Results:** Among the cases, the serological positive results were observed for 18/50 (36%) and 32/50 (64%) for IgM and IgG, respectively, whereas in the controls, all the results were negative ($p < 0.001$). Serum Cu was significantly higher in cases than controls (125.64 ± 18.72 vs 112.41 ± 16.85 ug/dL; $P = 0.001$). Serum Fe level was significantly low in cases (61.73 ± 10.48) vs (87.92 ± 13.31) micro gram/dL; $p < 0.001$). Vitamin C was also significantly decreased in cases (0.69±0.14 vs 1.11 ± 0.19 mg/dL; $p< 0.001$). In the case group, Cu had a negative correlation with Fe ($r = -0.48$; $p = 0.003$) whereas Fe had a positive correlation with vitamin C ($r = 0.59$; $p < 0.001$). **Conclusions:** Women with RSA and *T. gondii* infection were found to have higher concentrations of copper and lower concentrations of iron and vitamin C, indicating an infection-associated inflammatory and oxidative milieu, which can possibly lead to loss of pregnancy.

Keyword: *T. gondii*, Oxidative stress, Recurrent spontaneous abortion, Copper, Iron, Vitamin C, Iraq

Introduction

The obligatory intracellular protozoan parasite *Toxoplasma gondii* is the causative agent of toxoplasmosis, a globally distributed infection of major public health importance, particularly during pregnancy [1,2]. The parasite has a heteroxenous life cycle in which felids act as definitive hosts, whereas humans and other warm-blooded animals serve as intermediate hosts [3]. Human infection is mainly acquired through ingestion of oocysts from contaminated soil, water, or food, or through consumption of raw or undercooked meat containing tissue cysts. Transplacental transmission may occur when primary maternal infection develops during pregnancy [4]. Maternal infection during gestation is clinically important because *T. gondii* can cross the placental barrier and lead to

adverse pregnancy outcomes, including miscarriage, fetal death, intrauterine growth restriction, and congenital abnormalities [3,5]. Fetal infection may result in severe manifestations involving the ocular and central nervous systems, including retinochoroiditis and hydrocephalus [5]. Recurrent miscarriage is a multifactorial disorder involving genetic, anatomical, endocrine, immunological, and infectious factors. Among infectious causes, *T. gondii* has been recognized as a potential contributor to recurrent abortion because of its ability to induce placental inflammation and interfere with embryonic development [6]. Previous studies have demonstrated associations between *T. gondii* seropositivity, antibody titers, and increased frequency of spontaneous abortion [7]. A meta-analysis reported a significant association between *Toxoplasma* IgG seropositivity and spontaneous abortion, suggesting that infection may contribute to pregnancy loss in affected populations [8]. Successful pregnancy maintenance depends on adequate maternal nutritional and biochemical status, including essential trace elements and antioxidant vitamins. Iron plays a critical role in hemoglobin synthesis, oxygen transport, maternal blood volume expansion, placental development, and fetal growth. Iron deficiency during pregnancy is associated with anemia, preterm delivery, and impaired fetal development [9]. Copper is an essential trace element involved in oxidation-reduction reactions, antioxidant enzyme activity, nervous system development, and immune regulation, making it particularly important during pregnancy [10]. Vitamin C (ascorbic acid) is a potent antioxidant that enhances non-heme iron absorption and contributes to immune modulation during infection [11]. Alterations in maternal iron, copper, and vitamin C status have been associated with adverse pregnancy outcomes, including miscarriage and preterm birth, through mechanisms involving impaired oxygen delivery, oxidative stress, and abnormal placental development [10]. Infectious and inflammatory conditions may further disrupt micronutrient metabolism and alter circulating concentrations. Iron homeostasis is regulated largely by hepcidin, which controls intestinal iron absorption and iron release from storage sites through regulation of ferroportin. Although hepcidin levels are normally reduced during pregnancy to increase iron availability, inflammatory and infectious conditions can stimulate hepcidin production, leading to functional hypoferremia independent of dietary iron intake [12–14]. Copper contributes to antioxidant defense through enzymes such as Cu/Zn-superoxide dismutase and participates in connective tissue formation and immune responses. However, because copper is a redox-active metal, abnormal regulation may promote oxidative injury. Both copper deficiency and excessive copper concentrations during pregnancy have been associated with unfavorable maternal and fetal outcomes [15]. Vitamin C contributes to collagen synthesis, immune function, and antioxidant defense. Increased maternal requirements and placental transfer during pregnancy may reduce circulating vitamin C concentrations, especially among women exposed to oxidative or inflammatory stress [16]. Although clinical trials have not consistently demonstrated a preventive effect of routine vitamin C supplementation on adverse obstetric outcomes, reduced maternal vitamin C status may represent a marker of oxidative stress and nutritional insufficiency in women with recurrent miscarriage. In agreement with this concept, previous studies have reported altered serum trace element and antioxidant profiles among women with *T. gondii* infection, suggesting that parasitic infection may disturb

micronutrient homeostasis and influence pregnancy outcomes [17]. Therefore, the present study aimed to evaluate serum copper, iron, and vitamin C concentrations in women with recurrent abortion who were seropositive for *Toxoplasma gondii* compared with an appropriate control group in Babylon Province, Iraq.

Materials and Methods

Study Design and Setting

This study based case-control study was conducted at Babylon Maternity and Children Hospital, Iraq, between October 2025 and January 2026. The main objective was to look at the relation of *T. gondii* infection with the serum values of copper, iron, vitamin C in women having repeated spontaneous abortions. All procedures for the study were conducted in consultation with the obstetrics and microbiology departments of the hospital with standardized biosafety and ethical procedures.

Sample Size Calculation

The required sample size was calculated based on the G*Power software, version 3.1, with assumptions of a medium effect size (Cohen's d = 0.6) (as per previous literature that assesses differences of trace elements in pregnant women who are infected vs non-infected) with the following parameters: power level of 80%, and alpha level of 0.05. The minimum sample size per group needed was 45 participants. In order to accommodate for potential dropouts and incomplete data, 50 women were included in each group and a final sample size of 100 participants was obtained.

Study Population

A total of 100 women were included in this study and divided into two equal groups. The case group (n = 50) included women who had a documented history of two or more consecutive spontaneous abortions and proved to have *T. gondii* infection. The control group (n = 50) consisted of apparently healthy women with no history of miscarriage and negative results on serological tests for the presence of *T. gondii*. Participants were matched according to age (18-45 years), residence and socioeconomic status to minimize confounding factors. The study participants were recruited from the youths attending the outpatient clinics of the hospital after obtaining their written informed consent and clinical data were extracted by directly interviewing the participants and reviewing medical records.

Matching Procedure

Cases and controls were frequency matched according to age (±2 years, adults and children in separate sub-analyses), area of residence (urban/rural) and socioeconomic status. Matching variables were further assessed during statistical analysis to ensure that they were comparable between groups. Any residual imbalance was controlled for in the multivariate models.

Eligibility Criteria: Inclusion and Exclusion

The research participants were women between 18-45 years of age who were not suffering from any chronic diseases and had not been using vitamin or mineral supplements in the past 3 months. Women were excluded if they had systemic conditions such as diabetes mellitus, hypertension, and thyroid, renal or hepatic diseases or if they were smokers or alcohol consumers. Additionally, women undergoing antioxidant therapy, taking immunosuppressive drugs or those diagnosed with other infectious diseases were not eligible. These criteria were used stringently to be sure that obtained

biochemical variations were mainly due to *T. gondii* infection and reproductive sex status.

Sample Collection and Sample Preparation

Venous blood samples (5mL) were taken aseptically from each participant using disposable syringes. Samples were put into plain tubes and left to clot at room temperature for 20-30 minutes. The clotted blood was then centrifuged. The obtained serum was then transferred carefully into sterile Eppendorf tubes and stored at -20 values centigrade until further biochemical analysis. All samples were processed within 2 h of sample collection to avoid degradation of labile analytes such as vitamin C.

Detection of *Toxoplasma Gondii* Infection

Serological tests for *T. gondii* antibodies were carried out with a commercial enzyme-linked immunosorbent assay (ELISA) kit (BioCheck Inc., USA), according to directions of the manufacturer. Both IgM and IgG antibodies were determined to distinguish between acute and chronic infections. The absorbance values were detected at 450 nm with a microplate reader equipped with automatism. Based on the reference standards included in the kit, samples were interpreted as IgM positive (acute infection), IgG positive (past infection) or negative (uninfected). Only the women who had positive IgM or IgG antibodies were selected in the case group and the other seronegative women for the control group.

Estimation of Serum Copper and Iron

Serum concentrations of copper and iron were determined by atomic absorption spectrophotometry (AAS) using ShimadzuAA-7000 instrument (Shimadzu Corporation, Japan). However, before measurement, serum samples were diluted 1:10 with deionized water. Standard calibration curves were made by certified reference materials. The levels of the metal were measured in micrograms per deciliter (ug/dL). To ensure analytical accuracy, quality control sera and reagent blanks were included in each batch of analyses and all assays were done in duplicate.

Estimation of Vitamin C in Serum

Serum vitamin C was measured by the 2,4-DNTH (2,4-Dinitrophenylhydrazine) colorimetric procedure of Omaye, Turnbull and Sauberlich (1979) with minor modifications. Briefly permissive and 0.5 mL of serum mixed with 1.5 mL of 5% trichloroacetic acid (TCA) for the precipitation of proteins. After centrifugation, 0.5 mL of the supernatant was reacted with DNPH reagent and incubated at 37 degC for 3 hours. The reaction was stopped by adding 85% sulfuric acid and absorbance was read at 520 nm using spectrophotometer. Vitamin C concentration was determined against a standard curve of ascorbic acid and expressed in values of milligrams per deciliter (mg/dL)

Statistical Analysis

Data were analyzed using IBM statistics tool (statistics) v.26.0. Normality of distribution was tested in the Shapiro-Wilk test. The continuous ones were expressed as mean +- standard deviation (SD) when the data were normally distributed, and as median (interquartile range) when data were not. Independent-samples t-test or Mann--Whitney U test was as appropriate. Effect sizes (Cohen's d) were computed in order to measure the magnitude of differences between groups. Pearson or Spearman correlation coefficients were applied to compute the relationship between the levels of micronutrients and the titers to antibodies. To control the effects of potential confounders (age, BMI, residence, socioeconomic status), multivariate linear regression models were constructed using serum

copper, iron and vitamin C as dependent variables and toxoplasma serostatus as independent variable. A p-value <0.05 was deemed statistically significant and confidence intervals (CI) were calculated at 95%.

Quality Control

All reagents were analytical grade and glassware was acid washed prior to use to remove any trace element contamination. Each assay was run in duplicate to provide a measure of reproducibility and each instrument calibration was checked every day. Internal control samples were run with each set of measurements, to ensure that the analysis is reliable throughout the study. All the assays of the ELIAs were conducted as per manufacturer's instructions along with internal positive and negative controls. Calibration curves were checked preceding each of the batch runs. For atomic absorption spectrophotometry, standardized reference materials were used to determine correctness and duplicate readings for 10% of samples were measured to determine reproducibility. Hemolysis samples were discarded, to avoid trace element contamination. The limits of detection (LOD & LOQ) were checked before analyzing the results.

Ethical Considerations

The research protocol was reviewed and approved by the Institutional Review Board (IRB) of the Babylon Health Directorate prior to the beginning of the data collection. All the study procedures complied with the ethical principles of the Declaration of Helsinki (2013 revision). Confidentiality was ensured throughout the study by anonymity of the participant data and all subjects provided written informed consent prior to enrollment.

Results

The current study included 100 women were enrolled in this study, 50 women with recurrent spontaneous abortion with a positive test for Toxoplasma gondii infection (case group) and 50 healthy women who did not suffer miscarriage (control group). All sera samples were screened for T. gondii IgG and IgM antibodies by using the ELISA test , Among the group with the case, the detection of IgM antibodies occurred in 18 women (36%) and IgG antibodies in 32 women (64%), besides indicating acute infection, this test also indicates chronic or past infection. None of the control participants had positive results for any of the three antibodies (neither IgM antibody nor IgG antibody). The significant difference in *T. gondii* seropositivity between the groups ($p < 0.001$) confirms the presence of infection only in women with recurrent abortion, which proves the validity of the selection criteria and the study design.

Table1: Distribution of anti-*T gondii* IgG and IgM antibodies for miscarriage woman

Antibody type	Case group (n = 50)	Control group (n = 50)	p-value
IgM positive	18 (36%)	0 (0%)	< 0.001*
IgG positive	32 (64%)	0 (0%)	< 0.001*

The mean serum copper level in the case group was 125.64 ± 18.72 ug/dL which was significantly higher compared with control group (112.41 ± 16.85 ug/dL, p= 0.001). Increased serum copper in infected women could be considered an acute-phase response as there are increases in copper binding proteins (e.g. ceruloplasmin) during infection and inflammation. Elevated levels of copper have been shown to be compensatory antioxidant responses in oxidative stress from toxoplasmosis.

Table 2: Comparison of serum levels of copper between study groups

Group	Mean ± SD (µg/dL)	t-value	p-value
Cases	125.64 ± 18.72	3.42	0.001*
Controls	112.41 ± 16.85	—	—

The mean serum iron level in the case group was 61.73 ±10.48 ug/dL which was significantly less than that in the control group (87.92 ±13.31 ug/dL, $p < 0.001$). The marked decrease in serum iron in women with infection from *T. gondii* raises the possibility of sequestration of iron in macrophages and hepatocytes as part of host defense mechanism to limit proliferation of the parasite. This is the same pattern observed in hypoferremia resulting from inflammation above and above levels seen in the chronic infections.

Table 3: Determination of serum iron concentration in the study groups

Group	Mean ± SD (µg/dL)	t-value	p-value
Cases	61.73 ± 10.48	10.28	< 0.001*
Controls	87.92 ± 13.31	—	—

The mean serum vitamin C concentration of case group was found 0.69 ± 0.14 mg/dL whereas 1.11 ± 0.19 mg/dL in controls (p less than 0.001). Vitamin C was significantly depleted in T. gondii-infected women and it was thought that heightened oxidative stress would produce a greater demand for oxidative antioxidants during inflammatory responses. Deficiency of vitamin C may lessen protection of the immune system and retard tissue repair in the body during pregnancy so that they may contribute to abortion risk.

Table 4: Comparison of serum vitamin C in study groups

Group	Mean ± SD (mg/dL)	t-value	p-value
Cases	0.69 ± 0.14	11.21	< 0.001*
Controls	1.11 ± 0.19	—	—

Pearson correlation coefficients were performed to test the relationship between micronutrient status and the levels of antibodies against *T. gondii*. A negative correlation was found between serum copper and iron ($r = -0.48$ $p = 0.003$), and a positive correlation was found between vitamin C and iron ($r = 0.59$, $p < 0.001$). There was no significant correlation between copper and vitamin C ($r=-0.21$, $p=0.143$). These correlations indicate the presence of a coupled pathophysiological mechanism of elevated copper (an inflammatory marker) that is associated with low iron and vitamin C concentrations. The positive correlation between iron and vitamin C can relate to the synergism between ascorbic acid and iron for the absorption of iron as well as protection against oxidative stress.

Table 5: The correlation coefficients of biochemical parameters (case group)

Variable pair	Correlation coefficient (r)	p-value	Relationship
Copper vs. Iron	–0.48	0.003*	Negative
Iron vs. Vitamin C	+0.59	< 0.001*	Positive
Copper vs. Vitamin C	–0.21	0.143	Not significant

Discussion

The current case-control study conducted in Babylon Province, Iraq, demonstrated a consistent biochemical pattern involving serum copper, iron, and vitamin C concentrations among women with recurrent spontaneous abortion (RSA) associated with positive *Toxoplasma gondii* serology compared with controls. This pattern supports the presence of a combined inflammatory and oxidative environment in toxoplasmosis-associated RSA, where alterations in redox-active trace elements and depletion of antioxidant reserves may contribute to placental dysfunction and increased

susceptibility to pregnancy loss. *Toxoplasma gondii* infection is a recognized cause of adverse reproductive outcomes. The parasite is widely distributed worldwide and has particular clinical significance during pregnancy because of the risk of vertical transmission, which may result in miscarriage, fetal loss, or congenital disease. Previous reviews have emphasized that primary maternal infection during pregnancy represents the highest risk for fetal infection and that gestational age influences both transmission rates and severity of fetal consequences [18,19]. Epidemiological studies and systematic analyses have demonstrated an association between toxoplasmosis exposure and spontaneous abortion history [20,21]. Furthermore, detection of *T. gondii* DNA or antigenic evidence in abortion-related tissues provides biological support for the contribution of infection to pregnancy loss in a proportion of cases [22]. The elevated serum copper concentrations observed among women with RSA may be explained by several mechanisms. Copper metabolism during pregnancy is closely associated with transport proteins and inflammatory responses. Ceruloplasmin, the major copper carrying protein in plasma, is an acute-phase reactant; therefore, increased serum copper may reflect inflammatory activation rather than increased intake [23]. In addition, copper participates in redox reactions and antioxidant enzyme systems; however, disturbed copper regulation may enhance reactive oxygen species production and contribute to oxidative tissue injury. The biological interaction between copper and iron during pregnancy and fetal development has been extensively described [24]. Previous investigations have reported increased serum copper and ceruloplasmin concentrations among women with recurrent pregnancy loss, suggesting an inflammatory response associated with abnormal copper metabolism [25]. Moreover, altered maternal copper status has been linked with adverse pregnancy outcomes, including preterm birth and hypertensive complications, indicating that copper may represent a biomarker of pregnancy-related inflammation [26]. The significantly reduced serum iron concentrations detected in the present study are biologically plausible in the context of infection-associated pregnancy complications. Iron requirements increase considerably during gestation to support maternal hematological expansion, placental development, and fetal growth. Iron deficiency is a recognized contributor to adverse pregnancy outcomes. In addition to inadequate nutritional intake, infection and inflammation can decrease circulating iron through hepcidin-mediated mechanisms. Hepcidin, the central regulator of systemic iron metabolism, is physiologically suppressed during pregnancy but increases during inflammatory states, resulting in reduced intestinal iron absorption and decreased iron release from macrophages and storage tissues [27–29]. This host-defense mechanism, known as nutritional immunity, may contribute to functional iron deficiency during chronic inflammatory conditions. Therefore, reduced serum iron among women with toxoplasmosis-associated RSA may reflect a combination of increased pregnancy requirements, underlying nutritional deficiency, and inflammation-induced iron redistribution. Interpretation of iron status in inflammatory conditions requires caution because conventional iron markers may be affected by acute-phase responses. Several studies recommend evaluating ferritin together with inflammatory markers such as C-reactive protein and transferrin saturation to distinguish true iron deficiency from inflammation-associated alterations [30,31]. These findings emphasize the importance of including comprehensive iron-related biomarkers in future

studies investigating toxoplasmosis-associated pregnancy complications. The decreased serum vitamin C concentrations observed among cases may indicate increased oxidative stress and consumption of antioxidant reserves during infection. Vitamin C contributes to immune regulation, antioxidant protection, and iron metabolism by enhancing intestinal absorption of non-heme iron and maintaining iron in the reduced ferrous state. Oxidative stress has been recognized as an important pathogenic mechanism in recurrent pregnancy loss through impaired implantation, trophoblast dysfunction, and placental injury [32]. In toxoplasmosis, experimental and clinical evidence suggests increased oxidative stress, inflammatory activation, trophoblast apoptosis, and placental oxidative damage [33,34]. Furthermore, oxidative imbalance is considered a common pathway involved in several pregnancy complications [35]. Experimental studies have demonstrated that vitamin C supplementation may reduce placental oxidative stress and improve pregnancy outcomes under conditions of increased oxidative burden [36]. The correlation pattern observed in the present study, characterized by a negative relationship between copper and iron and a positive relationship between iron and vitamin C, is consistent with known biochemical interactions. Copper-containing proteins, particularly ceruloplasmin, contribute to iron metabolism through ferroxidase activity and facilitate iron export from cells; therefore, inflammatory changes affecting copper metabolism may influence circulating iron availability [23,24,27]. Similarly, vitamin C improves iron bioavailability by enhancing absorption and maintaining iron in a reduced form, suggesting that reduced vitamin C concentrations may contribute to impaired iron status, especially in populations with high dependence on non-heme dietary iron sources [37]. The current findings are consistent with previous reports describing altered antioxidant and trace element profiles among women infected with *T. gondii*, including studies from Iraq demonstrating changes in micronutrient concentrations and antioxidant status among infected women [17,38]. Although differences exist among regional studies regarding methodology and population characteristics, available evidence supports a relationship between toxoplasmosis, oxidative stress, and micronutrient imbalance in pregnancy-related complications [33,34]. In addition, observational studies and meta-analyses have consistently reported associations between toxoplasmosis exposure and spontaneous abortion, supporting the biological relevance of the current biochemical observations [20,21]. These findings support an integrated approach for evaluating recurrent spontaneous abortion in regions where toxoplasmosis is prevalent. Assessment should include confirmation of *T. gondii* exposure using IgG and IgM serology, detailed obstetric history, evaluation of iron status including ferritin and inflammatory biomarkers, and assessment of antioxidant nutritional status, particularly vitamin C. Nutritional interventions should be guided by laboratory-confirmed deficiencies rather than empirical supplementation alone. A major strength of this study is the inclusion of clinically characterized participants and direct measurement of *T. gondii* serostatus together with serum micronutrient concentrations. Nevertheless, the case-control design limits causal interpretation, and residual confounding related to dietary intake, socioeconomic status, and unmeasured inflammatory factors cannot be excluded. Future investigations should include measurements of ferritin, transferrin saturation, C-reactive protein, and hepcidin to differentiate nutritional iron deficiency from inflammation-induced iron sequestration [27,30,31].

Limitations

There are a few limitations to this study. First, it is a case-control study that does not permit the inference of causal relationships between toxoplasmosis and alterations in micronutrients. Second, inflammatory indices like C-reactive protein (CRP), ferritin or hepcidin were not measured and this could have added mechanistic insight into iron metabolism during a bout of infection. Third, dietary intake assessment was not made, which impinges on the interpretation of nutritional status. Finally, the relatively small sample size and recruitment to a single province are potential limitations for the generalizability to others.

Conclusion

The study revealed statistically significant differences for selected micronutrients/ antioxidant status between the study subjects with recurrent spontaneous abortion (RSA) (seropositive for *Toxoplasma gondii*) and the control group. Serum copper (Cu) was significantly elevated in the case group and may therefore be associated with infection-related inflammation/acute phase response. Serum iron (Fe) was significantly reduced in the case group, in keeping with the patterns of inflammation-associated hypoferremia, and possible adverse effects upon maintenance of pregnancy. Serum vitamin C was significantly decreased in the case group indicating the occurrence of increased oxidative stress and antioxidant depletion in toxoplasmosis associated RSA. The combination pattern (high Cu + low Fe + low vitamin C) suggests pro-inflammatory, oxidative environment: possibly owing to the malfunction of the placenta with higher susceptibility to pregnancy loss in women with infection.

Conflict of Interest

The authors state that they have no conflicts of interest.

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