

# The Impact of *Toxoplasma gondii* Infection on The Serum Zinc, Vitamin D and Malondialdehyde Levels among Recurrent Miscarriage Women in Babylon Province-Iraq

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## ABSTRACT

*Toxoplasma gondii* (*T. gondii*) is an obligate intracellular protozoan parasite causing toxoplasmosis in animals and humans. It causes serious problems to the pregnant women that lead to incidence an abortion. Essential vitamins and minerals (for example vitamin D and zinc metals) are support maternal health and fetal development throughout gestation through processes that are integrated across maternal, placental and fetal compartments. This study was aimed to investigate the alterations in the levels of vitamins D, element Zn and MDA in the sera of women with toxoplasmosis with a history of recurrent miscarriage, and compare them with the results of age matched healthy volunteers as control group. Sixty patients with positive anti-T gondii (IgG) antibodies and 28 healthy individuals were included in this study. The levels of serum Zn was measured by Atomic absorption spectrophotometry Systems, while serum vitamin D and MDA concentrations were measured by using enzyme-linked immunoassay (ELISA) technique. The level of serum zinc significantly decrease in women with age (34-43years) it was (37.73 µg/dl) in compare with control group, while level of Vitamin D was significantly decrease in women with age (24-33years) it was (146.3ng/mL). The present study also confirms that level of Malondialdehyde (MDA) in miscarriage women was slightly increase (183.249 ± 83.169ng/mL) in compare with control group (162.678±65.589 ng/mL) respectively in age group 24-33years. In conclusion, the results indicated that the levels of antioxidant vitamins, zinc and MDA may have an important role to increase possibility of exposure to toxoplasmosis in women.

**Keywords:** *Toxoplasma gondii*, miscarriage women, Zn, Vitamin D and MDA.

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## INTRODUCTION

*Toxoplasma gondii* is an obligate intracellular protozoan parasite causing toxoplasmosis in animals and humans, with approximately a third of the world's human population estimated to have been exposed to the parasite [1]. *Toxoplasma gondii* is the most serious results of infections and occurs when another contracts the infection during pregnancy, the organism can causes abortion of the fetus or can cause several malformation at birth, if the women is already infected prior to conception there is a little danger but the greatest risk of congenital toxoplasmosis occurs during the first trimester of pregnancy [2].

Essential vitamins and minerals (for example vitamin D and zinc metals) are support maternal health and fetal development throughout gestation through processes that are integrated across maternal, placental and fetal compartments. Deficiencies or excesses in these trace elements are usually related to human diseases. There is relationship between antioxidant vitamin D as well as trace element zinc with toxoplasmosis [3].

Zinc (Zn) occurs as an important trace metal in all living organisms and all tissues, also an essential trace element for immune function that plays a role immune response against parasites [4].

Vitamin D regulates responsiveness of the immune system and how serum status modulates the host defense against pathogens. It is necessary for the functioning of more than 300 different enzymes, which means it plays a role in a great number of bodily activities. Some of those activities are critical during pregnancy, because they involve embryo and fetal development as well as infant growth [5].

Pathogenesis of *T. gondii* is linked with induction of oxidative stress, Lipid peroxidation and oxidative stress have been regarded as the main factors responsible for the generation of free radicals. Malondialdehyde (MDA), is a biomarker of lipid peroxidation and oxidative stress, and increases in chronic acquired toxoplasmosis [6].

This study was aimed to investigate the alterations in the levels of antioxidant enzyme Zinc, non-enzymatic antioxidant vitamin D and lipid peroxidation (MDA) in the sera of women with toxoplasmosis with a history of miscarriage and compare them with healthy volunteers as control group for a better understanding of toxoplasmosis pathogenesis.

## Materials and Methods

The current study was conducted in the Department of Microbiology and Biochemistry, College of Medicine, University of Babylon. The study period extended from April 2019 to end of February 2020.

## Patients

One hundred and seventy-five (175) aborted women were selected randomly to determine the role of *T. gondii* in their abortions. Patients were from Obstetrics and Gynecology Clinic, Emergency and consultant for the obstetrics and gynecology hospital and Al-Hilla surgical hospital in Babylon city, Iraq. The age of patients ranged between (14 - 43) years old. Blood samples were taken from patients and control, Control group, those included 28 apparently healthy women without past or present history of spontaneous abortion and not suffer any disease. A general information about each patient were recorded, included age, living area, main job, number of children, number of miscarriages, week of miscarriage and level of education.

## Sample Collection

Five milliliter blood was collected from miscarriages women in the first and second trimester of pregnancy. Serum was separated immediately and divided into two parts. The first part was stored at  $-20^{\circ}\text{C}$  until assayed for level of to determine some biochemical parameters such as MDA, indicator of lipid peroxidation, enzymatic antioxidant zinc and non-enzymatic antioxidant vitamin D. The second part of serum was used immediately for detection of antibody.

## Detection of Toxoplasma Antibody

Toxoplasmosis usually is diagnosed on the basis of antibody detection. The test was done according to [7]. All sera samples were screened for *T. gondii* IgG and IgM antibodies by using rapid diagnostic immunochromatographic test (Tox IgG/IgM Rapid Test Cassette) according to the manufacturer's instructions (Egyptian company for Biotechnology, Egypt).

## Biochemical Tests

### Determination of serum zinc

The miscarriage women participating in this study were classified according to age into three groups:

- First Group: miscarriage women (n=20) aged 14-23, Control (n=16).
- Second Group: miscarriage women (n=27) aged 24-33, Control (n=9).
- Third Group: miscarriage women (n=13) aged 34-43, Control (n=3).

Atomic absorption spectrophotometry Systems (Milano, Italia) was used to (IgG Ab), Zinc reacts with the chromogen present in the reagent, forming a colored compound which color intensity is proportional to the zinc concentration present in the sample. determine Zinc level in serum in recurrent spontaneous aborted women with chronic infection

### Determination of serum Vitamin D

The kit is a solid phase enzyme-linked immunoassay (ELISA), based on the principle of competitive binding. Anti-Vitamin D antibody coated wells are incubated with Vitamin D standards, controls, samples, and Vitamin D-Biotin conjugate at room temperature for (90) minutes. During the incubation, a fixed amount of biotin-labeled vitamin D competes with the endogenous Vitamin D in the sample, standard, or quality control serum for a fixed number of binding sites on the anti-Vitamin D antibody. Following a wash step, bound Vitamin D-Biotin is detected with streptavidin-HRP (SA-HRP). SA-HRP conjugate immunologically bound to the well progressively decreases

as the concentration of Vitamin D in the specimen increases. Unbound SA-HRP conjugate is then removed and the wells are washed. Next, a solution of TMB Reagent is added and incubated at room temperature for (30) minutes, resulting in the development of the color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450 nm. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The color intensity will be inversely proportional to the amount of 25-OH Vitamin D in the sample. The assay measures both the 25-OH Vitamin D<sub>2</sub> and D<sub>3</sub>. The total assay procedure run time is 2.5 hours.

## Determination of the level of lipid peroxidation (Malondialdehyde)

The serum Malondialdehyde level, which is one of the main products of lipid peroxidation, has been estimated from During the interaction between the lipid peroxides, mainly monomalealdehyde and the thiobiotyric acid (TBA). The microtiter plate provided in this kit has been pre-coated with MDA. During the reaction, MDA in the sample or standard competes with a fixed amount of MDA on the solid phase supporter for sites on the Biotinylated Detection Ab specific to MDA. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) are added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450nm  $\pm$  2nm. The concentration of MDA in the samples is then determined by comparing the OD of the samples to the standard curve.

## Statistical Analysis

Data were analyzed by using Statistical Analysis System (SAS) - 2012 to study the effect of different factors in different parameters which were used in this study. Least significant difference test (LSD) ( $p$  value  $<$  0.05) was used to compare between means of different groups in this study [8].

## Results and Discussion

### Distribution of *T. gondii* Infection in Recurrent Miscarriage Women.

The current study included 175 miscarriage women were enrolled and screened for the presence of anti-toxoplasma IgG and IgM antibodies, plus 28 natural healthy women (who did not suffer miscarriage) as control group. Both case group and control group are involved in the sample of the study. Toxoplasmosis infection appears to affect pregnancy in infected group compared with control group.

The seroprevalence of *Toxoplasma gondii* IgG and IgM antibodies result revealed that 60 (34.28%) of 175 miscarriage women were positive for anti-toxoplasma-antibody IgG and 115 (65.71%) of 175 were negative for anti-toxoplasma-antibody-IgG, while revealed that there was no case of acute infection IgM antibody table (1).

The results of this study confirm that the prevalence of toxoplasmosis was 34.28% when used LAT test; this result was in accordance with the results of [9] who confirmed the infection rate was 49.7% when they used LAT method as a screening test for the detection of toxoplasmosis. LAT provides an excellent format for routine serological screening because of its high specificity, low cost, and easy to use.

**Table 1.** Distribution of anti-*T. gondii* IgG and IgM antibodies for aborted woman using rapid immunochromatographic method.

Anti-Toxoplasma antibodies	Examined NO.	Positive NO.		Negative NO.	
		No.	%	No.	%
IgG	175	60	34.28	115	65.7
IgM	175	0	0	0	0
IgM+IgG	175	0	0	0	0

The result of this study was agreeing with other such as study in Kirkuk, Iraq, proportion of the examined patients' sera showed evidence of infection (IgG), whilst no has evidence of current infection (IgM) [10]. In present study the highest seroprevalence was in anti-*T. gondii* IgG, this is due to that in *T. gondii* infection the maturation of antibody (*T. gondii* IgG) in response to infection generally are slow [11]. However, seroprevalence of toxoplasmosis in the present study was similar to many studies around the world and some Arabic countries in women with Bad Obstetric History (BOH), Although, the result of present study is agreement with other studies in Iraq, that were 37.5% for IgG in Erbil [12]. While, in Diyala province found high infection were 37 (46.26%) women out of eighty were infected with toxoplasmosis [13]. A recent study in Iraq showed a different result than present study in which the ratio of infected female with toxoplasmosis was 330 (98.51%) [14].

None of the women in our study were positive for Toxoplasma specific IgM antibodies, indicating that no one has acute infection, This is in accordance with the study done by [15,16,17] who reported *Toxoplasma gondii* seroprevalence of 13.8%, 15.67% and 16.47% respectively. and the study in Baghdad city shows that the median values of IgM -Toxoplasma antibodies titer was statistically significant .It is higher in the group of pregnant women with positive diagnosis (1.98±0.850), compared to those with negative diagnosis of toxoplasmosis (0.41±0.315) [18]. This relatively high percent of toxoplasmosis , in current study may be due to many factors including the sample size which was only 175 This indicates that is considerable number of females in this society harboring the parasite, transmitter to other people and it is representing a real problem that should not be neglected and must receive attention from health authorities.

#### Distribution of Zinc Among Recurrent Spontaneous Aborted Women according to Age Group

The results in table (2) confirm that the first group, Mean serum Zn level in the (20) seropositive toxoplasmosis miscarriage women aged (14-23 years), was (40.75±7.26) while in control group(16) the mean serum Zn level was (42.07 ± 10.21),( Sig. Value = 0.6677). In second group, Mean serum Zn level was (41.59 ± 11.39) while in healthy women(9) was (39.7 ± 11.17), the results in third group, Mean serum Zn level was (37.78 ± 2.96 ) while in healthy women(3) was (67.7 ± 7.778),( Sig. Value = **0.0001**).In this group the Mean serum Zn level in seropositive toxoplasmosis abortive women were significantly ( P<0.05) lower than in control group( 67.7 ± 7.778 ).

**Table 2.** Means ± SD of Zinc (µg/dL) in The Serum of Toxoplasmosis Recurrent Miscarriage women and Controls According to Age Group

Parameter	T test	1 <sup>st</sup> Group 14-23 years		2 <sup>nd</sup> Group 24-33 years		3 <sup>rd</sup> Group 34-43 years	
		Abortive	Control	Abortive	Control	Abortive	Control
Zinc	Mean	40.75	42.07	41.59	39.7	37.78↓	67.7↑
	SD	7.26	10.21	11.39	11.17	2.96	7.778
	No	20	16	27	9	13	3
Sig. Value		0.6534		0.6677		<b>0.0001*</b>	

This decrease of zinc may be due to deficiency because of redistribution of serum zinc into the liver and because of decrease in serum albumin concentration where over 70% of the serum zinc is bound to albumin [19], or this decrease in the level of zinc may be due to the leukocyte endogenous mediators ( interleukins), released from activated phagocytic cells causing a lowering of zinc levels resulting from increased synthesis of phagocytic cells causing a lowering of zinc levels resulting from increased synthesis of metallothionine in liver and other tissues [20,21]. a reduction in serum zinc this mean zinc necessary for immune function and play a role in immune response against parasites [22].

Our results was agree with study done in India, Found that in SAb women with the mean age (24.85 ± 0.32 ) the mean serum levels of Zn 1.430 ± 0.03 as compared to control with the mean age (23.65 ± 0.33 ) 1.463 ± 0.05. revealed that the mean serum levels of Zn were higher in control subjects as compared to SAb subjects [23]. Other study

recorded the mean concentration of serum zinc levels in RSA women aged (21-42 years) was 74.98 ± 11.88 µg/dl, Also, serum zinc level in healthy controls was 82.90 ± 12.36 µg/dl [24]. While study in Mosul city ,showed that in women aged (19 – 42) years the mean levels of serum zinc (12.1 ± 5.6 ) were significantly ( P<0.05) lower than in control group (13.5 ± 3.0 ) [25].

Because zinc is important for normal placental development, deficiency may result in impairment in utero a cquisition of maternal antibodies by the child as well [26]. Therefore, one of the potential beneficial approaches to improve the immune defense against *T. gondii* is zinc supplementation, since due to its catalytic and regulatory functions it can enhance resistance to infections [27].

### Distribution of Vitamin D Among Recurrent Spontaneous Aborted Women according to Age Group

Effects of vitamin D deficiency in pregnancy have been associated with some adverse pregnancy outcomes. As shown in table (3). In the first group which included women aged (14-23 years), the mean level of Vitamin D in miscarriage aborted women with chronic infection (IgG Ab) was (148.9±43.76), while in healthy women the level of Vitamin D was (165.5±37.58) there was a non-significant difference in the spontaneous abortive women infected with *T.gondii* when compared with control group (Sig. value = 0.2374).

As for the second group of women participating in the study who are aged (24-33), the mean level of Vitamin D

in miscarriage women with chronic infection (IgG Ab) was (146.3±38.82), while in healthy women the level of Vitamin D was (181.9±17.51). there was significant decrease in the level of Vitamin D among 2<sup>nd</sup> group (Sig. value=0.0123) .

As for the third group, which includes women of ages (34-43), the mean level of Vitamin D in miscarriage women with chronic infection (IgG Ab) was (134.1± 64.03), while in healthy women the level of Vitamin D was (194.2±29.74), there was a non-significant difference in the miscarriage women infected with *T.gondii* when compared with control group (Sig. value = 0.145).

**Table (3):** Means ± SD of Vitamin D (ng/mL) in The Serum of Toxoplasmosis Recurrent Miscarriage women and Controls According to Age Group

Parameter	T test	1 <sup>st</sup> Group 14-23 years		2 <sup>nd</sup> Group 24-33 years		3 <sup>rd</sup> Group 34-43 years	
		Abortive	Control	Abortive	Control	Abortive	Control
VitD	Mean	148.9	165.5	146.3↓	181.9↑	134.1	194.2
	SD	43.76	37.58	38.82	17.51	64.03	29.74
	No	20	16	27	9	13	3
Sig. Value		0.2374		0.0123*		0.145	

Studying the difference between different age groups for miscarriage women and healthy women, the results revealed significant decrease in the level of Vitamin D Parameter among 2<sup>nd</sup> group (Sig. value=0.0123). the reason of decrease in vitamin D in infected miscarriage women might be due to the production of oxidants such as free radicals by the parasite, thus vitamin D as an antioxidant prevents the body from these roots by the oxidation itself, in addition to several Factors such as geographic location and latitude, ethnicity, skin phenotype and individual response to UV, age, physical activity and poor diet have to be interpreted cautiously [28,29,30].

The results of the present study were agreeing with [31] study in Riyadh, Saudi Arabia who showed that the women with vitamin D deficiency were mostly in the middle age group 25–35 years. [32] explained that was a strong association between low vitamin D levels and pregnancy loss (PL).

These results were supported by [33] and also by [34] showed that a strong association between low vitamin D levels and PL among women aged 20 to 35 years. [35] supported this association, when he found that Vitamin D insufficiency is associated with increased risk of miscarriage. [36,37] not find any association between vitamin D deficiency and miscarriage. This difference may be explained not only by differences in study design, but also by differences in our study population relative to other published studies, including the difference in maternal age, sample size and gestational age of specimen collection.

The risk of vitamin D deficiency increases during pregnancy due to the increase in maternal and fetal demands [38]. Moreover, vitamin D have effect on several pregnancy outcomes including fetal skeletal outcome, hypertensive disorders, and gestational diabetes mellitus (GDM) [39,40] . [41] founded that The relationship between vitamin D3 deficiency, toxoplasmosis, and women's age was significant; within which women were aging from 15 to 25 years, their sera reveal high rates of deficiency 98.46% compared to other age groups. While

vitamin D3 insufficiency highest recorded among women aging from 26 to 35 years, P < 0.05.

It can be speculated that factors other than vitamin D can determine maternal and neonatal outcomes. Furthermore, vitamin D action is also dependent on its interaction with its binding protein and its receptor [42]. genetic variations (e.g. Vitamin D receptor polymorphisms) can be involved in vitamin D metabolism and in disease susceptibility [43]. Finally, the increased oxidative stress associated with any placental dysfunction causes an alteration in the expression of vitamin D-binding protein and vitamin D receptors [44] that subsequently can alter the vitamin D action.

### Distribution of Malondialdehyde (MDA) Level Among Recurrent Spontaneous Aborted Women according to Age Group

This study intended to evaluate OS status in the women with spontaneous abortion and those with normal women by measuring MDA serum level .Table(4) showed that the mean ± SD of serum MDA in first group of seropositively miscarriage women aged (14-23years) was 176.6 ± 54.94 and in the normal healthy women was 178.8 ± 63.83 (P= 0.912). There is non-significant differences between the level of MDA and age factor between seropositively miscarriage women and healthy women . Likewise, the mean ± SD of serum MDA level in second group of seropositively miscarriage women aged (24-33years) was 194.9 ± 95.33 and in the normal healthy women was 132.7 ± 63.54 (P= 0.0779), in this age group show there is non-significant differences between the level of MDA and age factor between seropositively miscarriage women and healthy women. Also there is non-significant differences between the level of MDA and age factor between seropositively miscarriage women and healthy women in third group which include women aged (34-43 years), Going back to Table 3-15 show the mean ± SD of serum MDA level in seropositively miscarriage women was 183.3 ± 94.07 and in the healthy women was 166.4 ± 72.33 (P= 0.7783).

The results revealed no significant changing in the level of MDA among all age groups of cases and control , Our result



agree with the results of study in Baghdad- Iraq , shows that no significant difference in the mean serum lipid peroxid (MDA) value seropositive group from those of the control group within the age group 16-25 years and 36-45 years[45]. But this result not agree with the results of [46] in Turkey,how found that A statistically significant difference was found between patients aged 40.3 ± 5.8 yr and the control group aged 40.3 ± 5.8 yr in terms of MDA level, (41.32 ± 2.05, 9.18 ± 1.21 ) Respectively. Study also in Turkey shows that increased mean serum MDA levels in toxoplasmosis spontaneous aborted women aged 25.0±5.1(66.4±13.7nmol/m) compared with healthy women aged 27.2±4.8 (40.3±16.1nmol/m) [47]. As for the study of [48] in Mosul city, showed that a marked increase in MDA level in patients with toxoplasmosis aged range of 18-45 years in comparison with the control group.

Placenta is one of the source of MDA this lipid peroxides are secreted toward the fetal side and this could explain why the maternal circulating blood peroxide level was decreased significantly after delivery of the placenta MDA which formed as a breakdown product of fatty acid peroxides , are involved in aging and other pathological disorder such as atherosclerosis, oxygen toxicity , and liver injury.

The significant negative correlation between serum antioxidant vitamins level and maternal age indicates that with advancing age ,serum antioxidants decreases ,This change could be due to depletion of those antioxidant vitamins as a result of increased free radicals production which is supported by the positive correlation between age and MDA level . Advancing maternal age was associated with increasing oxidative stress and decreasing antioxidant activity

**Table 4.** Means ± SD of MDA (ng/mL) in The Serum of Toxoplasmosis Abortive Women and Control According to Age Group.

Parameter	T test	1 <sup>st</sup> Group 14-23 years		2 <sup>nd</sup> Group 24-33 years		3 <sup>rd</sup> Group 34-43 years	
		Abortive	Control	Abortive	Control	Abortive	Control
MDA	Mean	176.6	178.8	194.9	132.7	183.3	166.4
	SD	54.94	63.83	95.33	63.54	94.07	72.33
	No	20	16	27	9	13	3
Sig. Value		0.9122		0.0779		0.7783	

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