

# The Relationship of Vitamin D3, D-Dimer, and Antinuclear Antibody Levels with Toxoplasmosis

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## Abstract

**Background:** *Toxoplasma gondii* is an obligatory parasite that causes a zoonotic infection known as toxoplasmosis, which produces a wide range of clinical symptoms in humans and is considered the major cause of abortion in women. Immunocompromised patients are more likely to develop *T. gondii* infections due to parasite transmission over the placenta. **Objective:** The aim of this study was the determination of antinuclear antibody (ANA) and some biochemical parameters, such as vitamin D3 and D-dimer. **Materials and Methods:** This study was conducted in Kirkuk City extended from November 1, 2022 to March 21, 2023 included 200 blood samples from aborted women at Gynecological and Pediatric Hospital and Kirkuk General Hospital, Kirkuk, Iraq. The patient's serum was assessed for ANAs, vitamin D3, and D-dimer. **Results:** Forty-seven percent of 200 women who had abortions tested positive for toxoplasmosis. Serum vitamin D3 levels were found to be substantially lower in *Toxoplasma*-positive cases than in *Toxoplasma*-negative cases, which was an unexpected finding. In addition, ANA results were statistically significant ( $P = 0.034$ ). In our study, there was no discernible difference in the levels of D-dimer between the groups. **Conclusion:** This study investigated that there were significant differences in the level of vitamin D3 and the results of ANA between the groups in aborted women, whereas there were no obvious differences in D-dimer level between our study groups.

**Keywords:** Aborted women, antinuclear antibody, D-dimer, *Toxoplasma gondii*, vitamin D3

## INTRODUCTION

Toxoplasmosis is a parasitic disease caused by the protozoan parasite *Toxoplasma gondii* that is, transmitted from person to person. It is estimated that 20%–90% of the world's adult population has had contact with the parasite in various places.<sup>[1]</sup> The definitive hosts are felines and other members of the Felidae family, whereas intermediate hosts include a variety of vertebrates, including humans and other animals.<sup>[2]</sup> The majority of cases of toxoplasmosis are caused by consuming raw or undercooked meat containing alive tissue cysts, in addition to consuming contaminated food or water. Blood transfusion or organ donation from infected individuals are rare ways to contract toxoplasmosis.<sup>[3]</sup> The stomach enzymes disintegrate the cyst wall when cats ingest small animals or raw meat contaminated with tissue cysts, allowing bradyzoites to multiply, and form oocysts in the small

intestine, the oocysts are then expelled by the small intestine wall cells, which burst. The oocysts develop within 1 to 5 days of being excreted in cat faces, remain in typical ambient conditions for a long time, and contaminate environmental media including drinking water.<sup>[4]</sup> As this coccidian parasite may cause serious infections in people and animals by ingesting a single oocyst from polluted water, soil, fruits, or vegetables, finding *T. gondii* oocysts in environmental samples is an important issue.<sup>[5]</sup> The human becomes infected after consuming raw meat containing bradyzoites, which, under the influence of enzymes and bile salts,

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**Submission:** 21-Jun-2023 **Accepted:** 05-Dec-2023 **Published:** 24-Sep-2024

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**How to cite this article:** Aziz MJ, Ali AA, Haddad MF, Abdullah B. The relationship of vitamin D3, D-dimer, and antinuclear antibody levels with toxoplasmosis. *Med J Babylon* 2024;21:556-9.

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10.4103/MJBL.MJBL\_812\_23

transform into sporozoites and settle in macrophages of the small intestine. Later, it exits the intestine and multiplies asexually in the endothelial cells of blood vessels to produce the first generation of schizonts and merozoites. The latter infiltrate the endothelial cells of capillaries to produce the second generation of schizonts and merozoites, which encyst as bradyzoites within the muscle fibers as a result of increased immunity. As mobile and dispersed throughout the body, tachyzoites and sporozoites ultimately transformed. Tachyzoites proliferate rapidly, causing tissue injury and the spread of infection.<sup>[6]</sup> Toxoplasmosis symptoms include fever, anorexia, or dyspnea, as well as evidence of neurological, respiratory, cutaneous, and corneal involvement.<sup>[7]</sup> The indirect hemagglutination antibody, the latex agglutination test, the indirect fluorescent antibody test (IFAT), the modified agglutination test (MAT), the Western blot, and the enzyme-linked immunosorbent assay (ELISA) are the serological techniques used to detect antibodies against *T. gondii* in serum. MAT, IFAT, and ELISA are the most popular and well-established methods.<sup>[8]</sup> A global health issue, vitamin D3 insufficiency is linked to a higher risk of developing several disorders.<sup>[9]</sup> The vitamin D injection enhances nitric oxide generation by activating macrophages, which in turn reduces the replication of *T. gondii*.<sup>[10]</sup> The presence of antinuclear antibodies (ANAs) during pregnancy suggests an underlying autoimmune process that may disrupt placenta development and increase the risk of miscarriage. Autoantibodies, such as ANAs, are frequently seen in the blood of patients with unexplained recurrent abortion.<sup>[11]</sup> Circulating D-dimers, which result from fibrin degradation and are an expected intravascular coagulation dissemination feature (disseminated intravascular coagulation and consumptive coagulopathy), are nevertheless an outbreak marker of infections.<sup>[12]</sup> This study aimed to determine the level of ANA and some biochemical parameters, such as vitamin D3 and D-dimer, in two groups Toxo IgM +ve and Toxo IgG +ve.

## MATERIALS AND METHODS

### Study population

This study was conducted in Kirkuk City in two hospitals (Gynecological and Pediatric Hospital and Kirkuk General Hospital, Kirkuk, Iraq) on 200 aborted women aged 15–45 years from November 1, 2022, to March 21, 2023.

### Sample collection

Approximately 5mL of venous blood was collected using a sterile syringe from each aborted woman, and we put 3mL of that blood sample into sterile gel tubes. Test tubes were left at room temperature for 20–30min to allow the blood to clot, then centrifuged at 3000rpm for 5–10min.

The serum was next divided into four Eppendorf tubes and kept in a deep freezer (-20°C) until it was required. Two milliliters of blood were put into citrate-containing tubes that needed to be filled, mixed, and delivered to the laboratory for the D-dimer test.

### Enzyme-linked immunosorbent assay

This test was used for the detection of ANA using (Antinuclear Antibody ELISA Kit, Orgentec, Germany) according to the protocols designed by the manufacturer of the diagnostic kits.

### Cobas e 411 immunoassay analyzers

Elecsys and Cobas e411 immunoassay analyzers, the electrochemiluminescence binding assay were designed to estimate vitamin D3 and D-dimer, which was done by using Vitamin D3 Cobas Kit (Roche, Germany) and D-Dimer Kit (Korea) according to the protocols designed by the manufacturer of the diagnostic kits.

### Ethical approval

At the beginning of the study, we obtained official ethical approval to conduct the study from the Directorate of Health (Kirkuk, Iraq). The participants were explained the purpose and procedure for taking information from aborted women who attend hospitals according to document number 964 dated October 25, 2022.

### Statistical analysis

The data were analyzed according to the Minitab statistical program with ANOVA test and *t* test. Duncan's multiple range test was used to compare the differences between means under the probability level 0.05 ( $P < 0.05$ ).<sup>[13]</sup>

## RESULTS

Out of 200 aborted women included in the study 28 (14%) was IgM +ve, more than 1.0 cut-off index (COI) considered as positive. Whereas 66 (33%) was IgG +ve, more than 30 IU/mL was considered positive, the total was 94 (47%) positive for toxoplasmosis [Table 1].

Statistical analysis of the obtained results showed that the mean  $\pm$  SD vitamin D3 level in aborted women who had

**Table 1: Vitamin D level in Toxo IgM +ve and Toxo IgG +ve**

Study groups	No.	Vitamin Level (ng/mL) (Mean $\pm$ SD)	P value
Toxo IgM +ve	28	23.36 $\pm$ 6.63	0.042
Toxo IgG +ve	66	26.51 $\pm$ 6.90	

Toxo IgM: *Toxoplasma* immunoglobulin M, Toxo IgG: *Toxoplasma* immunoglobulin G, SD: standard deviation

**Table 2: Antinuclear antibody (ANA) level in Toxo IgM +ve and Toxo IgG +ve**

Study groups	No.	Antinuclear antibody (ANA) COI level (Mean ± SD)	P value
Toxo IgM +ve	28	0.2570 ± 0.2940	0.034
Toxo IgG +ve	66	0.1470 ± 0.0762	

Toxo IgM: *Toxoplasma* immunoglobulin M, Toxo IgG: *Toxoplasma* immunoglobulin G, SD: standard deviation, ANA: antinuclear antibody, COI: cut-off index

toxoplasmosis IgM +ve was  $23.36 \pm 6.63$ , whereas vitamin D3 level in aborted women who had toxoplasmosis IgG +ve, the mean  $\pm$  SD was  $26.51 \pm 6.90$ , which indicates significant differences with a *P* value of 0.042. The normal range of vitamin D3 is 30–100 ng/mL and the low level is <20 ng/mL.

The result in Table 2 revealed the mean  $\pm$  SD of ANA in aborted women who had toxoplasmosis IgM +ve was  $0.2570 \pm 0.2940$ , whereas in aborted women who had toxoplasmosis IgG +ve the mean  $\pm$  SD was  $0.1470 \pm 0.0762$ , which indicates significant differences with a *P* value of 0.034. The positive results of ANA >1 COI and negative result <1 COI.

The results showed that the mean  $\pm$  SD of D-dimer level in aborted women who had toxoplasmosis IgM +ve was  $0.448 \pm 0.187$ , whereas in aborted women who had toxoplasmosis IgG +ve, the mean  $\pm$  SD was  $0.495 \pm 0.381$ , which indicates no significant differences with a *P* value of 0.420. Normal range of D-dimer is <0.55 mg/L) and mild-moderately elevated range of D-dimer is 0.55–5.5 mg/L.

## DISCUSSION

Toxoplasmosis, a parasitic infection caused by *T. gondii*, is one of the leading causes of abortion and congenital abnormalities in affected women.<sup>[14]</sup> In the present study, anti-*T. gondii* IgG antibodies were recorded in 66 (33%) aborted women. Whereas anti-*T. gondii* IgM antibodies were recorded in 28 (14%) aborted women, there was a significant difference at *P* value of 0.05. Toxo IgG antibodies were high, indicating that all of the women in the current research had previously had *T. gondii* and had an anti-*Toxoplasma* antibody. The seroprevalence of Toxoplasmosis varies from region to region. The IgG seroprevalence varies from 30% to 50% in Europe, and it is approximately 9.1% in American women of reproductive age. The frequency ranges from 30% to 80% throughout South America, reaching 100% in the oldest age categories of the poorest communities. With statistically significant variations between World Health Organization regions, the global IgM and IgG seroprevalence in pregnant women is 1.9% and 32.9%, respectively.<sup>[15]</sup> Additionally, vitamin D insufficiency in Iraq today is confusing given the great prevalence

of vitamin D in Iraqi communities. Less exposure to sunlight, which allows for adequate penetration for the production of sufficient amounts of vitamin D, maybe the cause of that; climatic changes may also contribute to this deficit. In this study, we found aborted women who were positive for IgG antibody had lower serum vitamin D3 levels, which is lower than 20 ng/mL, vitamin D3 deficiency was found to be substantially linked with the possibility of *T. gondii* infection. Our results agree with.<sup>[16]</sup> To illustrate the link between vitamin D and *T. gondii* infection, serum vitamin D3 levels were measured in women and found to be considerably lower in persons positive for toxoplasmosis, some studies have found a link between vitamin D3 deficiency and toxoplasmosis seroprevalence in different female populations. This could be explained by the concept that vitamin D insufficiency reduces immune system performance, putting people at risk for toxoplasmosis agreed with.<sup>[10]</sup> Vitamin D3 is an immune modulator<sup>[17]</sup> that has shown therapeutic immunoregulatory benefits in treating a variety of infectious illnesses. 1,25-dihydroxyvitamin D3, the hormonal form of vitamin D3, is well known for its immunosuppressive, anti-proliferative, and pro-apoptotic properties.<sup>[18]</sup> The current study observed the prevalence and significance of antinuclear antibodies in women who had unexplained recurrent miscarriages. According to certain research, *T. gondii* infection has a significant influence on the development of numerous autoimmune disorders. A study<sup>[19]</sup> confirmed the presence of a high incidence of ANA in persons with *Toxoplasma*, which is compatible with our findings, which show an increase in the amount of ANA in aborted women with *T. gondii*. Previous research has identified several potential processes through which ANAs contribute to miscarriage. First, ANAs may directly impair the quality and development of oocytes and embryos, lowering the likelihood of pregnancy and implantation. The zone pellucid did not include nuclear antigens, but *in vitro* research showed that ANAs could bind to the embryos directly, and it was hypothesized that ANAs might recognize the glycerol moiety or protein cofactor.<sup>[20]</sup> ANAs are one of the autoimmune variables thought to be responsible for 30% of occurrences of fetal rejection. Pregnant women who contract toxoplasmosis run the risk of having an abortion. Several earlier studies have documented the link between *T. gondii* infection and unexplained abortion.<sup>[21]</sup> D-dimer, a tiny protein fragment that is, discovered in the blood after the blood clot has been broken down by fibrinolysis, is a fibrinolysis product. One might conclude that its goal is to aid in the blood clots' diagnosis. Because of this, we decided to conduct a study to see whether there is a connection between *Toxoplasma* parasite infection in pregnant women and abortion rates.<sup>[22]</sup> D-dimer is substantially lower in non-*Plasmodium falciparum* malaria, which is an intracellular parasite like *T. gondii*. In this way, D-dimer levels seem to correspond to the various degrees of endothelium

**Table 3: D-dimer level in Toxo IgM +ve and Toxo IgG +ve**

Study groups	No.	D.dimer level (ng/mL) (Mean ± SD)	P value
Toxo IgM +ve	28	0.448 ± 0.187	0.420
Toxo IgG +ve	66	0.495 ± 0.381	

Toxo IgM: *Toxoplasma* immunoglobulin M, Toxo IgG: *Toxoplasma* immunoglobulin G, SD: standard deviation

activation or damage.<sup>[23]</sup> This agrees with our study. The results of our study show no significant differences in the results of D-dimer in both groups of Toxo IgG +ve and Toxo IgM +ve, with a *P* value of 0.420 given in Table 3.

## CONCLUSION

Toxoplasmosis is one of the most prevalent infectious diseases. Due to the parasite's transmission across the placenta, the mother's illness during pregnancy increases the possibility of congenital infection in the fetus. In our results, there were significant differences in vitamin D3 levels in aborted women, as well as significant differences in ANA results. There were no clear differences in the results of D-dimer.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

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