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Original Research Article

The Association of Interleukin Levels with Toxoplasmosis in Recurrent Abortion among Iraqi Women

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Abstract: *Toxoplasma gondii* is a widespread zoonotic parasite and a known factor contributing to recurrent abortion. This cross-sectional study, conducted in Tikrit City, Iraq from November 2023 to June 2024, aimed to evaluate the relationship between *T. gondii* infection and serum interleukins IL-7, IL-17, and IL-27 in women with recurrent abortion. Sixty women aged 20-45 years were enrolled, including 30 seropositive cases and 30 healthy controls. The overall seroprevalence of toxoplasmosis was 28.3%, with chronic IgG positivity being the most common. Serum IL-7, IL-17, and IL-27 levels were significantly elevated in infected women compared with control ($p<0.005$). These findings suggest that increased cytokine levels may contribute to immune dysregulation associated with recurrent abortion. Routine screening and immunological monitoring may help reduce pregnancy loss among high-risk women.

Keywords: *Toxoplasma Gondii,* Recurrent Abortion, IL-7, IL-17, IL-27, Cytokines, Women.

INTRODUCTION

Warm-blood vertebrates, including humans, can contract the obligatory intercellular protozoan parasite *Toxoplasma gondii,* with causes toxoplasmosis [1]. The parasite's life cycle is complicated because it requires two different kinds of hosts: intermediate hosts, which include many homoeothermic animals like humans and birds, and final hosts, are members of the felidae family particularly cats [2]. People can contract toxoplasmosis by eating raw or undercooked meat that contains viable tissue cysts, drinking contaminated water, or ingesting oocysts from contaminated fruits, vegetables, or soil [3]. The active phase of the infection, known as tachyzoite, is transferred from the mother to the fetus directly through the placenta. Rarely, blood transfusions or the transplantation of afflicted organs are used to spread the infection [4]. Dietary practices, national geographic conditions, and host immunity all have a significant impact on the seroprevalence of toxoplasmosis in humans [5]. The host has a strong immune system and shows no symptoms of the infection [6]. In most situations, the host's immune system can prevent the parasite 's growth and the development of tissue cysts in the majority of body tissue, which are primarily found in the central nervous system, skeletal muscles and cardiac muscles, without causing any symptoms [7]. Primary toxoplasmosis in pregnant women can seriously harm the fetus and result in server neurodevelopmental abnormalities like microcephaly and as well as irreversible conditions like abortion [8]. Pregnant women who are immune-competent typically have asymptomatic acute infections, but 10% may also exhibit various symptoms, such as fever, headache, flu-like lines, lymphadenopathy, chorioretinitis, and myositis [9]. The control of several intracellular pathogens, including the obligatory intracellular parasite *T. gondii*, depends on the development of an efficient CD8+ T cell response [10]. While acute *T.gondii* infection induces both CB4+ and CD8+ T cell responses the CD8+ T cell subset is largely responsible for long-term protection against the parasite. IFN-c, a cytokine essential for survival against both acute and chronic phases of infection, is produced by immune CD8+ T cells from *T. gondii* infection hosts [11]. Furthermore, *T. gondii* infected hosts CD8+ T cells can demonstrate cytotoxic activity against parasite infected targets in vitro [12]. According to reports, these antigen-specific CD8+ T cells cytotoxic infection is crucial for controlling chronic infection [13]. IFN-c or CD8+ T cells depletion eliminates the host defenses against *T. gondii* infection, which can result in morbidity or death. Despite the well-established significance of CD8+ T cell immunity against *T. gondii* infection.it is unclear which specific cytokines are responsible for producing this response [14]. IL-15 is thought to be

essential for promoting the basal proliferation of memory CD8+ T cells, whereas IL-7 is important for giving survival signals to both naïve and memory CD8+ T cells [15]. One of the pro-inflammatory cytokines that Th 17 cells produce is IL-7. Inflammatory responses are also influenced by other cells, such as CD4+ T cells and natural killer cells. Through the stimulation of proinflammatory and neutroph-promoting chemicals, IL-7 plays a variety of role in development of autoimmune, allergic and neoplastic disease. It helps the host defend against a variety of pathogens, such as parasites, fungi and bacteria [16]. IL-17 mediated signaling is necessary for the early stimulation of neutrophil produce during toxoplasmosis. It has been noted that neutrophils are essential for the innate immune response to be lines [17]. Antigen-presenting cells secrete IL-27, which has been demonstrated to control inflammation during pregnancy, reduce proinflammatory cytokine production through STAT (Signal transducer and activator of transcription) transcription factors, and induce Th1 differentiation of CD4 Tcell [18]. Additionally, in *T. gondii* infection, IL-27 signaling improves a subset of T regulatory cells that limit intestinal pathology and improve host survival [19]. This study aimed to measured serum levels of IL-7, IL-17 and IL-27 in women who frequently had abortions in comparison to a healthy control group.

MATERIALS AND METHODS

In Tikrit, Iraq, This cross-sectional study was carried out between November 2023 and June 2024. 60 women between ages of 20 and 45years were included in the study and spit into two groups:

- 30 women with a history of recurrent abortion (defined as two or more consecutive pregnancy losses) and toxoplasmosis (seropositive comprise the study group (cases).
- 30 healthy (seronegative) women without a history of repeated abortion made up the control group.

To ensure that the findings more accurately reflect the association between toxoplasmosis infection and abortion, women with known hormonal disorders, chronic systemic disease (such as uncontrolled diabetes), or those undergoing immunosuppressive therapy were excluded.

Informed Consent and Ethical Issues

After thoroughly outlining the purpose and nature of the study, the researcher obtained each participant's written informed consent before starting any procedures, participants received assurances that their involvement was completely voluntary and that their healthcare would not be impacted if they decided to stop at any point.

All personal information, including the identities of the participants, including the identities of the participants, was kept completely private.

Serological Analysis and Sample Collection

Each participant had sterile 5 m L venous blood sample taken, which was then put into serum tubes. Centrifugation was used for five minutes at 3000 rpm to extract the serum from the samples. For short-term use, the serum was kept at 4-8 C° storage, it was kept at -20 C°.

Commercially available enzyme-linked immunosorbent assay (ELISA) kits from Sunlong, China were used to detect IgM and IgG antibodies to the parasite *Toxoplasma gondii*, carefully following the manufacturer's instructions.

Level Measurement of Cytokine

Using commercially available ELISA kits, the serum concentrations of interleukins (IL-7, IL-17and IL-27) were measured using (USA) kits, and IL-17 and IL-27 were measured using Komabiotech (India) kits.

Statistical Analysis

The statistical program SPSS was used to conduct the statistical analysis Differences between categorical variables (like incidence rates) were measured using the Chi- square test. The t- test was used to compare the means of continuous values (such as cytokine levels) between two groups. Statistical significance was defined as a p-value of less than 0.05.

RESULTS

Of the 60 women in this study, 30 had a history of repeated abortion ages and were infected with the parasite *Toxoplasma gondii*, while the remaining 30 were sero-negative and in good health. The participants were between the age of 20 and 45.

Table 1: Prevalence of *Toxoplasma gondii* infection among Groups

Group	N	+ve *Toxoplasma gondii* N(%)	x^2(p-value)
All	60	17(28%)	
Control	30		
Patient	30		
Acut (IgM)		3(10%)	**Chi-Square = 4.941 P-Value = 0.055**
Chronic (IgG)		9(30%)	
IgM+IgG		5(16.66%)	

The distribution of *T. gondii* serological markers among participants is displayed in Table 1. The overall infection rate was 28.3%, the most common seropositivity was chronic IgG (30%), followed by mixed IgM and IgG (16.7%), and the last common was acute IgM positivity (10%) between infection categories, no significant difference was found (p=0.055).

Table 2: Association between Abortion Rates and Age Groups

Age (years)	**Abortion N (%)**	**x^2(p-value)**
20-29	10 (33.33%)	**
30-39	5 (16.66%)	**Chi-Square = 8.647 P-Value = 0.013**
40>	2 (6.66%)	

According to Table 2, women between the ages of 20 and 29 had the highest abortion rates (33.3%), followed by those between the ages of 30 and 39 (16.7%) and those over 40 (6.7%). Age groups and the incidence of abortions were found to be statistically significantly correlated (p=0.013).

Table 3: Comparison between Frequency Abortion Frequency and Age

Group	**Age**	**N (%)**	**x^2(p-value)**
Abortion less than <3	**30-39**	**4 (13.33%)**	**
	40>	**2 (6.66%)**	**Chi-Square = 11.824 P-Value = 0.003**
Abortion more than>3	**20-30**	**11 (36.66%)**	

Similarly, women between the ages of 20 and 30 had the largest percentage of recurrent abortion case (36.7%), indicating a significant correlation between age and recurrent abortion frequency (p=0.003) in Table 3.

Table 4: Distribution of Serum Interleukins Levels among Study Groups

Group/ (IgM+IgG Postive)	**Mean±SD**		
	IL-7 Pg/ml	**IL-17pg/ml**	**IL-27pg/ml**
Abortion	**270.4 ± 20.3**	**351.8 ± 37.8**	**575.2 ± 20.3**
Control	**20.652 ± 0.966**	**27.59 ± 1.36**	**53.85 ± 2.29**
T-test	**21.25 ** **	**19.15 ** **	**57.15 ** **
p-value	**0.0008**	**0.0007**	**0.00002**

Table 4 displays the mean serum cytokine levels. Women with recurrent abortion and T. gondii infection exhibited significantly elevated concentrations of IL-7 (270.4 ± 20.3 pg/ml), IL-17 (351.8 ± 37.8 pg/ml), and IL-27 (575.2 ± 20.3 pg/ml) compared with healthy controls (20.65 ± 0.97, 27.59 ± 1.36, and 53.85 ± 2.29 pg/ml, respectively). These differences were all statistically significant ($p < 0.05$).

DISCUSSION

The results of the study show that, with a total rate of 28%, *Toxoplasma gondii* infection is still widespread in the community being studied. Chronic infections and mixed infections were more common than acute infections. Since the parasite usually remains in the body in a dormant state after an initial infection, this distribution is consistent with its epidemic nature. These findings are consistent with several studies conducted in Iraq. IgG seroprevalence rates among reproductive age women varied from 30% to 45%, according to early Iraqi studies [20, 21]. More recent studies like Rasheed *et al*., (2024) in Zakho and Al-Dabbgh *et al*., (2023) in Mosul, which found similar results with IgG rates of 31.7-32.4% and IgM rates between 3-9% [22, 23], support the persistence of chronic infection patterns across different regions and time periods. Similar findings 34.5% seroprevalence [24], and Ahmed *et al*., (2022) in Baghdad, who reported 29.8% overall positivity [25], confirmed the wide distribution of *T. gondii* in Iraq. Globally, similar rates have been reported, including 32% in Egypt [26]. 27% in Turkey [27], 29% in Iran [28], and 36.6% in meta-analysis among pregnant women

worldwide [29]. These consistent results from various geographic locations emphasize that toxoplasmosis is endemic especially in areas where oocyst survival is favored by climatic conditions, animal contact, and food hygiene. The current study's comparatively low percentage of IgM antibodes (10%) might point of increased public awareness and health education initiatives. All things considered, this study confirms earlier findings that toxoplasmosis is still common in Iraq, consistent with regional and worldwide patterns, and emphasized the necessity of ongoing surveillance and preventive measures, particularly for high- risk populations like expectance mothers and people with weakened immune systems.

The findings show a statistically significant correlation between age and abortion, with young women between age (20-29) having the highest abortion rates. Similar local studies have produced similar findings in Iraq. The high rate of abortion among women in their (20-30) may be related to environmental and health factors, such as inadequate nutrition, parasitic infections like *Toxoplasma gondii*, and inadequate medical follow-up during pregnancy, according to a study conducted in the Baghdad Governorate by Ali *et al*., [30]. According to a study by Hussein *et al*., (2022), *T. gondii* infection and a lack of knowledge about prevention are the main causes of abortion among young women [31]. The interpretation of the current findings as having environmental and immunological influence rather than purely age-related is supported by Al-Mousawi *et al*., (2022) from Basra, which showed that viral and parasitic infections, including HIV, clearly contribute to the development of AIDS, regardless of age [32]. However, these findings diverge from many reports from around the world. According to an Ireland study by Kenny *et al*., (2013), chromosomal abnormalities and deteriorating egg quality cause the risk of abortion to progressively risk after the age of 35 [33]. According to Chinese study by Zhu *et al*., (2019), advanced age and genetic factors cause abortion to rise dramatically in women over 40 [34]. On the other hand, Singh *et al*., in India suggested that younger women with active parasitic infections or chronic reproductive tract infections might have higher rates of abortion, which is in line with the pattern indicated by the current study's findings [35]. As indicated in Table 3, the current study's results are in line with those of a recent Iraqi study [36]. Which found that the majority of women with recurrent abortion were in the 30 to 39 year old age range and that chromosomal and immunological factors are important in this group's recurrence of pregnancy loss. Due to declining egg quality and a higher incidence of chromosomal abnormalities, the risk of pregnancy loss increases gradually after the age of 30, reaching its highest levels after the age of 40, according to Magnus *et al*., (2019) study published in the BMj. Along with chromosomal, immunological, and hormonal abnormalities, age is one of the most significant risk factors linked to recurrent abortion [37], according to the American Counseling Grouo (ACOG, 2018) [38]. The concentrations of the IL-7, IL-17 and IL-27 in serum of *Toxoplasma gondii* infected women who experienced repeated abortion were significantly higher than those in the control group, according to Table (4). This suggests that different immune-inflammatory processes were not present after pregnancy. Increase in T-cells and their proliferation may be the cause of rise in IL-7. Th-17 stimulating cells then produce IL-17, which lowers intrauterine inflammatory mediators and weakens and prevents pregnancy. These findings are in line with those of a study carried out in Iraq by [39], which found that women with toxoplasmosis who experienced repeated abortion had significantly higher levels of IL-17 and IL-27, confirming that these cytokines significantly contribute to abortion by inducing uterine inflammation [39]. Additional research conducted in Iraq has also demonstrated that genetic variations in the IL-17A gene may raise the risk of recurrent abortion by making a person more susceptible to an overabundant inflammatory response [40]. The current findings that increased serum IL-7 may reflect local immune activity that impedes implantation are supported by [41], international study, which demonstrated that local expression of IL-7 in the endometrium was elevated in cases of recurrent abortion. Additionally [42, 43], showed that increased IL-17 and IL-27 are linked to Th17 cell hyper activity and an imbalance between Th17 and Treg cells, which results in the loss of immune to lerance required for pregnancy to succeed.

CONCLUSION

Through significant alteration in the levels of specific immune cytokines, the study's findings suggest that infection with the parasite *Toxoplasma gondii* may undoubtedly contribute to the immune disruptions that lead to recurrent abortion in women. Compared to the control group, women infected with Toxoplasma who had abortion had significantly higher serum levels of interlukin-7(IL-7), interlukin-17(IL-17). And interlukin-27(IL-27), indicating a complex inflammatory and immune role in causing early pregnancy termination.

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