



## Study of Some Immunologic Parameters in Aborted Women Infected with *Toxoplasma Gondii*

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### Abstract

The demographic data of the patients was collected using a pre-designed questionnaire that included information on age and exposure to animals. Written permission was obtained from all participants in this research. The research was conducted in Karbala Maternity and Pediatric Hospital as well as other private labs in Kerbala city, Iraq, from November 2022 to May 2023. The blood samples underwent centrifugation, and the levels of serum IgM, IgG, interleukin-27 (IL-27), and Monocyte Chemoattractant Protein-1 (MCP-1) were measured using the enzyme-linked immunosorbent assay (ELISA) technique. The findings indicated that a total of 53 female individuals had abortion as a direct consequence of toxoplasmosis infection. The age group with the greatest incidence rate (56.6%) of toxoplasmosis was 18-26 years, whereas 79.2% of the patients were found to have had contact with animals. The blood immunoglobulin level analysis revealed that 98.1% of patients exhibited positive IgG findings, while 1.9% tested positive for IgM. In contrast, all healthy women in the control group tested negative for both IgM and IgG. The study found that women infected with toxoplasmosis had significantly higher levels of serum IL-27 (645.95 pg/ml) compared to the control group (88.26 pg/ml). Similarly, the infected women had significantly higher levels of MCP-1 (272.20 pg/ml) compared to the control group (48.83 pg/ml). These differences in interleukin levels were highly significant ( $P < 0.0001$ ). The present investigation indicates that women with recurrent abortion who were infected with *T. gondii* had elevated levels of IgG, IL-27, and MCP-1.

## Introduction

Toxoplasmosis is a contagious illness that may be transmitted between animals and humans. It is caused by a microscopic parasite called *Toxoplasma gondii*, which lives within cells. Pregnant women who are particularly sensitive to *Toxoplasma* infection may have abortion or congenital abnormalities such as hydrocephalus and retinochoroiditis (Chen et al., 2017). Various variables contribute to the development of toxoplasmosis and the ability of the host to survive. The reasons contributing to this phenomenon include the diverse genetic composition of various strains of *T. gondii*, the intricate immunological characteristics of hosts, the biochemical interplay between certain cytokines, the invasion tactics used by the parasite, and the immunogenicity of antigens encountered by the host's immune cells. The strain of *Toxoplasma* determines the kind of cytokines that are produced (Sana et al., 2022).

While toxoplasmosis often does not show any symptoms, it has the potential to produce serious and perhaps fatal diseases in some instances. Acute toxoplasmosis manifests after a few days of incubation and exhibits symptoms like the flu, such as fever, exhaustion, muscular soreness, sore throat, and headache. It may also present with mononucleosis-like symptoms, typically accompanied by myalgia (muscle pain) and adenopathy (swollen lymph nodes). Vulnerable

populations consist of pregnant women and those with weakened immune systems, such as organ transplant recipients, cancer patients, and HIV-positive patients (Šušak et al., 2023). To prevent *T. gondii* infection, it is important to implement basic epidemiological measures. These include raising awareness about personal hygiene, washing hands and surfaces after handling raw meat, thoroughly washing fruits and vegetables, and regularly cleaning cats and their environments (Hill & Dubey, 2002).

The majority of persons with a healthy immune system, over 80%, do not display any symptoms or only have mild flu-like symptoms when they encounter an infection (Robert-Gangneux & Dardé, 2012). A seroprevalence metric might indicate the level of exposure to pathogens. Seroprevalence refers to the measurement of the proportion of persons in a community who have antibodies against a certain infectious disease, determined by analyzing their blood serum. The positive results of the samples indicate past exposure to the specific disease, as shown by the presence of the designated antibodies (Centers for Disease Control and Prevention, 2020). Detecting *Toxoplasma* infection may be indicated by the presence of particular IgG and IgM antibodies. Performing repetitive serological screening for IgG and IgM allows for differentiation between acute and chronic infections (Robert-Gangneux & Dardé, 2012). Immunity against *T. gondii* is achieved by a sophisticated immune response that involves several types of cells, such as inflammatory cells, lymphocytes, macrophages, and cytokines (Filisetti & Candolfi, 2004).

The cytokine profile in response to toxoplasmosis seems to differ based on several factors, including the stage of infection, the underlying clinical conditions, and notably, the strain of the parasite (Mantilla-Muriel et al., 2020).

## Methods

### Samples

In the present case-control study, (5) ml of venous blood samples were taken from 115 women aged 18-47 years suffering from repeated abortion, and from 30 apparently healthy women as a control group. The demographic information of patients was recorded in a questionnaire form prepared in advance including age and contact with animals. A written consent was taken from all participants in this study. The study was carried out in Karbala Maternity and Pediatric Hospital and other private laboratories in Kerbala city, Iraq during November 2022 to May 2023.

### Immunological assay

The blood samples were put in gel tubes, and centrifuged at 300 rpm for 10 minutes to obtain serum which were stored at -20°C until use. The levels of serum IgM, IgG, interleukine-27 (IL-27) and Monocyte Chemoattractant Protein-1 (MCP-1) were estimated by the enzyme-linked immunosorbent assay (ELISA) method, using the Human Mini ELISA kits manufactured by ELK Biotechnology Company, USA. The ELISA method adopted the Capture antibody principle.

### Statistical analysis

For statistical analysis of data, the Statistical Package for Social Sciences (SPSS-25) was used, which was presented as Mean + SE. Statistical differences between means was estimated by analysis of variances, followed by least difference test or Duncan test. The difference was considered significant at ( $p < 0.05$ ) (Al-Bayati et al., 2023).

## Results and Discussion

The results of the current study showed that the highest rate of infection 30 (56.6%) with *T. gondii* parasite was among the age groups 18-26 years, and the lowest infection rate was 6 (11.3%) among the age groups 37-47 years, with a statistically highly significant difference ( $P < 0.0001$ ), (Table 1).

Table 1. Distribution of toxoplasmosis patients according to age

Factor	Group	No.	%	Chi square	P value
Age (years)	18-26	30	56.6	16.340	0.0001
	27-36	17	32		
	37-47	6	11.3		
<b>Total</b>		53	100	-	-

In regard to animal contact with toxoplasmosis infected women, the results of the study showed that the incidence of toxoplasmosis was higher in aborted women who kept animals 42 (79.2%) when compared with infected women who were not in contact with animals 11 (20.7%), with a highly significant difference ( $P < 0.0001$ ) as shown in table (2).

Table 2. Distribution of toxoplasmosis patients according to animal contact

Factor	Group	No.	%	Chi square	P value
Animal contact	No	11	20.7	18.132	P=0.0001
	Yes	42	79.2		
<b>Total</b>		53	100	-	-

When the sera were examined using the ELISA technique, it was found that 52 (98.1%) were positive for IgG antibodies, while only 1 (1.9%) was positive for IgM antibodies, while no IgG or IgM antibodies were detected in the healthy control group, with a highly significant difference  $p < 0.0001$  as shown in table (3).

Table 3. IgG and IgM rates in the study groups

Study groups	IgG	IgM	Chi square	P value
Toxoplasmosis patients	52 (98.1%)	1 (1.9%)	49.075	0.0001
Control group	0.00	0.00	-	-

The mean serum level of the cytokine IL-27 increased in the sample of patients aborted due to toxoplasmosis 645.96pg/ml compared to the control women 88.26pg/ml, with a highly significant difference ( $p < 0.0001$ ) as demonstrated in table (4).

Table 4. IL-27 levels in the study groups

Variable	Study group	No.	Mean	SE	P value
IL-27 (pg/ml)	Toxoplasma patients	53	645.96	33.98	0.0001
	Controls	30	88.26	3.14	

The mean serum level of the cytokine MCP-1 increased in the sample of patients aborted due to toxoplasmosis (272.20) pg/ml compared to the control women (48.83) pg/ml, with a highly significant difference ( $p < 0.0001$ ) as demonstrated in table (5).

Table 5. MCP-1 levels in the study groups

Variable	Study group	No.	Mean	SE	P value
MCP-1 (pg/ml)	Toxoplasma patients	53	272.20	15.36	0.0001
	Controls	30	48.83	2.62	

The research revealed that 98.1% of participants tested positive for IgG antibodies, whereas just 1.9% of patients tested positive for IgM antibodies using the ELISA method. Seroprevalence refers to the measurement of the proportion of persons in a community who have antibodies against a certain infectious disease, determined by analyzing their blood serum. Positive samples for certain antibodies indicate prior exposure to the corresponding pathogen (Centers for Disease Control and Prevention, 2020). Detecting Toxoplasma infection may be indicated by the presence of particular IgG and IgM antibodies. The use of repetitive serological screening for IgG and IgM allows for differentiation between acute and chronic illnesses (Robert-Gangneux & Dardé, 2012). The detection of *T. gondii*-specific IgM by serological identification implies a recent or ongoing/acute infection, whereas the presence of *T. gondii*-specific IgG indicates a previous or dormant infection (Filisetti & Candolfi, 2004).

The study conducted by Satti et al. (2011) revealed a prevalence rate of 38.9% for ELISA IgG in Khartoum state. Similarly, Mohamed et al. (2014) reported a prevalence rate of 73.1% for ELISA IgG in rural parts of Sudan. It may also contradict the findings of (Maha, 2006), who demonstrated a prevalence of 35.1% positive IgG antibodies to *T. gondii* in Sudanese pregnant women using ELISA. However, the outcome was consistent with the findings of Saeed et al. (2014), which demonstrated that 20.2% of pregnant women tested positive for IgG. A further study revealed that out of the 797 women of reproductive age who were examined, only 23.46% had IgG antibodies against *T. gondii*. The seroprevalence rate of 23.46% observed in their research closely aligns with the 33% prevalence reported in a meta-analysis done among Iranian women of reproductive age (Mizani et al., 2017). The reduced occurrence of IgM, which serves as a marker for recent infestation, is likely due to the absence or little symptoms of the illness and limited testing for *T. gondii* during the first phase of infestation (Liu et al., 2015).

The findings of the present investigation revealed that the age group between 18 and 26 years exhibited the greatest prevalence of *T. gondii* infection, while the age group between 37 and 47 years had the lowest prevalence. The findings of the present research align to some extent with the previous study, which indicated that there is a higher occurrence of seroprevalence among individuals aged 15-30 years. The research did not indicate a correlation between age and seropositivity. Prior studies have shown that the occurrence of parasite infection, as indicated by seroprevalence, rises with advancing age, namely in individuals between the ages of 35 and 38, as well as those older than 48 (Tagegne et al., 2016; Yohanes et al., 2017). The observed fluctuations in infection rates may be ascribed to the age categorization of research participants in the present investigation. This might also be attributed to inadequate personal hygiene. This highlights the need of persistently educating women in their reproductive years on the prevention of toxoplasmosis. The seropositivity did not provide any statistically significant results (Okojoku et al., 2023).

Unlike the findings of the present study, earlier researchers have shown an age-related rise in the seroprevalence of the parasite, with younger women exhibiting lower rates. This relationship may have arisen as a result of prolonged exposure to risk factors linked to infection, such as interaction with animals that serve as carriers of the parasite, such as cats (Peyron et al., 2016; Olariu et al., 2020).

This analysis did not find any evidence of a connection between owning a cat and being seropositive for *T. gondii*. Research conducted in Egypt Mandour et al. (2017) and Burkina Faso Bamba et al. (2017) yielded comparable findings. The variation in findings between research suggests that the likelihood of acquiring a *T. gondii* infection is not only linked to the presence of cats in one's household, but also to the exposure to cat feces containing oocysts when engaging in gardening activities. The assessment of the relationship between cats and toxoplasmosis is challenging due to the complexity of epidemiological surveys. The increased chance of toxoplasmosis transmission might be attributed to frequent exposure to cat excrement or a lack of prophylactic measures. In Jos, cats are not confined inside and are allowed to wander freely, causing significant pollution to the environment. No significant association was seen between seropositivity and the ownership of a dog, pig, or any other animal in connection to *T. gondii* infection. Additional studies conducted in Ethiopia have shown same findings (Fenta, 2019).

The findings of our study indicate that the average blood concentration of IL-27 was higher in patients who had abortion owing to toxoplasmosis, as compared to the women in the control group. This finding is in line with several previous investigations. For instance, a study discovered that the levels of IL-27 were higher in women with toxoplasmosis who had recurrent miscarriage, as compared to healthy women (Baquer et al., 2021).

Research found that women with recurrent miscarriage had elevated levels of IL-27 compared to healthy pregnant women. Additionally, there was a concurrent increase in both anti-inflammatory and pro-inflammatory cytokines. This phenomenon may be elucidated by enhancing the immunological response and activating the defensive system of the fetus. An imbalance in cytokinesis results in pregnancy loss in women with recurrent miscarriage (Malyshkina et al., 2020).

IL-27 significantly contributes to the suppression of the immunological response to *T. gondii*. When IL-27 is not present, mice infected with toxoplasmosis have a dangerous and overactive immunological response, which may result in death (Aldridge et al., 2024).

The blood samples of abortion patients exhibited elevated levels of MCP-1 in comparison to the control sample in the present investigation.

Chemokines, including MCP-1, IP-10, and MIP-1, have a role in mobilizing and activating many types of white blood cells, such as monocytes, macrophages, and PMNs. They also facilitate the movement of lymphocytes to sites of infection. The immunological response to toxoplasmosis infection activates phagocytic cells and lymphocytes that secrete MCP-1.

## Conclusion

It can be concluded from the current study that the levels of IgG, IL-27 and MCP-1 were increased in women with repeated abortion who were infected with *T. gondii*

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