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Original Article

Association of Some Pro-and Anti-Inflammatory Cytokines Response Pattern against Toxoplasmosis in Newly Married Couples

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Abstract

Background: The study aimed to investigate the association between cytokine profiles and toxoplasmosis in newly married couples, with identifying the immunological markers to minimizing fertility complications through early detection and intervention.

Methods: From May 2024 to February 2025, blood samples of 480 newly-wed couples in Zakho City, Iraq, were evaluated for Anti-*Toxoplasma* G (IgG) and M (IgM) antibodies, as well as serum cytokines like Interleukin 12 (IL-12), Tumer Necrosis Factor α (TNF-α), Interleukin10 (IL-10), and Transforming Growth Factor ß1 (TGFß1), using ELISA. Additionally, C Reactive protein (CRP) and Erythrocyte Sedimentation Rate (ESR) were also estimated.

Results: The overall seroprevalence of Anti-*Toxoplasma* antibodies were 25.8% (124/480), constituting 18.2% (88/480) for IgG, and 7.5% (36/480) for IgM, with highly significant difference ($P< 0.001$). Rates were higher in males, with age-related differences observed for both IgG and IgM. While statistically significant differences were reported for IgM alone relates to gender. Regarding cytokines, seropositive individuals showed significantly higher IL-10 and IL-12 levels than controls: IL-10 (IgG: 85.88±9.67 vs 37.88±5.06; IgM: 80.95±13.19 vs 51.72±6.19 pg/mL) and IL-12 (IgG: 22.43±3.05 vs 10.08±1.03; IgM: 29.25±5.46 vs 11.83±1.06 pg/mL). Besides, TNF-α elevated in the IgM+ group only.

Conclusion: Elevated IL-10 and IL-12 levels in seropositive individuals suggest active immune modulation, while TNF-α dynamics point to stage-specific responses, higher in acute (IgM) infections. These immune patterns may influence reproductive health, underscoring the importance of early diagnosis and immune monitoring to reduce potential fertility complications, particularly in early marriage.

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Introduction

The intracellular zoonotic parasitic protozoan *Toxoplasma gondii* has a worldwide distribution infecting humans and all warm-blooded vertebrates. This parasite has evolved a variety of defense mechanisms against immune response (1). The ability of *T. gondii* to live for long period inside host cells is one of the reasons that make it a successful parasite (2). Toxoplasmosis mainly occurs through ingestion of raw or undercooked meat containing tissue cysts or food contaminated with sporulated oocysts from cat feces. In healthy individuals, infection is usually asymptomatic, but it can be severe or fatal in immunocompromised people (3). The most common cause of toxoplasmosis in immunocompromised individuals is the reactivation of a latent infection, which manifest neurological symptoms such as disorientation, sleepiness, convulsions, headache, altered reflexes, and hemiparesis (4, 5). In immunocompromised patients, recent toxoplasmosis can affect multiple organs, with encephalitis being the most common, followed by pneumonia, retinochoroiditis, and other systemic manifestations (6). Furthermore, several variables, such as the number of cats in the vicinity, the climate, age, dietary and related factors, cultural and ethnic customs, can be blamed for the variation in the seroprevalence of toxoplasmosis among the community (7).

There are some indications that female animals can contract *T. gondii* through semen (8), which can lead to serious issues such as infertility (9). In addition to its effects on female reproduction, *T. gondii* infection has been shown to temporarily impair and deteriorate human male reproductive parameters and lower hormonal levels, which may result in low male productivity, fertility issues, damaging the reproductive system, and causing sexual dysfunction (10,11). The parasite multiplies inside the cell after entering the host's body, impacting the reticuloendothelial system (12). Tachyzoites are highly contagious and can cause severe, potentially fatal systemic toxoplasmosis in immunocompromised individuals (13). Toxoplasmosis elicits a strong immune response characterized by humoral immunity and Th1 cytokine profiles, leading to the production of specific anti-*Toxoplasma* antibodies. Cytokines, are crucial mediators in both the innate and adaptive immune systems, coordinate immune cell responses against the infection. The initial immune reaction against *T. gondii* is marked by rapid type 1 inflammatory cytokine activity, prompting polymorphonuclear leukocytes to swiftly migrate from circulation to the infection site (14,15).

When it comes to parasite-host interaction, the parasite may try to boost the host's defensive immunity; to prevent such a response, the parasite quickly overwhelms and kills the host (16). The clinical consequence of toxoplasmosis depends on host genetic factors and parasite virulence. Cytokines play a key role in initiating and regulating immune responses, being protective when controlled, and potentially harmful if unregulated (17). Anti-inflammatory cytokines like IL4, IL10, and TGFβ1 and pro-inflammatory cytokines like IL1β, IL12, IL18, TNFα, and IFNγ all produce a cytokinome that responds to immunological stimuli to develop an immune response against toxoplasmosis susceptibility or resistance (15). Additionally, previous studies (18, 19) have shown that women positive for *T. gondii* antibodies showed variable cytokine expression, which can reveal the immune consequences of recent or latent infection. The balance between pro- and anti-inflammatory cytokines influences pregnancy outcomes, varying from immune imbalance and severe fetal effects in early pregnancy to immunological equilibrium in multiparous women.

The current study was adopted due to the unavailability of any information among the population in Zakho City related to the rela-

tionship of the effect of toxoplasmosis on the reproductive system and its spread through sexual contact between couples in addition to its relationship to the host's immune system. These findings will provide valuable insight into the local epidemiological and immunological profiles of *Toxoplasma* infection in this region among newlywed couples.

Materials and Methods

Ethics approval and informed consent

Verbal and written consents were obtained from enrolled individuals, and the study received agreement from the ethical committee of Zakho University (College of Medicine) (MAY2024/E06) and Duhok General Health Directorate (Ref. number: 26062024-5-2).

Study design

Is a cross-sectional study, performed from May 2024 to February 2025. Involved a random selection of 480 newlywed couples (240 males and 240 females) aged 18 to 55 years, who visited a pre-marriage counselling center in Zakho City, Duhok Province, Iraq. All participants were screened through detailed questionnaires regarding antimicrobial use, as well as the presence of acute and chronic illnesses. Samples from individuals reporting other diseases were excluded to ensure the reliability of immunological comparisons.

Sample collections

From each participant, 5mL of venous blood was withdrawn using a sterile syringe, each blood sample was separated into two portions. One portion was placed into a fully labelled test-tube with the participants complete information containing anticoagulant for the ESR test, using the Westergren's tube method (China). The ESR test was used to determine the seroprevalence and frequency of inflammation caused by toxoplasmosis, in comparison with pertinent control values. The remaining blood was placed in a different labelled test-tube with clot activator for serum separation, centrifuged for five minutes at 4000 rpm, and the serum was kept for serological testing. Each serum sample was transferred into two separate 2-mL Eppendorf tube, and all sample were retained in the laboratory's deep freezer at -20 °C for subsequent processing.

Serological testing

The ELISA kits (Bioactive Diagnostica, Germany) were used for the detection of anti-*Toxoplasma* IgG and IgM antibodies for all participants. While for the immunological profiles such as pro-inflammatory cytokines (IL-12, TNF-α) and anti-inflammatory cytokines (IL-10, TGFß1) the DRG, Biocheck Company (Germany) were used for the serological tests of some seropositive and seronegative cases (as control). To maintain cytokine stability, each serum sample was used once for thawing and avoiding repeated freeze-thaw cycles. Following IgG and IgM testing all cytokine assays were performed on the same day using freshly thawed aliquots. Additionally, titer kits from MISPA-i3/AGAPPI DIAGNOSTIC (Switzerland GmbH) were used to determine the C-Reactive Proteins (CRP) agglutination test to assess the level of inflammation. Each procedure has been carried out in compliance with the guidelines enclosed with each test kit.

Statistical investigation

The evaluation of data was operated using SPSS version 27. The Pearson chi-squared test assessed the seroprevalence of IgG and IgM antibodies. When more than 20% of cells in a table had expected values less than five, Fisher's exact test was applied. For paired data involving IgG and IgM cross-tabulation, the McNemar test was utilized. Statistical significance differences* was expressed through a *P*-value of 0.05 or less.

Results

Table 1, demonstrates the overall seroprevalence of anti-*Toxoplasma* antibodies, with the presence of highly significant differences between antibody types ($P<0.001$).

Table 1: Total seroprevalence of anti-*Toxoplasma gondii* IgG and IgM Antibodies among Premarital Couples

Study group	Total positive		IgG +		IgM +		P-value	Total tested no.
	No.	%	No.	%	No.	%		
Premarital couples	124	25.8	88	18.3	36	7.5	<0.001*	480

Males showed non-significantly higher seroprevalence of IgG antibodies compared to females. However, for IgM antibodies, males had a significantly higher seroprevalence than females (Table 2).

Table 2: Seroprevalence of anti-*Toxoplasma gondii* IgG and IgM Antibodies among both genders

Genders	Total no.	IgG+		*P*-value	IgM+		P-value
		No.	%		No.	%	
Male	240	48	20.0	0.345	24	10.0	0.038*
Female	240	40	16.7		12	5.0	
Total	480	88	18.3		36	7.5	

Concerning age (Table 3), the highest seroprevalence of anti-*Toxoplasma* IgG antibodies was observed among the ages of 36–55 years. For IgM, the highest seroprevalence was found among ages older than 45 years, with a highly significant differences regarding both tests.

Table 3: Seroprevalence of anti-*Toxoplasma gondii* IgG and IgM Antibodies within different ages

Age in years	Total no	IgG+		*P*-value	IgM+		P-value
		No.	%		No.	%	
18 - 25	244	36	14.8	0.017*	8	3.3	<0.001*
26 - 35	200	40	20.0		20	10.0	
36 - 45	32	12	37.5		4	12.5	
> 45	4	0	0.0		4	100.0	
Total	480	88	18.3		36	7.5	

The relationships between, both inflammatory markers, ESR and CRP, (Table 4), didn't show any significant differences with anti-*Toxoplasma gondii* IgG and IgM antibodies.

Table 4: Relationship of the seropositivity of anti-*Toxoplasma gondii* IgG and IgM Antibodies with ESR and CRP levels

Inflammatory Test	IgG + (n= 88)	IgG – (n= 392	P-valu	IgM + (n= 36)	IgM – (n= 444)	P-valu
	Mean±SI	Mean±SI		Mean±SI	Mean±SI	
ESR mm/hr	13±1.5	13±0.6	0.908	15±2.2	13±0.6	0.333
CRP mg/dl	18±2.0	16±0.9	0.268	14±3.0	16±0.9	0.517

Relating to immunological parameters, the IgG antibodies displayed significant differences relating to TNF-α, IL-10, and IL-12 mean concentrations, whereas the IgM+ antibodies exhibited significant differences relating to IL-10 and IL-12 mean concentrations (Table 5).

Table 5: Association between seroprevalence of anti-*Toxoplasma gondii* IgG and IgM Antibodies and immunological parameters

Immunological Parameters pg/mL	IgG		*P*-value	IgM		*P* -value
	Positive (n = 24)	Negative (n = 36)		Positive (n = 11)	Negative (n = 49)	
	Mean±SE	Mean±SE		Mean±SE	Mean±SE	
IL-10	85.88±9.67	37.88±5.06	< 0.001*	80.95±13.19	51.72±6.19	0.048*
TGFβ	127.00±21.4	138.78±21.1	0.708	103.45±23.5	140.94±17.8	0.345
IL-12	22.43±3.05	10.08±1.03	< 0.001*	29.25±5.46	11.83±1.06	0.010*
TNF-α	54.91±7.71	87.61±4.82	< 0.001*	91.94±18.39	70.62±3.92	0.281

Discussion

Toxoplasma gondii is a zoonotic intracellular protozoan with worldwide seroprevalence (20). The study showed an overall seroprevalence of anti-*Toxoplasma* antibodies of 25.8% (18.2% IgG and 7.5% IgM). A previous study in this city reported higher seroprevalence of anti-*Toxoplasma* antibodies among aborted women (32.46% for IgG and 8.86% for IgM), from which around 15% were residing in the Cham Mishko displacement camp (19). The high seroprevalence of anti-*Toxoplasma* antibodies was attributed to participants' living conditions, the presence of stray cats contaminating the environment, and other factors such as poor hygiene practices, food habits, behavioral risks, environmental and socioeconomic conditions (20, 21).

Concerning gender, males showed non-significantly higher anti-*Toxoplasma* IgG antibodies seroprevalence compared to females (20% vs 16.7%). While they showed statistically significant (*P*= 0.038) differences in the seroprevalence of anti-*Toxoplasma* IgM antibodies (10% vs 5%). This outcome regarding to IgG antibodies is relevant with a study performed in Zakho City that showed the highest non-significant seroprevalence (16.4%) of IgG antibodies among males. While for IgM antibodies it contradicts with it because the higher non-significant seroprevalence was recorded among females (4.8%) (16). These variations might be attributed to increased exposure to *T. gondii* sporulated oocysts due to prolonged

contact with cats or soil over time or to eating infected undercooked meat (22).

Relating to age, highly significant differences for both antibodies were reported among different ages with the maximum among ages of 36-45 years (37.5%) and >45 years (100%) for IgG and IgM respectively. Similarly, a much higher seroprevalence (45.3%) among nearly same ages (30-35) years and a minimum seroprevalence (14.6%) among ages of 16- 20 years were reported in Duhok City (23). While, the present study contradicts with another study also, conducted in Duhok (24), that recorded the highest seroprevalence among younger ages (18-25) years. The higher seroprevalence in the current study among older ages might be attributed to getting contact for longer time with the infectious agents and to a lower immune resistance (16).

As for ESR, equal mean values were recorded among IgG seropositive and seronegative cases (13±1.5 vs 13±0.6 mm/hr). Regarding, IgM antibodies, higher non-significant mean value of 15±2.2 vs13±0.6 mm/hr was observed among seropositive in comparison to seronegative cases. The current study results contradict with the study of Mizuri et al. (16) that stated patients with high levels of ESR, presented the maximum seroprevalence of anti-*Toxoplasma* IgG and IgM antibodies (16.1% vs 12.7%, 8.1%vs 1.7%) respectively, which were highly significant (P =0.049) for IgM antibodies only. While in the current study only participants who were IgM seropositive showed non-significantly higher ESR mean levels. The current study results, also contradict with other studies performed in Egypt and Kirkuk City that revealed the maximum seroprevalence of anti-*Toxoplasma* antibodies among individuals with high ESR level as compared to those with low ESR levels (25,26). These findings are verified by medical and experimental studies revealing that microbial infections can trigger or worsen inflammation, potentially leading to arthritis (16).

The results of CRP showed non-significant higher mean concentration among IgG seropositive cases compared to seronegative once (18±2.0 vs 16±0.9 mg/dl). While for IgM antibodies, seronegative group showed a non-significant higher mean concentration compared to seropositive group (16±0.9 vs 14±3.0 mg/dl). Similarly, another study stated that CRP mean concentrations were non-significantly higher among seropositive cases compared to control, with an average CRP mean concentration in the seropositive cases of 8.84 ± 15.26 mg/L in comparison to control group (3.66 ± 4.87) mg/L (27). This may be due to parasitic infections that modulate the immune system leading to these imbalanced changes (25). Another study reported non-significantly higher ESR and CRP values in anti-*T. gondii* IgG and IgM seropositive individuals, suggesting a possible association between toxoplasmosis and inflammation in both acute and chronic stages (28). In the present study, the lack of significant CRP and ESR changes may be related to younger aged participants that might result in lower systemic inflammation because the levels of both ESR and CRP are influenced by non-inflammatory factors like age and gender (29). Furthermore, age may exert several effects on the tissues and organs of the immune system (30).

Regarding anti-inflammatory cytokines, seropositive cases had significantly higher mean IL-10 concentrations than control (85.88±9.67 vs 37.88±5.06 pg/mL for IgG and 80.95±13.19 vs 51.72±6.19 pg/mL for IgM). This is in line with a study in Wasit city that also, indicated the presence of statistically significant (P=0.003) differences in the mean concentration of IL-10 between seropositive toxoplasmosis and control groups of 248.76 vs 177.18 pg/mL (31). While another study reported a 5 folds higher mean concentration of IL-10 among toxoplasmosis seropositive individuals in comparison to healthy controls (32). Since it has been demonstrated that parasite invasion promotes liberation of IL-5,

TNF-α IL-8, and IL-10 which is attended by inflammatory state and eosinophilia (33). Therefore, IL-10 is crucial to the inflammatory response during an infection with *T. gondii* (34).

However, for TGF-β, higher non-significant mean concentration was recorded among control than seropositive cases (138.7±21.1 vs 127.00.±21.4 for IgG antibodies and 140.94.± 17.8 vs 103.45.± 23.5 for IgM antibodies). The current study contradicts with (35) who reported higher mean concentration of TGF- β among seropositive than control group at 75% vs 60% pg/mL. The discrepancy between our findings and those of (35), who reported elevated TGF-β values following *T. gondii* exposure, may be attributed to differences in sample source and experimental design. Since the analyzed sera in our study were from infected human subjects, while Bakr's used murine immune cell cultured *in vitro*, in such cases the immune responses can differ significantly from those of *in vivo* human systems (35,36). Alsailawi et al. (37) stated that the higher TGF-β mean concentration is attributed to the placental expression of TGF-β which show significant (P<0.05) correlation during infection with toxoplasmosis. Recent studies showed the production of immunological balance with the anti-inflammatory reaction during persistent chronic infection. Furthermore, TGF-β values rose mostly during acute infection (38). While (39) stated that TGF-β possibly plays a dual role against *Toxoplasma*, firstly, inducing immune responses by the development of Th17 lymphocytes and mucosal immunity and secondly suppressing the immune responses through direct and indirect pathways. In general, TGF-β decreases during toxoplasmosis because the immune system shifts toward a pro-inflammatory state to eliminate the parasite. Suppressing TGF-β helps in strengthen this response, although it increases the risk of inflammation (36).

Moreover, concerning pro-inflammatory cytokines, compared to control, seropositive cases had significantly higher mean concentrations of IL-12 (22.43±3.05 vs 10.08±1.03 pg/mL for IgG and 29.25±5.46 vs 11.83±1.06 pg/mL for IgM). This finding aligns with a study in Kufa City, which reported that toxoplasmosis significantly ($P \leq 0.05$) affects mean IL-12 concentration compared to controls which was 21+2.4 vs 8+1.04 pg/mL(39). Most studies stated that chronic toxoplasmosis significantly enhances the cellular immune response, increasing IL-10 and IL-12 values in seropositive individuals (40).

As regard to TNF-α, the control group exhibit highly significant higher mean concentration than the IgG seropositive group (87.61±4.82 vs 54.91±7.71 pg/mL), while the IgM seropositive group had a non-significant higher mean concentration in relation to the control group (91.94±18.39 vs. 70.62±3.92 pg/mL). These results are in accordance with a study performed in Erbil City, that reported higher values of TNF-α among control group in comparison to those seropositive for toxoplasmosis (350 vs 120 pg/mL) (35). While the study performed in Baghdad (41) showed significant differences (P< 0.05) among seropositive and control rheumatoid arthritis (RA) patients regarding TNF-α. Toxoplasmosis can disrupt the immune response, allowing the parasite to evade detection and persist in the host (12).

Most of the seropositive cases in the current study were chronic that is why the mean values of these mediators were lower among seropositive cases than control, because the inflammatory mediators IL-1β, IL-17A, IL-18, CSF3, and TNF-α were much lower in chronically infected individuals than in noninfected once, but they stayed high or constant during acute infection (42). Acute *T. gondii* infection triggers a pro-inflammatory response, reflected by increased TNF-α in IgM-positive cases, while chronic infection shows a more regulated immune state with decreased TNF-α in IgG-positive individuals (43). In immunocompetent hosts, a robust innate and adaptive

immune response eliminates the majority of *T. gondii* parasites; though, the parasite can evade the immune response and remain as a chronic infection (44).

Conclusion

The present study reveals a predominance of IgG over IgM, indicating a higher rate of chronic infections. Importantly, elevated mean concentrations of IL-10 and IL-12 among IgG and IgM seropositive individuals, suggest an active modulation of the host immune response during *T. gondii* infection, that potentially affect fertility. The elevated TNF-α in the IgM group may indicate its involvement in the early immune response to acute infection, while its reduction in the IgG group could reflect a regulatory mechanism that prevents prolonged inflammation during chronic infection. Collectively, these outcomes suggest that toxoplasmosis and related immune responses may have implications for reproductive health, specifically in early marriage. Early diagnosis and immunological monitoring may serve as significant tools in decreasing fertility complications associated with toxoplasmosis.

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Conflict of Interest

The authors declare no conflicts of interest.

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