

Correlation of interleukin-10, interleukin-17 and micro-RNA-938 expression profiles in some Iraqi women with toxoplasmosis

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ABSTRACT

Toxoplasmosis is an infectious parasitic illness resulting from *Toxoplasma gondii*, which involves intricate crosstalk with host immune pathways. Pathogenic progression includes both innate and adaptive immune mechanisms. miRNAs are an epigenetic factor crucial for regulating immune activity by interfering with cytokine signaling pathways. This study was conducted to determine the relationship between Anti-inflammatory cytokines (interleukin-10) and Pro-inflammatory cytokines (interleukin-17) in chronic toxoplasmosis cases. Serum samples were obtained from a total of 200 women, comprising 100 controls and 100 toxoplasmosis cases, aged 18–50 years, collected between November 2023 and September 2024. Before use, every sample was kept. For molecular study, miRNA-938 was measured in 100 whole blood samples, 50 patient samples, and 50 control samples utilizing Quantitative Real-Time PCR. Statistically, the findings exhibit no meaningful differences in microRNA-938 expression among the patient group (1.2368 ± 0.65981 ng/L) and the reference (healthy) group (1.239 ± 0.792 ng/L). The serum IL-10 levels in the patient group decreased by (13.658 ± 2.115 ng/L), more than those in the control (33.404 ± 5.973 ng/L). Whereas patients appeared elevated serum IL-17 levels (609.639 ± 45.184 ng/L) compared to control (207.778 ± 4.794 ng/L). Generally, our investigation revealed that the miR-938 expression remains unchanged during *T. gondii* infection, therefore, we suggest that miRNA-938 does not participate in

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changing immunological pathways, and the parasite has evolved strategies that have successfully manipulated host immunity. Additionally, dysregulation of the expression of both markers IL-10 and IL-17 gene could serve as an indicator for women infected with toxoplasmosis.

Keywords: miRNA-938, cytokines, interleukins, toxoplasmosis, qRT-PCR

INTRODUCTION

It is a globally encountered parasitic disease caused by infection with *Toxoplasma gondii* in humans and animals. Felids, involving domestic cats are considered a definitive host¹. Despite the disease being mild, such as self-limiting influenza-like symptoms or asymptomatic in immunocompetent individuals, toxoplasmosis is a greater risk in immunocompromised patients, who can develop potentially lethal encephalopathy², psoriasis³, and diabetes patients⁴. Additionally, primary infections acquired during pregnancy can lead to various antagonistic outcomes, including cranial and neurologic deformities, blindness, stillbirth, fetal ocular, and miscarriage⁵. An estimated eleven percent of the U.S. population, more than 6 ages are seropositive for toxoplasmosis, based on analysis of sera taken by the National Health and Nutrition Examination Survey between 2011 and 2014⁶. The microRNA-938 molecules have not been reported in Iraqi patients. Furthermore, *Toxoplasma gondii* has a composite life cycle comprising two sequential phases. During the acute phase, tachyzoites actively replicate and spread through the host, whereas in the chronic stage, featuring slow-growing bradyzoite cysts that are responsible for the persistence of the parasite and can reactivate in immunocompromised people. Moreover, the parasite employs numerous mechanisms to circumvent host defenses, where bradyzoites are observed to encapsulate themselves in cysts that enable *T. gondii* persisting lifelong within the host, resistant to host immunological reactions and anti-parasitic medicines⁷. Additionally, the immune status of the intermediate host plays a pivotal role in shaping host-parasite dynamics. Parasites secrete excretory compounds that modulate human immune effector cells, enabling them to evade host immune responses. Through this process, they have evolved diverse strategies that facilitate their persistence within the host and ultimately contribute to the establishment of chronic infection⁸.

Cytokines are the central agents of intracellular communication and modulators of immune regulation, which are secreted by various cell types in response to many stimuli. The intricate nature of cytokine activity in experimental infections complicates the interpretation of host immune responses⁹. Interleukin-10

(IL-10) is a regulatory cytokine with anti-inflammatory properties, it has demonstrated to suppress dendritic cells and macrophages after an inflammatory trigger, in acute *Toxoplasma gondii* infection, IL-10 contributes to limiting immune reactions and preventing tissue damage. In addition, it acts as a feedback regulator to control excessive cytokine release during the initial stage of infection¹⁰.

Following infections via *Toxoplasma gondii*, Natural killer (NK) cells serve as negative regulators and are identified as the dominant origin of IL-10 secretion. This cytokine can mitigate immune-mediated tissue damage triggered by pathogens. For example, Natural Killer cells induced by IL-15C to produce IL-10 have been revealed to prevent severe pathology and mortality in experimental cerebral malaria models¹¹. Bragato et al. (2022) demonstrated that decreased expression levels of miR-21 lead to diminished IL-10 levels in culture supernatants from splenic leukocytes in Canine leishmaniasis¹². Notably, in IL-10-deficient mice, *T. gondii* infection contributes to unchecked IL-12, IFN- γ , and TNF- α responses, resulting in severe immunopathology and death. However, sustained IL-10 production during chronic infection may also facilitate parasite persistence by dampening effective immune clearance¹³.

A decrease in IL-10 levels during chronic toxoplasmosis disturbs the equilibrium between Pro-inflammatory and Anti-inflammatory responses, thereby increasing inflammation and tissue damage¹⁴. Understanding the strategies behind interleukin-10 regulation through chronic toxoplasmosis is essential advancement of selective therapies to prevent complications and control disease progression. Interleukin-17, is a proinflammatory cytokine secreted by T-helper-17, mast, and myeloid cells populations. Moreover, IL-17 can stimulate several cell types, including osteocytes, chondrocytes, macrophages, and synovial fibroblasts, to secrete and synthesize additional pro-inflammatory cytokines. The sequential release of cytokines amplifies the inflammatory milieu during toxoplasmosis¹³. Furthermore, elevated IL-17 is associated with progression and development of various persistent inflammation and Auto-immune illnesses. New research indicates that accurate and effectively regulated IL-17 signaling has the potential to successfully manage the most majority of these conditions¹⁵.

Micro-RNAs (miRNAs), are small noncoding RNA molecules, approximately 18 to 22 nucleotides in length that modulate gene expression post-transcriptionally by binding to the 3' untranslated region (UTR) of numerous mRNA targets. On occasion, microRNAs are called miRNAs, are key regulators in several pathophysiological processes, including oxidative stress, the immune system,

and inflammation¹⁶. Zeiner et al. (2010) exhibited that *Toxoplasma gondii* infection specifically modulates host miRNA expression: for instance, the abundances of (miR-17/92 and miR-106B-25) clusters rise significantly at infected primary human cells via upregulation of their promoters¹⁷. Furthermore, in acute ocular toxoplasmosis, certain miRNAs such as (miR-155/5p and miR-29c/3p) significantly increased, whereas (miR-21/5p and miR-125b/5p) downregulated compared to asymptomatic control¹⁸. More recent studies show alterations in other miRNAs (e.g., miR-146a, miR-155) during pregnancy-associated toxoplasmosis and neuro-infection, with potential effects on host cell function and disease outcomes¹⁹.

In contrast, miRNA expression in host cells is altered dynamically; some miRNAs are upregulated, others downregulated, depending on the infection context and tissue type. These changes reflect the complex interplay between cytokine signaling and genetic regulation of infection by *T. gondii*²⁰. According to various studies' findings, miR-938 is predominantly characterized in cancer studies, especially for its role in targeting SMAD3 within the TGF-β pathway, e.g., in colorectal cancer²¹ and pituitary adenomas²². In addition, Qian et al. (2020) validated that elevated levels of miR-938 facilitated lung adenocarcinoma cell proliferation. Conversely, its reduced expression has an opposing effect²³. Moreover, in immune-related diseases (e.g., autoimmune disorders represented by Systemic Lupus Erythematosus, Reperfusion damage and Asthma elevated levels of multiple miRNAs (miR-106a, let-7, miR-98, miR-142-3p/5p) capable of binding directly to the 3' untranslated region (UTR) of IL-10 mRNA, causing decreased IL-10 expression in relation to immune-mediated and inflammatory conditions²⁴. Moreover, as reported in a different study, the dysregulation of miRNA-146a in Rheumatoid Arthritis (RA) patients relative to the control leads to a rising IL-17 levels in the patients. Therefore, IL-17 may contribute substantially to chronic inflammatory processes and can be utilized as a useful biomarker for patient diagnosis²⁵.

Following toxoplasmosis, the parasite disrupts the tightly controlled miRNA profile in a manner that promotes parasite persistence and facilitates disease progression. Remarkably, this altered expression landscape is beneficial for distinguishing between Acute and Chronic illness states and elucidating underlying pathogenic mechanisms²⁶. As noted above, previous studies have shown that miR-938 expression has a beneficial intervention in malignant conditions therapy, but its function during *Toxoplasma gondii* or other parasitic infection remains poorly understood, with no existing publishing has been documented.

To the best of our understanding, this represents the first investigation aimed to assess the expression of microRNA-938 molecules in toxoplasmosis patients. In addition, detect its relations with both Anti-inflammation interleukin-10 and Pro-inflammation interleukin-17 profiles in the serum of Iraqi females infected with toxoplasmosis. Moreover, evaluate the function of miRNA-938 in the immunological responses and disease severity during chronic toxoplasmosis.

METHODOLOGY

Specimen acquisition

A 5 milliliters vein blood sample was obtained from volunteers by utilizing a disposable syringe; the blood was extracted from each woman (patient and control), whose ages ranged from 16 to ≥ 50 years old, between November 2023 until late September 2024 in Al Kawashif- Lab and Al-Mass. The TORCH rapid diagnostic test was used to detect toxoplasmosis. The blood was divided into two aliquots; the first aliquot was drawn in an Eppendorf tube for molecular study, and the second aliquot was distributed in a plain tube to collect serum. After clotting, the blood sample was centrifuged at 3000 revolutions per minute of 5 minutes at ambient temperature. Following, serum layer was separated, portioned into Aliquots, and saved at -20°C until the immunological analysis.

Serum procurement

One milliliter of serum was acquired and preserved in an Eppendorf tube and then used for the assessment both of IL-10 and IL-17 levels by Enzyme linked immunosorbent assay (ELISA). Samples were preserved at -20°C until analysis.

Whole blood collection

One milliliter of Whole blood was drawn using a disposable syringe. Subsequently, using a pipette, 250 mL of whole blood was transferred via pipette into an Eppendorf tube containing 750 mL of Triazole reagent, thoroughly mixed, finally stored at -20°C for later MiR extraction.

Serological tests

***Toxoplasma* IgM / IgG rapid detection assay serum/ plasma; flow chromatographic methods**

Toxo- IgM and IgG Rapid assay employs lateral flow chromatographic immunoassay technology to simultaneously detect and distinguish Anti-Toxoplasma IgM and IgG antibodies in human serum. The kit is a qualitative detection diagnostic device.

Quantification and purity analysis of RNA

In accordance with the manufacturer's protocol, RNA quantity and purity were assessed using the Qubit™ RNA HS Assay Kit (Q32852, Thermo-Fisher®, USA). All samples exhibited mi-RNA concentrations ranging from 10 pg / mL to 100 ng / mL species.

Quantification of micro-RNAs

Qubit™ mi-RNA Assay Kits (Thermo-Fisher®, USA) were utilized to measure tiny RNA fragments (20 nucleotides or base pairs) based on the manufacturer's guideline.

Specific primer of miRNA-938

The micro-RNA-938 primer sequences utilized in Laboratory work were custom-designed and synthesized by Applied Biological Materials (Macrogen), South Korea, exhibiting melting temperatures between 60°C and 95°C. The lengths of PCR amplicons range between 75 and 150 bp, whereas the lengths of primers are 22 nucleotides. The following is the name and order (Table 1).

Table 1. Specific primers of miRNA-938

miR-938	TGCCCTTAAAGGTGAACCCAGT	22
miR-U6 FP.	AGAGAAGATTAGCATGGCCCT	22
Universal r. transcription.p	CAGGTCCAGTTTTTTTTTTTTTTVN	26
miRNA- universe. r.p	GCGAGCACAGAATTAATACGAC	22

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 29 to evaluate the influence of various factors on study parameters. The normality of the data was assessed using the Shapiro-Wilk test, which is recommended for samples of this size (n=200). The test indicated a non-significant deviation from normality (p>0.05). This was supported by a histogram showing a symmetric distribution. Therefore, the data were considered normally distributed, supporting the use of parametric statistical analyses²⁷. Mean comparisons were conducted using the t-test²⁸. Graphical representations were generated with Graph-Pad Prism version 9. Continuous variables are presented as mean ± standard error of the mean (SEM). Pearson correlation analysis was applied to examine associations, while receiver operating characteristic (ROC) analysis was employed to determine the area under the curve (AUC). ROC analysis

was performed as a comprehensive way to assess the accuracy of the studied markers. The area under the curve (AUC) provides a useful tool to compare different biomarkers. Whereas an AUC value close to 1 indicates an excellent diagnostic and predictive marker. Statistical significance was defined as a $p < 0.05$ ²⁹.

RESULTS and DISCUSSION

Seroprevalence of toxoplasmosis

Statistical analysis of TORCH test results across affected versus unaffected groups of toxoplasmosis samples demonstrated markedly significant disparities ($p \leq 0.01$) among the study cohorts. We noticed the patient's group was (3.35 ± 0.08) while the healthy group was (0.452 ± 0.02), as indicated in Figure 1.

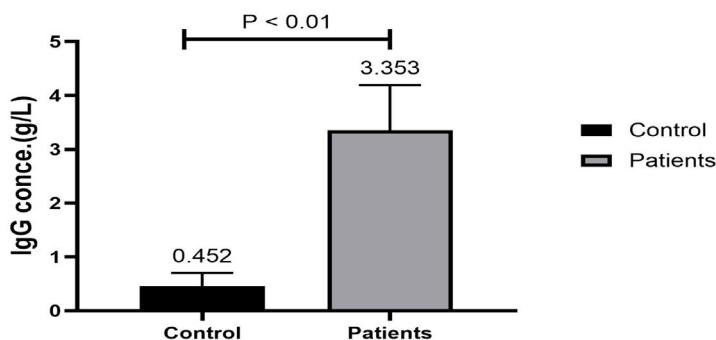


Figure 1. Comparison between control and patient groups in IgG concentration

Quantification of miRNA-938 expression in individuals with toxoplasmosis and healthy controls

We evaluated the expression level of miRNA-938 in the whole blood of the 50 patients with *T. gondii* and 50 healthy controls. As presented in Figure 2, the levels of miR-938 expression showed no statistically significant differences between the patient's group (1.236 ± 0.659 ng/L), when compared to the healthy control group (1.239 ± 0.792).

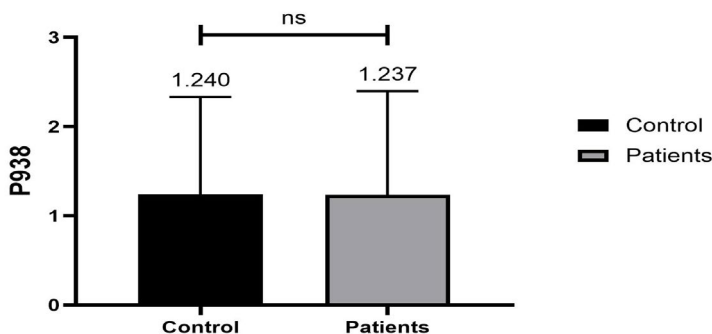


Figure 2. The average miR-938 gene expression levels across control and cohorts' groups

As illustrated in Figure 3, The Receiver Operating Characteristic (ROC curve) analysis indicates that miRNA-938 yielded an area under the curve (AUC) of 0.53 at a defined cut-off value of 1.335, with a sensitivity 34%, and specificity 76%.

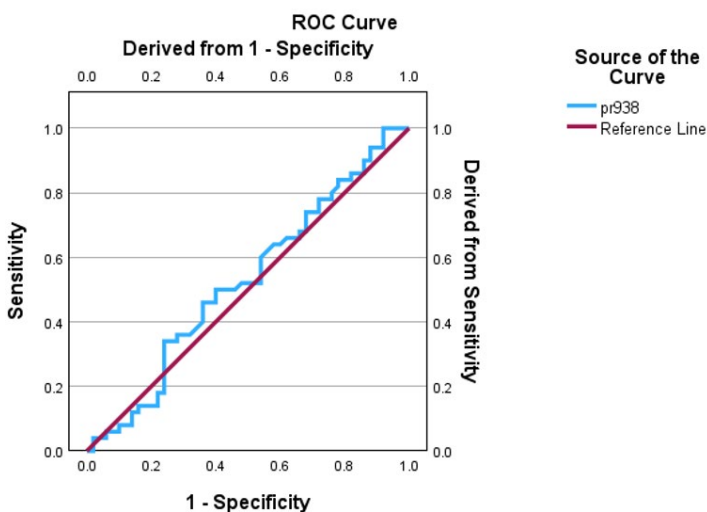


Figure 3. ROC- curve analysis of MiRNA-938 expression fold- change distinguishes via healthy and patient groups

Measured interleukin-10 concentration in serum

Comparative investigation of serum IL-10 levels between the toxoplasmosis individuals and healthy group. Statistically, the findings revealed that reduced levels of IL-10 in patients' serum with *T. gondii* was significant ($p < 0.001$) comparative with the healthy controls. As depicted in Figure 4, the average IL-10 concentration in patients was 13.658 ± 2.115 ng /L, while 33.404 ± 5.973 ng /L in the control group.

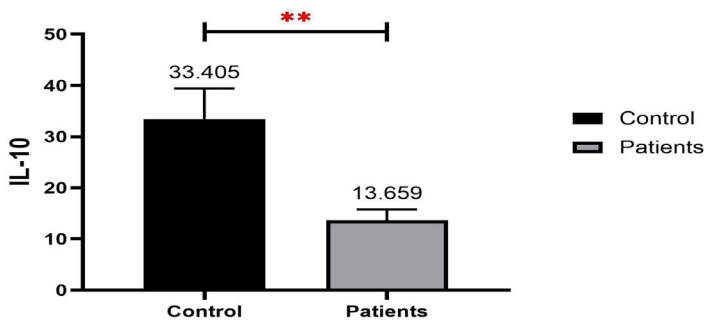


Figure 4. Comparative interleukin-10 levels in control versus patient samples

Furthermore, ROC analysis indicated that the expression of IL-10 in the samples was a good predictor through a cut-off value of 20.228 U/mL, an area under the curve (AUC) of 1.000. In addition, both specificity and sensitivity reached 100% (Figure 5).

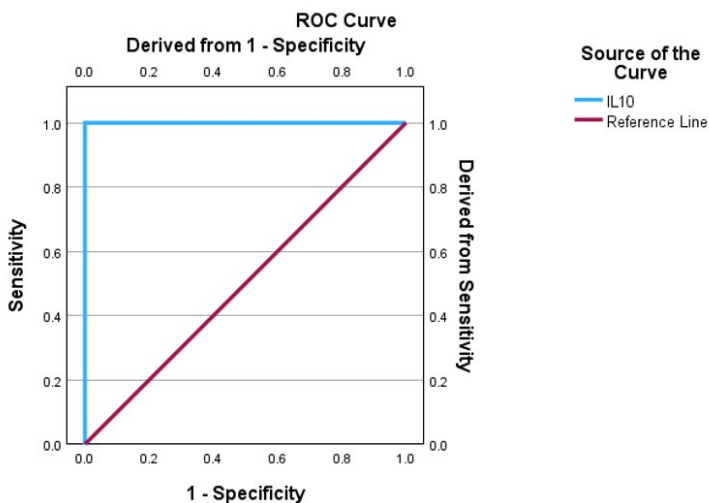


Figure 5. ROC curve illustrating interleukin-10-fold expression as a discriminator between patient and healthy groups

Measured interleukin-17 concentration in serum

The serum IL-17 concentration in toxoplasmosis patients was statistically compared to that of healthy controls. As shown in Figure 6, patients infected with *T. gondii* exhibited a significantly elevated IL-17 level 609.639 ± 45.184 ng/L relative to the control group 207.778 ± 4.794 ng/L, with a $p < 0.001$.

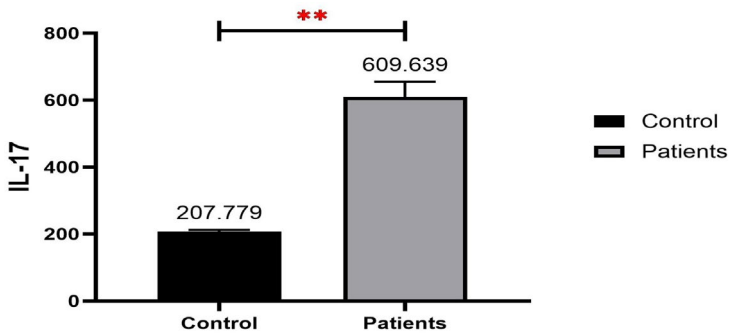


Figure 6. Serum IL-17 in controls versus patients

And sequentially, as illustrates in Figure 7, ROC curve analysis demonstrated that IL-17 expression served as a strong predictive marker, using a threshold value of 373.582 U/mL, an area under the curve (AUC) of 1.000, and perfect specificity and sensitivity of (100% each).

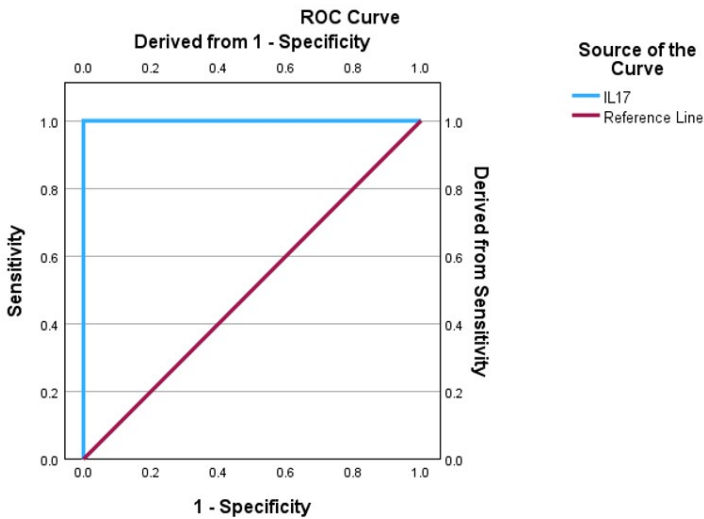


Figure 7. ROC curve analysis of IL-17-fold expression for differentiating between patient and healthy groups

Correlation between Fold miRNA-938 level with both IL-10 and IL-17

In this study, Pearson's correlation coefficient was used to examine the relationship between MicRNA-938 with IL-10 and IL-17 levels. The results indicated that miR-938 has statistically significant double correlation, negatively with IL-10 ($r = -0.061$) and positively with IL-17 ($r = 0.025$). Additionally, IL-17 has higher significant negative correlation ($p < 0.01$) with IL-10 ($r = -.895^{**}$) as demonstrated in Table 2.

Table 2. The correlation between fold MicRNA-938 level, and both IL-10 and IL-17

		miR-938	IL-10	IL-17
miR-938	Pearson correlation	--	-0.061	0.025
	p-value (two-tailed)		0.545	0.802
IL-10	Pearson correlation		--	-.895**
	p-value (two-tailed)			<.001
IL-17	Pearson correlation			--
	p-value (two-tailed)			

Toxoplasmosis is an overlooked infectious disease characterized by variable incidence and symptomatology across patient populations and geographic regions. Among immunocompetent hosts, Toxoplasmosis can be established as either acute or chronic infection. A developmental switch occurs as the parasite progresses from tachyzoites to bradyzoites, which is the hallmark of this phase, enabling long-term survival within the host. This transition is facilitated via the host's immunological defenses³⁰.

The parasite's ability to maintain a chronic infection is related to its capacity to evade immune detection through modulation of Th1/Th2/Th17 axis, which might otherwise promote inflammation and clearance of the parasite³¹. In the context of infectious diseases, the cytokine profile reflects the body's immune response to pathogens. Acute inflammatory responses are marked by elevated levels of pro-inflammatory cytokines, including IL-1 β , TNF- α and IL-6 as part of the body's defense mechanism. Nonetheless, overexpression or Dysregulated cytokine release may drive chronic inflammation, contribute to tissue injury, and facilitate immune escape by the pathogen. In the case of toxoplasmosis, altered cytokine profiles are thought to contribute to the severity of infection and pregnancy outcomes³².

In our study, the TORCH test that is utilized to perform comparative analysis between *T. gondii* infected individuals and the healthy group, which demonstrated statistically robust differences ($p \leq 0.01$) between groups. The patient's group was (3.35 ± 0.08) while the healthy group was (0.452 ± 0.02). This finding was explained by Montoya et al. (2002) who observed limitations in diagnostic approaches for *T. gondii*, emphasizing that IgG antibodies typically emerge within two weeks post- infection, reach peak levels within 1-2 months, decline variably, and remain detectable for life. Consequently, it cannot reliably detect acute or recent infections or distinguish between recent and post exposures. Although IgM antibody detection is widely available through commercial kits, these assays often suffer from low specificity and are prone to misinterpretation³³.

Numerous research has demonstrated that miRNAs are pivotal regulators of both innate and adaptive immunity. By modulating cytokine expression, miRNA- driven post- transcriptional control orchestrates key processes such as the maturation, proliferation, differentiation, and activation of T and B Lymphocytes. To our knowledge, this is the first investigation in Iraq to explore the involvement of miRNA-938 and its targets- IL-10 and IL-17- in the chronic pathogenesis of *Toxoplasma gondii*. This report investigates the potential involvement of miR-938 expression in inflammatory diseases, based on prior evidence of its role in modulating inflammatory responses. In chronic *T. gondii*. The current study revealed that the miR-938 expression was 1.236 ± 0.659 ng/L in *T. gondii* patients, while for the control, this was 1.239 ± 0.792 ng/L. The findings show no statistically significant variation between patient and healthy group. The current result is corroborated by another finding, the research of Chan et al. (2025), which has observed miRNA alterations in various parasitic infections. In cane toads infected with *Rhabdias pseudosphaerocephala*, transcriptional changes were limited to only one gene differentially expressed, emphasizing the subtlety of host responses³⁴. Similarly, *Trypanosoma cruzi* infection induced miRNA shifts in epithelial and cardiac cells, while macrophages exhibited broader changes³⁵, underscoring the importance of cell-type selection in detecting miRNA dynamics.

Multiple factors can interplay and cause miRs to fail to exert a detectable effect on immune response during inflammation, such as in hidradenitis suppurativa. MiR-125b-5p expression was markedly reduced in Lesional skin relative to Perilesional areas, but its overall low abundance limited its potential as a reliable biomarker³⁶. Similarly, in spite of miR-146a being considered a well-characterized inflammatory regulator in studies of Genini et al. 2014, showed often exhibits low

expression levels in normal conditions, making it difficult to detect meaningful changes during inflammatory responses³⁷. As other studies have also found, Parasites that alter miRNAs within mucosal or circulating sections can reprogram the microbiota or systemic signaling, contributing to persistent inflammation or tolerance conditions that ultimately lead to chronic illness³⁸.

Collectively, these findings suggest that low miR-938 expression limits its diagnostic³⁹ and functional impact⁴⁰ in chronic toxoplasmosis. Besides that, Arif et al. (2020) confirmed that DNA methylation and chromatin modifications can influence miRNA accessibility to target sites. The research indicates that epigenetic changes can alter chromatin, potentially affecting the accessibility of miRNA binding sites⁴¹. As well, RNA-binding proteins (RBPs) interfere with miRNAs' function. Some RBPs directly with miRNAs for overlapping binding sites, whilst others bind to adjacent regions, leading to reduced miR-induced mRNA⁴². Based on the findings, IL-10 levels in serum were significantly decreased during *T. gondii* infection, 13.658 ± 2.115 ng/L, compared to those in healthy controls, 33.404 ± 5.973 ng/L. Conversely, IL-17 levels were elevated by three times in the *T. gondii* patient group, 609.639 ± 45.184 ng/L, relative to 207.778 ± 4.794 ng/L in the control group. Accordingly, IL-17 was increased (pro-inflammatory Th17 axis), whereas IL-10 was decreased (loss of anti-inflammatory control). This data indicates that the duo turned toward sustained inflammation needed to contain latent *T. gondii*, coupled with insufficient counter-regulation by IL-10⁴³. Additionally, these results supported that disease severity is influenced by factors beyond parasitic burden, but also to the imbalance of cytokines⁴⁴.

On the other hand, the Receiver Operating Characteristic analysis (ROC curves) to miR-938 reveals that the AUC of 0.53 at a cut-off value of 1.335, 34% sensitivity, and 76% specificity. These data are considered statistically unacceptable. Also, it cannot be regarded as a novel diagnostic biomarker for toxoplasmosis as it has significantly less recognized performance standards for parasitic infection diagnostics, which classically require sensitivity ($\geq 80-90\%$) and specificity ($\geq 90-95\%$)⁴⁵. With respect to the ROC curve evaluation for IL-10 and IL-17 in the samples revealed the cut-off values of 20.228 U/ml and 373.582 U/ml respectively, with an AUC of 1.000, and both markers showed 100% specificity and sensitivity. As evidenced by the results, both IL-10 and IL-17 can be considered good and excellent diagnostic markers for chronic toxoplasmosis. The high specificity of these cutoff values indicates that positive results would be highly reliable for confirming toxoplasmosis. Additionally, the combination of both biomarkers may provide enhanced diagnostic accuracy compared to individual cytokine measurements⁴⁶.

Consequently, the current report demonstrated that Pearson correlation coefficient was utilized to examine the association between miRNA-938 with IL-10/ IL-17 levels. The results indicated that miR-938 has statistically significant correlations, a negative correlation with IL-10 ($r = -0.061$) while a positive correlation with IL-17 ($r = 0.025$). Additionally, IL-17 has higher negative correlation ($p < 0.01$) with IL-10 ($r = -0.895^{**}$). The study concluded that miRNA-938 expression has no impact on immune response during toxoplasmosis. Furthermore, elevated pro-inflammatory cytokine-17 and decreased anti-inflammation-10 caused by infection with *T. gondii*. Moreover, inverse correlation may be attributable to the excessive increase in IL-17 production, which resulted in a diminished IL-10 response during chronic toxoplasmosis, and it can lead to severe immunopathology and tissue damage. Interestingly, chronic parasitic infection characterizes a significant universal health problem, affecting millions globally and causing substantial illness and mortality. These infections include complex host-parasite interactions with the pathogen and the host immune system⁴⁷. Several studies have illustrated that immune responses can change through IL-17 elevation and IL-10 reduction during chronic parasitic infections. Gonzalez et al. (2013) established that IFN- γ and IL-17 levels were elevated during IL-10-deficient *Leishmania* mice, with IL-17 specifically contributing to increased neutrophil infiltration and tissue pathology⁴⁸.

IL-17 has a significant role during parasitic infection. In chronic *Toxoplasma cruzi*, the severity of cardiac weakness in children has been related to high IL-6 and IL-17 levels, accompanied by elevated IFN γ production together with profibrotic issues such as IL-13⁴⁹. Resemblance, in another study, showed that *Trypanosoma brucei* infection triggers an accumulation of interleukin -17A producing cells represented by (Th17 and V γ 6⁺ T cell in the fat tissues under the skin. Interestingly, mice that lacked the IL-17 receptor were spared from the tissue damage typically caused by the infection, suggesting that excessive IL-17 activity may drive damaging effects⁵⁰. In relation to IL-10 serves as a regulatory cytokine throughout the chronic phase of infection. Interleukin-10-secreting regulatory Bregs cells are activated during toxoplasmosis as a component of parasite-driven immunological activity, leading to chronic phase of infection creation⁵¹. Moreover, some specific virulence strains of *Toxoplasma gondii* can reduce IL-10 production or favor IFN- γ expression, leading to highly virulent strains driving stronger IL-10 reduction, excessive production of inflammatory cytokines exemplified by IFN- γ , TNF- α , and IL-12 that result in persistent inflammation⁵². Study conducted by Doherty et al. (2024) revealed that the *Toxoplasma* dense granule protein GRA-24 triggers substantial secretion of

IL-12, and if IL-10 from the cell is absent, this gives rise to mortal immune pathology. All of the above highlights that the interaction between parasite and host regulatory cytokines limits whether infection is controlled or becomes immune-pathologic⁵³.

The diagnostic performance of miR-938 falls substantially below the established standards for parasitic biomarkers and would not be considered clinically useful for chronic toxoplasmosis diagnosis without significant improvement or combination with other biomarkers. Although this study advances our understanding of how infection status relates to cytokine expression IL-10 and IL-17, yet several limitations must be considered. This encompasses that variation in the duration of participants' injury periods may have influenced cytokine dynamics, with observed differences potentially reflecting distinct stages of infection rather than a consistent immune response. Immunological factors, including pregnancy. In addition, immunosuppression, and other health conditions were not rigorously controlled, which could confound cytokine levels.

Another factor to consider is genetic heterogeneity among participants (such as variation in genes or micro-RNAs regulating cytokine expression) may contribute to inter-individual variability, limiting the generalizability of our conclusions. Furthermore, variability in the sensitivity and specificity of molecular detection methods may have affected measurement accuracy. Moreover, other unmeasured influences, including age, nutrition, comorbidities, and environmental exposures, may have contributed to immune heterogeneity. To overcome current limitations, future research should refine study design by recruiting more uniform and well-defined cohorts, expanding sample sizes to capture greater genetic and environmental diversity, and employing highly standardized molecular assays to improve accuracy. Longitudinal designs will be especially valuable for monitoring cytokine changes over time and clarifying how genetic, immunological, and environmental influences shape immune responses.

In conclusion, our findings are that miR-938 does not have a clear and effective role in *T. gondii* patients; therefore, it cannot be considered a novel biomarker for toxoplasmosis. In addition, excessive IL-17 level production in contrast reduction of IL-10, this dysregulation in both cytokines and *T. gondii* has evolved strategies that have successfully manipulated host immunity, promoted inflammatory persistence, and played a role in *Toxoplasma gondii* pathogenesis.

STATEMENT OF ETHICS

This study was approved by the College of Science for Women in University of Baghdad Scientific Committee (Decision Number: 22/7902, Date: 21.12.2023).

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

The authors contributed equally to the article.

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