

## Interleukin -10, Lactic Acid Dehydrogenase, Total Protein, Albumin and Globulin in Infected Women with Toxoplasmosis

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### Abstract

**Objective:** The aim of the study was to identify biomarkers in women diagnosed with Toxoplasmosis. **Materials and Methods:** The study was conducted between February 2022 and March 2023, and it focused on detecting *Toxoplasma gondii* in 100 female patients with a history of repeated abortion, aged between 18 and 43 years. The patients receiving treatment were present at two educational hospitals located in Baghdad. The diagnosis was established through the utilization of Immunochromatography and ELISA techniques. Blood samples were gathered from every individual, along with 30 healthy controls matched for age. The study measured the concentrations of Interleukin-10, Lactic acid Dehydrogenase, Total protein, Albumin, and Globulin in the patients' and controls' sera. **Results:** The findings revealed that 45 cases exhibited the presence of anti-*Toxoplasma* IgG, while 76 cases displayed the presence of anti- *Toxoplasma* IgM, out of the 100 women with a history of abortion and *Toxoplasma* infection. This was determined using immunochromatography methods, on the other hand, utilizing the ELISA methods, anti-*Toxoplasma* IgG was detected in 40 cases, and anti-*Toxoplasma* IgM was detected in 42 cases. **Conclusions:** The findings revealed elevated levels of IL-10, Lactic acid Dehydrogenase, Total protein, and Globulin in the patients' sera, along with decreased levels of Albumin compared to the healthy controls.

**Keywords:** *Toxoplasma gondii*, IL-10, Dehydrogenase, Albumin, Globulin.

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## INTRODUCTION

*Toxoplasma gondii*, a parasitic organism, possesses the capacity to invade various warm-blooded animals and humans [1, 2]. It has been acknowledged as a noteworthy foodborne parasite and a prominent opportunistic pathogen worldwide. In intermediate hosts like humans and warm-blooded animals, the parasite has the ability to exist in two distinct forms: rapidly multiplying tachyzoites and slowly dividing bradyzoites [3].

*T. gondii* actively penetrates host cells and takes up residence within specialized membranous structures known as parasitophorous vacuoles (PVs). It can multiply within these PVs, but if the PVs are disturbed, *T. gondii* cannot survive inside the host cells. The host's

immune cells perform a dual function, where they identify *T. gondii* components to trIgGer acquired immunity and simultaneously eliminate the PVs (parasitophorous vacuoles) to hinder the spread of proliferating *T. gondii* within the host.

However, *T. gondii* utilizes various virulence factors that it releases into host cells to obstruct both acquired and innate immune responses. The current work focuses on recent findings concerning the host's defense mechanisms against *T. gondii* and the strategies employed by the parasites to evade these defenses [4].

Upon infection of the host by *T. gondii*, dendritic cells (DCs) and macrophages become activated, trIgGering the creation of a range of cytokines including IL-1 $\beta$  and TNF- $\alpha$  [5- 7]. In mice, the

management of IL-1 $\beta$  and TNF- $\alpha$ , or simultaneous administration of IL-1 $\alpha$ , offers protection against *T. gondii* infection, with the presence of IL-12 playing a crucial role [8].

In the context of the innate immune response, IL-12 is produced by DCs (dendritic cells), plasmacytoid DCs, and macrophages. This prompts the proliferation of NK cells, CD4<sup>+</sup> T cells, and cytotoxic CD8<sup>+</sup> T cells, resulting in significant IFN- $\gamma$  production [8, 9]. IFN- $\gamma$ , a crucial component generated by activated immune cells like NK cells, ILC1 (innate lymphoid cells type 1), and T cells, plays a vital role in defending against *T. gondii* infection [10].

In Iraq, there has been a significant increase in the focus on Toxoplasmosis, as evidenced by several recent studies [11]. Researchers have taken an interest in studying the impact of serum biochemical parameters on individuals infected with parasites, these factors encompass cholesterol levels, triglycerides, high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), total protein, total globulin, total albumin, Vitamin D, and Dopamine [12- 15]. In vitro research has demonstrated that these parasites can thrive in lipid-rich environments, even without the presence of serum [16]. The objective of the present study was to assess the levels of specific biochemical parameters and biomarkers in women diagnosed with Toxoplasmosis.

## MATERIALS AND METHODS

The study was carried out at two teaching hospitals in Baghdad, involving women aged between 20 and 40 years. The participants were divided into two groups: one comprised of suspected patients, and the other consisted of healthy individuals serving as controls. Blood samples were obtained from 100 women who were clinically suspected of being infected with *T. gondii* and confirmed by specialist doctors.

### Blood Samples

The participants' blood samples were drawn from their veins and then placed into sanitized plastic tubes. Subsequently, each sample was left standing in serum separator tubes at room temperature for 30 minutes before being centrifuged at 3000 rpm for 5 minutes. The resulting serum from each sample was collected using Eppendorf tubes and stored at -20 °C.

### Immunochromatographic Test

Approximately 100  $\mu$ l of serum from each sample was placed in the designated area of the kit. The intensity of color corresponds to the level of antibodies present. After 10 minutes, the appearance of colored bands confirms the correct execution of the test. This CerTest-Toxoplasma kit is used to qualitatively detect

*Toxoplasma* in blood samples. The membrane was pre-coated with mouse monoclonal antibodies specifically targeting *Toxoplasma* antigens. During the test, the samples were mixed with colored conjugates (red antibodies of mouse monoclonal microspheres against *Toxoplasma*) and dried. Then, the mixture migrated through capillaries to reach the membrane as the samples passed through the membrane, the colored particles moved along. In positive results, certain antibodies on the membrane captured these particles, resulting in the appearance of a clearly observable red line. On the other hand, negative results (representing control samples) showed a green line.

### Measurement of IgM Antibodies against Toxoplasma and Assessment of IgG Levels

In order to conduct a qualitative and quantitative analysis of IgG and IgM antibodies against *Toxoplasma gondii*, two types of test kits were utilized to detect these antibodies in the sera of infected women. The *Toxoplasma* IgM enzyme immunoassay (EIA) and the *Toxoplasma* IgG EIA (enzyme immunoassay) kits manufactured by ACON Laboratories, Inc., located in San Diego, USA, were utilized. The quantification of *Toxoplasma* antibody levels was conducted using the ELISA technique. Antibody levels below 0.9 IU/ml were classified as negative, while levels ranging from 0.9 to 0.99 IU/ml were considered equivocal and required further verification. A positive result was indicated by a level equal to or above 1.0 IU/ml.

### Estimation of IL-10 Level

The IL-10 levels were measured in 42 patients who tested positive for anti-*Toxoplasma* antibodies using Cusabio's manual procedure.

Biochemistry examination Sera samples were assessed for LDH using kits from Randox Laboratories Ltd., UK, while Total protein, Albumin, and Globulin levels were measured using kits from Human, Germany, following the manufacturer's instructions.

### Statistics

Statistical analysis of the gathered data involved the utilization of the T-test in Statistical Package for the Social Sciences (SPSS) version 18.

## RESULTS

### T. Gondii Diagnosis

In Table-1, out of 100 cases analyzed using the immunochromatography method, the presence of anti-*Toxoplasma* IgM was detected in 76 cases, accounting for 76% of the total. Similarly, the presence of anti-*Toxoplasma* IgG was observed in 45 cases, representing 45% of the total. Interestingly, both anti-*Toxoplasma* IgM and IgG were found together in only 12 cases, making up 12% of the total.

**Table 1: The prevalence of anti-Toxoplasma gondii IgG and IgM antibodies among women with a history of recurrent abortion**

Anti-Toxoplasma antibodies	Total	Positive		Negative	
		No.	%	No.	%
IgG	100	45	45	55	55
IgM	100	76	76	24	24
IgM+IgG	100	12	12	88	88

In Table-2, the ELISA method was used to analyze a total of 100 cases. Out of these cases, anti-Toxoplasma IgM was detected in 42 cases, accounting for 42% of the total. Similarly, anti-Toxoplasma IgG was

found in 40 cases, representing 40% of the total. Interestingly, the presence of both anti-Toxoplasma IgM and IgG antibodies was observed in only eight cases, making up 8% of the total.

**Table 2: The presence and distribution of anti-Toxoplasma gondii IgG and IgM antibodies were examined.**

Anti-Toxoplasma antibodies	Total	Positive		Negative	
		No.	%	No.	%
IgG	100	40	40	60	60
IgM	100	42	42	58	58
IgM+IgG	100	8	8	92	92

In Table-3, the levels of IL-10 pg/ml exhibited a significant increase in the patient groups when compared to the healthy control group.

**Table 3: IL-10 levels in healthy control and infected women and**

Groups	Total	IL-10 Pg/ml	
		Mean	SD
Infected women	42	7.23	1.66
Control	30	1.38	0.48

Table-4 demonstrates a significant increase in the activity of lactate dehydrogenase (LDH) in infected

women with Toxoplasmosis compared to the healthy control group.

**Table 4: The activity of lactic acid dehydrogenase (LDH) in women infected with Toxoplasmosis was compared to that of the healthy control group**

Groups	No.	LDH Activity (IU/L)	SD
Infected women	42	241.6	6.8
Control	30	164.5	12.3

The concentration of Total protein increased significantly in infected women with Toxoplasmosis in comparison with control group (Table-5).

**Table 5: comparison between women infected with Toxoplasmosis and the healthy control group**

Groups	No.	Total protein(gm/dl)	SD
Infected women	42	8.26	0.52
Control	30	6.29	0.78

The concentration of Globulins increased significantly in infected women with Toxoplasmosis in comparison with control group (Table-6).

**Table 6: The concentration of globulin (g/dl) was compared between women infected with Toxoplasmosis and the healthy control group**

Groups	No.	Globulin (g /dl)	SD
Infected women	42	2.98	0.62
Control	30	2.18	0.95

In Table-7, a significant decrease in the concentration of albumins was observed in infected

women with Toxoplasmosis when compared to the healthy control group.

**Table 7: The comparison between women infected with Toxoplasmosis and the healthy control group**

Groups	No.	Albumin (g /dl)	SD
Infected women	42	3.21	0.36
Control	30	5.81	0.48

## DISCUSSION

The majority of acquired infections in healthy individuals tend to be benign, regularly asymptomatic or presenting with unclear symptoms. The prevalence of positive *T. gondii* infections among females, reaching 46%, clearly demonstrates the parasite's ability to spread within different populations. It is predictable that around one-third of the global people carries *Toxoplasma* infection, with significant variations in prevalence observed among different countries (17). In Table-1, the immunochromatography method revealed that 45 cases (45%) tested positive for Anti-*Toxoplasma* IgG, while 76 cases (76%) showed positive results for IgM. Similarly, in Table-2, the ELISA method indicated that 40 cases (40%) were positive for Anti-*Toxoplasma* IgG, while 42 cases (42%) tested positive for IgM.

The prevalence of infection is influenced by various factors, such as dietary habits, exposure to soil, age, living in urban or rural areas, regularity of get in touch with with domestic animals, climatic surroundings (e.g., moisture), and pregnancy. Furthermore, the ELISA test demonstrates higher specificity and sensitivity in comparison to alternative methods [18]. The anti-*T. gondii* antibodies in this study is consistent with seroprevalence data reported in earlier studies carry out in Iraq. Out of the 226 pregnant women surveyed in Salah-Adden government, 66 cases (29.2%) had *Toxoplasmosis*. Among these cases, 59 (26.1%) tested positive for Anti-*Toxoplasma* IgG, while 7 (3.1%) tested positive for IgM antibody [11].

IL-10, known as an important human factor named cytokine synthesis inhibitory factor (CSIF), acts as an anti-inflammatory cytokine. Research findings indicate that IL-10 is linked to a higher susceptibility to *T. gondii* infection, supporting reference 19. IL-10 assumes a vital role in the inflammatory response when encountering acute *T. gondii* infection. This cytokine exerts diverse and pleiotropic special effects on inflammation and immunoregulation, leading to a reduction in of Th1 cytokines expression; Macrophages exhibit MHC class II antigens along with co-stimulatory molecules. Additionally, this fosters B cell survival, proliferation, and the generation of antibodies, as mentioned in reference 20. IL-10 deactivates macrophages, trIgGers IFN- $\gamma$  release by *T. gondii*, and aids in the intracellular survival of the parasite. During *T. gondii* invasion, IL-10 induces immunosuppression, benefiting both the host and the parasite, as explained in reference 21. The elevated levels of lactic acid dehydrogenase (LDH) in infected women with *toxoplasmosis* could be attributed to LDH involvement in the lysis of cells infected with the *Toxoplasma* parasite

or the disruption of cells caused by the parasites, as noted in reference [22].

The rise in total protein and globulin levels observed in women with *toxoplasmosis* can be linked to elevated levels of specific immunoglobulin antibodies produced by the immune system in reaction to *T. gondii* infection. *T. gondii* stimulates the generation of diverse immunoglobulins, such as and IgE, IgM, IgG, and IgA, antibodies, targeting both membrane and excretory-secretory antigens, as referenced in [23]. However, the reduction in albumin levels in infected women with *toxoplasmosis* is most likely a consequence of liver damage.

## CONCLUSIONS

- 45 cases are anti-*Toxoplasma* IgG, 76 cases anti-*Toxoplasma* IgM, while in using the ELISA methods, anti-*Toxoplasma* IgG only 40 cases are identified, and only 42 cases are with anti-*Toxoplasma* IgM.
- Additionally, the results demonstrated elevated levels of IL-10, lactic acid dehydrogenase, total protein, and globulin in the patients' sera, while the level of albumin was decreased comparing with control group.

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