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Biomarker Evaluation in *Toxoplasma gondii*-infected Patients

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ABSTRACT

Back ground: This study used Toxoplasma IgG/IgM Antibody Rapid test to detect Toxoplasmosis in pregnant women and evaluate their lipid profile compared to a healthy control group.

The aim of the study: Serodiagnosis of parasite infection in aborted pregnant women using the rapid detection method. Evaluating the level of some immunological parameters in infected women such as IL17-A, TNA-, lactoferrin, shedding light on the lipid profile of parasite-infected women.

Patients and Methods: blood samples 240 were collected from aborted pregnant women attending some governmental hospitals and private medical clinics in some areas of Salah al-Din Governorate during the period from 1/7/2022 to 1/2/2023 and the required information was recorded according to a special questionnaire form.

The results: were analyzed statistically using the IBM SPSS Statistics (version 23) program, according to the t-test, at a significant level of p≤0.05. The results of Rapid Test (93 positive samples) 38.7% (divided into 80) 86.02% (positive samples for IgG, while 13 sample) 13.97% (positive for IgM and 147 negative samples) 61.25%. (There is a positive correlation between (LTF, IL-17A, TNF-a) (highly significant). The lipid profile analysis revealed a non-significant increase for each of cholesterol and high-density lipoprotein (HDL) in infected women, as Mean ± S.E concentration was (168.4±8.2 Pg/ml); (33±1.5pg/ml) respectively, compared to the control group, where the Mean ± S.E concentration was (141.6±15.5 Pg/ml); (28±3.09Pg/ml) respectively. While there was a non-significant decrease in each of LDL, very low-density lipoprotein (VLDL), and triglycerides in pateints group, as Mean ± S.E concentration was (80.6±3.7pg/ml); (45.4±1.07 pg/ml); (159.7±4 pg/ml), respectively, compared to the control group, where the average concentration was (84.3±5.3pg/ml); (48.6±2pg/ml) 161.3±6.7pg/ml) respectively.

Keywords- Toxoplasma gondii, Lipid profile, Aborted women, Cholesterol, Rapid test.

I. INTRODUCTION

Toxoplasmosis is a disease with a worldwide spread that affects approximately one third of the population. It is caused by Toxoplasma gondii, an obligate intracellular protozoan. [1]. This parasite can be infecting a broad range of warm-blooded vertebrates, humans and animals as intermediate hosts, while Felidae (cats) serve as intermediate, and definitive host [2]. The primary mode of transmission of this parasite to humans is through the swallowing of food or water that has been contaminated with oocysts discharged by felines, or through the consumption of undercooked or raw meat harboring tissue cysts. [3]. Tachyzoites have been identified in many sources such as blood components, tissue transplants, and fresh milk. Various hazards, such as the level of education attained, exposure to cats, debris, and the intake of vegetables and fruits fresh , have an impact on the spread of *T. gondii*. [4]. The involvement of pro-inflammatory cytokines is crucial in the development of Toxoplasmosis [5]. The production of cytokines is contingent upon the cellular kinetics, wherein TM1 cells are responsible for manufacturing tumor necrosis factor alpha (TNF-α) or beta (TNF-β), whereas TH17 cells are responsible for producing Interleukin-17A (IL-17A) [6]. The generation of acute inflammatory reactions, as well as the activation of the adaptive immune response and the immediate immunological response against the

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Toxoplasma parasite, can be attributed to TNF-α. Furthermore, it induces the production of interferon-gamma (IFN-Y) by natural killer cells in the context of acute Toxoplasma infection. TNF-α is involved in the initiation of the initial immune response through its ability to induce the cellular killing mechanism of macrophage cells in the context of acute toxoplasmosis infection [7]. The cytokine IL-17A is implicated in the immune response of the host against infections, and it is believed to have a significant impact on the development of protective immunity against T. gondii through the activation of neutrophil cells in areas of inflammation. Furthermore, it has been observed that IL-17A plays a role in the promotion of neuroinflammation in cases of chronic toxoplasmosis infection [8]. Lactoferrin (LF) is a glycoprotein that is present in a diverse range of body fluids, including milk, external secretions, saliva, tears, vaginal fluids, semen, nasal secretions, bronchi, bile salts, intestinal fluids, urine, and neutrophil cell granules. The intricate molecular mechanism behind the effectiveness of LF against parasite infestation involves the disruption of the parasite's iron intake process, which is essential for its development. Lactoferrin (LF) has been observed to possess the ability to decrease and impede the activity of Toxoplasma gondii [9].

T.gondii is parasite obligately intracellular, showing its dependence on an intracellular source of vital nutrients. The process of replication within the parasitophorous vacuole (PV) requires an adequate amount of lipids to facilitate the formation of membranes. *T. gondii* may create phospholipids on its own, but it needs the host's lipids converted intactly for membrane assembling because it lacks the necessary enzymes for sterols molecule synthesis. Clearly, *T. gondii* possesses a mobilization function that transfers lipids from the host into the parasite's PV membrane (PVM)[10].T. gondii has such a profound effect on host lipids that it's important to investigate how lipid levels relate to infection in humans. However, T. gondii's lipid metabolism and absorption have not been sufficiently studied. [11]. Thus, the association between T. gondii seropositivity and blood lipid levels is yet unknown, particularly in healthy adults.The occurrence of alterations in the lipid metabolism of the host has been observed in during acute infection, as substantiated by a growing body of research.

II. MATERIALS AND METHOD

2.1 Sample collection

From July 2022 to February 2023, blood samples were collected from abortion patients who visited hospitals and private medical clinics in Salah al-Din, Iraq. 5 ml of venous blood was drawn from each patient using a syringe made of pyrogen-free, disposable, pyrolysis-free plastic. Transported blood into the clot activator tubes. The containers containing the clot activator were allowed to coagulate for 15 to 30 minutes at room temperature before being centrifuged at 2000 rpm for 10 minutes to obtain the serum.

2.2 Toxoplasma IgG/IgM Antibody Rapid test

The rapid test detection involved the application of a small amount of patient serum onto the designated area of the cassette. The test result was then recorded and observed within a time frame of 15 to 30 minutes, following the established protocol for the qualitative detection of T. gondii IgG/IgM antibody in serum samples. This diagnostic method utilized immunochromatography and was conducted using a kit manufactured by Qinodao Hightop Biotechnology, China.

2.3 Human TNF-α, IL-17A, Lactoferrin ELISA test

The serum concentrations of TNF-α, IL-17A, and lactoferrin were assessed using the enzyme-linked immunosorbent assay (ELISA). The ELISA plates were precoated with human TNF-α, IL-17A, and lactoferrin antibodies. The samples were added to the plates, and the color development in the substrate solution correlated with the level of TNF-α, IL-17A, and lactoferrin. The process was stopped by adding a stop solution, and absorbance was measured at 450 nm.

2.4 Lipid profile tests

Cholesterol, triglycerides, and HDL levels were quantified using a conventional enzymatic Test provided by Linear Chemicals, Biosystem-Barcelona, Spain. The levels of LDL and VLDL were indirectly assessed using Friedewald's equation [12].

LDL = Total cholesterol – HDL – (TG/5);
VLDL = Triglyceride/5

2.5 Statistical Analysis

The statistical analysis was conducted using IPM SPSS version 22. Categorical information was presented using frequencies and percentages. The convention for representing continuous variables in statistical analysis typically involves reporting the means along with their corresponding standard deviations (SD). Significance was attributed to p-values that were equal to or less than 0.05.

III. RESULTS

3.1. Diagnosis of Toxoplasma gondii By Method Toxoplasma IgM/IgG Rapid test kit

A total of 240 pregnant women were enrolled and screened for the presence of anti-toxoplasma IgG and IgM antibodies.The seroprevalence of Toxoplasma gondii IgG and IgM antibodies result revealed that 80 (33.3 %) of 240 pregnant women were positive for anti-toxoplasma-antibody IgG and 13 (5.41 %) of 240 were positive for anti-toxoplasma antibody-IgM. not recorded any mixed IgM+IgG in any samples.show a table(1).

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Table 1: Prevalence of anti-Toxoplasma antibodies in aborted women.

Method	samples examined	IgG ve+)%(	IgG ve- (%)	IgM ve+ (%)	IgM ve- (%)
Toxo - IgG/IgM Rapid test kit	240	80(33.3%)	160(66.6)	13(5.41%)	227(94.5%)

The results of the current study agreed with the results of both [13] in Sulaymaniyah, [14] in Salah al - Din, [15] in Tikrit, The researchers observed a higher prevalence of IgG antibodies in pateints compared to the prevalence of IgM antibodies. While the results of the current study did not concur with the [16] in the northern Tarmiyah district of Baghdad, the percentage of IgM was higher than the percentage of IgG antibodies in pregnant women.

3.2 Correlation between immunological parameters LTF,IL-17A,TNF-α

The correlation between biomarkers in women with toxoplasmosis, there is a positive correlation between (LTF, IL-17A, TNF-a) (highly significant).

Table 2: Correlation between immunological parameters LTF,IL-17A,TNF-α

Parameters		**LTF**	**IL-17A**	**TNF-α**
LTF	r		0.733	0.810
	P. value		0.001	0.004
IL-17A	r	0.733		0.721
	P. value	0.0021		0.0021
TNF-α	r	0.810	0.721	
	P. value	0.002	0.003	

The study show A positive correlation between LTF , IL-17A and TNF-α , A direct correlation appeared between IL-17A , LTF and TNF-α , Also, our study showed a moral rise with TNF-α , IL-17A and LTF.

3.3 Lipid profile in women infected with toxoplasmosis and control

Table (3) show The results of lipid profile in patients revealed a non- significant increase for both Cholesterol and high -density lipoprotein level HDL, as the Mean concentration (168.4 ± 8.2pg/ml); (33.6 ±1.5pg/ml), respectively, compared to the control group where the Mean concentration (141.6 ± 15.5pg/ml); (28.2 ± 3.09pg/ml) respectively. While a non - significant decrease appeared in both low density lipid LDL, very density lipoprotein VLDL and triglycerides in patients, as the mean concentration (80.6 ± 3.7pg/ml); (45.4 ± 1.07pg/ml); (159.7 ± 4.0pg/ml), respectively, compared to the control group, with mean concentration (84.3 ± 5.3pg/ml); (48.6 ± 2pg/ml); (161.3 ± 6.7pg/ml) respectively.

Table 3: Lipid profile values of prevalence in women infected with toxoplasmosis and control

Standard	**Patient group N=93 Mean ± S.E**	**Control group N=30 Mean ± S.E**	**P.value**
Cholesterol	**168.4** $\pm 8.2 pg/ml$	**141.6** $\pm 15.4 pg/ml$	**0.099**
HDL	**33.6** $\pm 1.5 pg/ml$	**28.2** $\pm 3.09 pg/ml$	**0.91**
LDL	**80.6** $\pm 3.7 pg/ml$	**84.3** $\pm 5.3 pg/ml$	**0.574**
VLDL	**45.4** $\pm 1.07 pg/ml$	**48.6** $\pm 2.1 pg/ml$	**0.136**
Triglycerides	**159.7** $\pm 4.0 pg/ml$	**161.3** $\pm 6.7 pg/ml$	**0.830**

IV. DISCUSSION

The results of cholesterol and HDL agreed with Al-Kuraishi etal., Taher, and Abed [17] , [18] , [19] , but did not agree with [20], The results of the current study for all LDL,VLDL and Triglycerides agreed with Taher [18],and did not agree with Al-Kuraishi etal [17] and Abed, [19] ,The current study agreed to decreased the level of triglycerides with the results of Taher [18] , Ali, and Al-Warid [20] , but the results of the study did not agree on the high level of triglycerides with Al-Kuraishi etal., and Abed [17] , [19].

Immunochromatography (rapid test) was used during this study as a diagnostic tool for determining antibodies produced in response to T. gondii antigens.The presence of IgG antibodies indicates chronic infection with counterattacks, whereas the presence of IgM antibodies indicates an acute infection; the presence of IgG and IgM antibodies together indicates the occurrence of stimulation of the chronic infection and its transformation into an acute infection; and the cause of the presence of IgG is greater than the IgG rate. Adding IgM until the IgM disappears or decreases to extremely low levels, followed by the appearance of IgG that persists for a prolonged duration [21]

The correlation of LTF with IL-17A and TNF-α parameters of the current research can provide more

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details about the physiological and clinical importance of LTF in diseases. Positive correlation indicates that the two parameters are positively correlated both of them increase together. But negative correlation denotes that the two parameters are inversely correlated, it means an increase in one variable leads to a decrease in the other.

Fats organic compounds play an important role in almost all aspects of life, they are structural components in cells and participate in metabolic and hormonal pathways [22]. It also has importance in defending against the parasitic infections, as it is considered important in the host's defenses during the acute phase of innate immune response, as infection and inflammations clearly change the level of metabolism lipids of the host [23]. Many viral, bacterial and parasitic infections can change or affect the level of lipid in the blood of patients, as many studies recorded the occurrence of fat changes during the infection with intracellular parasites, for example, malaria and visceral leishmaniasis [18].

Another study demonstrated the occurrence of a reverse change of HDL in the blood of patients with the severity of three species of worms [24]. People infected with Entamoeba Histolytica and Giardia lamblia are observed a decrease in the level of HDL [25]. Another study conducted on patients with Plasmodium falciparium has a difference in the level of lipids compared to the uninfected control group [26]. Also with visceral leishmaniasis ,a decrease in cholesterol and a high triglycerides [27]. A experimental study on the female laboratory mice infected with T.gondii's parasites recorded changes in the level of fat and fat with the progress of the disease from acute to chronic stage, as well as the mice infected naturally with Trypanosoma Cruzi and Schistosoma mansoni appears a change in the level of fats [28]. The fat is characterized by its special importance for pathogens that search for stations or places inside the host rich in fat, as they enhance the availability of fat by stimulating and directing the guns, as the intracellular pathogens possess complex mechanisms to interfere in the process of acting and the nutritional ovation of fats in the host cells and benefit from them, Within the cells of the parasite host, it is inside special vacuoles, as the transmission and flow of lipids between the host and the components of the parasitic vacuole are the key that determines the secret of the parasite's success [18]. Many previous studies have revealed the role of membranous cholesterol in interaction and connection between the parasite and the host [29]. It is an important component of cellular membranes in real -core organisms and plays a critical role in the function and regulation of membranous proteins and receptors [30]. T. gondii is unable to synthesis a new cholesterol because it does not have certain enzymes and depends on its creation on taking lipoproteins and a low-density lipid cholesterol LDL from the host cells, by the Endocytosis process with the help and mediation of the future protein of the low density lipid cholesterol LDL [31]. Other studies that the mechanism, with the host cholesterol, suggested that the Toxoplasma into the target host cells appeared, and here the important role of cholesterol appeared in the pathogenic toxic disease [32]. The growth and replication of the parasite inside the (PV) in the host cell requires large amounts of specific lipids for the vital manufacturing of the membranes [33]. In any case, the information and data on the source of the parasite lipids are few and rare, and the molecular mechanism used by the parasite to acquire the host's fat is not well clear [34].

T. gondii induces specific T-cell-mediated immune response. Th17 and Treg cells are two important CD4+ T cell subsets that mediate immune response for normal pregnancy. Both Th17 and Treg cells differentiate from the same naive CD4+ T cells, but have the opposite effects on immunological self-tolerance and inflammation. The Th17/Treg imbalance has been demonstrated to be linked with adverse pregnancy [35]. Regarding TNF-α, we observed lower levels in both groups of infected pregnant women, contrary to the findings of Marchioro et al., who did not find differences in the levels of this cytokine in infected pregnant women when compared with controls, and this difference in the immune response could be attributed in part to the different genotypes of the parasite[36]. Recently, Dziadek et al. (2005) reported the presence of a common receptor for bovine lactoferrin, transferrin, and ovotransferrin on T. gondii, which is the membrane protein of 42 kDa. In this study, it was determined that in the case of human iron-transporting proteins only lactoferrin was bound by both live tachyzoites and TLA crude antigen and this binding was not inhibited by human transferrin. On the basis of these observations we presume that T. gondii tachyzoites possess speciWc surface receptors for human lactoferrin. The presence of such receptors could play an important biological role in the parasite invasion and Wnally in replication [37].

V. CONCLUSION AND RECOMMENDATIONS

Toxoplasma gondii played a role in altering the lipid profile values of infected women, as evidenced by an increase in cholesterol and HDL and a decrease in LDL, VLDL, and Triglycerides. Further research is required to maintain the effect of toxoplasmosis duration (acute or chronic) on lipid profile changes. That the collection between parameters positive collation between immunological parameters in the woman infected with toxoplasma. Conducting a study that includes the collection of blood samples from the harbors of women with women and investigating some of the factors of virility and parasite antigens, as well as conducting a study that includes evaluating standards under study with other abortion nurses. further studies must be done to identify the parasite human lactoferrin receptors and

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explain their biological role not only for tachyzoites, but also other stages of T. gondii in humans. Th17 cells and TNF-α play avital role in the immune response in women with toxoplasmosis

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