THE EFFECT OF CHRONIC INFECTION WITH *TOXOPLASMA GONDII* ON SOME IMMUNOLOGICAL PARAMETER IN BABYLON PROVINCE WOMEN/IRAQ

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ABSTRACT

Since 1908, *Toxoplasma gondii* (*T.gondii*) has been discovered and intensively studied with the host's immune response. *T.gondii* is a parasite presented inside cells and affects more than a quarter of the world's population, it is very important to study this parasite especially for the patient with immunodeficiency or immunosuppressive diseases and pregnant women. Basically, the aim of this study is investigation of the chronic toxoplasmosis in Babylon's province women using the VIDAS technique to get patients' titer IgG, as well as studying the relationship between a chronic toxoplasmosis infection and some immunological parameters D4, Kininogenase, and PAF using the ELISA technique. Furthermore, this study conducted on 255 women, where 50 (19.6%) women were diagnosed with chronic toxoplasmosis, while 90 samples were randomly selected to be the control group, and the collection of blood serum samples lasted six months. The distribution of titer IgG for these immunological parameters showed that the highest percentage (72%) for titer IgG was in infected women with titer (<100). The low density of titer IgG indicates the poor immune response in infected women with chronic toxoplasmosis. The results showed that the concentrations of immunological parameters D4, Kininogenase, and PAF in the blood of women with chronic toxoplasmosis (3.124ng/ml, 27.320ng/ml, and 4.543ng/ml) respectivelyis higher than the concentrations of the control group (2.781 ng/ml, 23.342 ng/ml, and 4.116 ng/ml) respectively, and this confirms the immunological criteria role in immune response against the *T.gondii* parasite, as it works to resist and inhibit the parasite through many mechanisms and substances that stimulate it to kill the parasite. Also, the results showed the highest concentrations of immunological parameters for women aged 20-30 years.Generally, the presented study demonstrates the effective role of immunological parameters (D4, Kininogenase, and PAF) in the immune response against the T.gondii parasite through its resistance and inhibition.

Keywords:*T.gondii*, D4, Kininogenase, PAF, titer IgG.

I. INTRODUCTION

T.gondii is an intracellular parasite that is capable of infecting all warm-blooded animals including humans (Dubey, 2009). Mostly, it could cause abortion, immunocompromised individuals, encephalitis, and death (Halonen and Weiss, 2013). Transmission in humans are usually occurs via the ingestion of undercooked or raw meat containing oocysts or consumption of contaminated food or water with oocysts, in addition to congenitally when the mother got the infection during pregnancy, where *T.gondii* transported to the fetus via the placenta and then affected the fetus(Tenter *et al.*, 2000). Spontaneous abortion is among the most gestational complications that occurred in responding to *T.gondii* parasite infection (Lyu *et al.*, 2013). Extensive studies have been conducted to investigate seroprevalence percentages in different parts of the world. The greatest importance comes from the prevalence among women of childbearing age.

The immune system works to protect the body and expel foreign bodies in two ways: natural immunity and acquired immunity.Natural immunity is somewhat undeveloped in response to preventing germs and pathogens from reaching the body, and this is an innate immunity that occurs automatically without the body having been exposed to pathogens in advance.This type of immunity activates automatically during pregnancy and is represented by an increase in the number of granulated white blood cells, monocytes, and others. Either adaptive or acquired immunity consists of two types of immune responses: 1) Humoral immunity, which produces

antibodies. 2) Cellular immunity, in which cells are degraded by specialized lymphocytes (cytotoxic T cells), and this type is characterized by a memory response, meaning that the immune cells can remember the type of foreign antigen and in return produce the appropriate antigen in a faster time and stronger, Cytokines are widely used in this type of immunity, which can maintain the continuity of pregnancy through immune balance during pregnancy(Herbert *et al.*, 2010).

Platelet-activating factor (PAF) is a phospholipids potent activator and mediator of numerous leukocyte functions, platelet lysis and aggregation, inflammation, and anaphylaxis. It seems apparent that PAF has different physiological roles in animals, plants, and unicellular organisms, such as apoptosis, physiological inflammation, wound healing, reproduction, angiogenesis, long-term potentiation (Maclennan*et al.*, 1996; Birkl *et al.*, 2019).. It is considered the most potent lipid mediator known to date(Siafaka-Kapadai et al., 1986; C A Demopoulos, 2000).

Leukotrienes D4 are lipid mediators with potent inflammatory activities, they have a part in controlling protozoan and helminth infections by modulating the immune system through direct cytotoxicity to parasites. Interestingly, some proteins from the saliva of insect vectors that transmit protozoans and secreted protein from helminth could bind Leukotrienes and may consequently modulate the course of infection or pathogenesis (Rogerio and Anibal, 2012).

Kininogens are precursor proteins for kinins, biologically active polypeptides involved in blood coagulation, vasodilation, smooth muscle contraction, inflammatory regulation, and the regulation of the cardiovascular and renal systems (Duchene and Ahluwalia, 2011).

Due to the important roles that this immune factors (D4, Kininogenase, and PAF) play in many biological activity, including inflammation.The present study aimed to investigate the concentration of this factors in the sera of infected women with chronic toxoplasmosis and compare them with their counterparts in uninfected women.

II. MATERIALS AND METHODS

1. Collection of Information

The present study was conducted on women who are suspected of chronic Toxoplasmosis and have been referred by the specialist doctor at, Gynecology & Children Babylon Hospital and the Laboratories were conducting the necessary examinations to determine this infection. From the beginning of October 2020 to the end of March 2021.

2. Samples collection

Blood samples (5 ML) were collected from all enrolled women. The blood was placed in tubes without EDTA for obtaining the serum. Serum was separated from the whole blood by centrifugation at 2000 R.P.M for 5 minutes. In addition, it has been used the VIDAS technique to determinate the concentration and titer of IgG in order to diagnose the patient's condition whether or not was infected with chronic toxoplasmosis, then collected serum was kept at -20ºC until being used.

3. Immunological Tests

The concentration of immune parameters (D4,Kininogenase, and PAF) was measured using ELISA technique according to the method supplied by the manufacture company (BT LAB /China).

III. RESULTS

This study was conducted on 255 samples of women, where the examination results showed that 50(19.6%) sera out of 255 were positive for chronic toxoplasmosis, as shown in Table(1).

Table (1): Prevalence of chronic toxoplasmosis in Babylon province women

No. of examined	No. of infected	Percentage %	No. of non-infect	Percentage %

			ed	
255	50	19.6	205	80.4

The results of present study showed a significant increase ($p<0.05$) in the concentration of immunological parameters (D4, Kininogenase, and PAF) in sera of infected women compare with non-infected women with chronic toxoplasmosis (Table 2).

Table (2): The concentrations of D4, Kininogenase, and PAF among control group and infected women with chronic toxoplasmosis

Immune parameters	Infection	Descriptive Statistics			ANOA Comparisons sig.
		Mean	S.D.	Mean Difference	
D4 ng/ml	Infected	3.124	0.571	0.343*	0.002
	Control	2.781	0.475	-0.343*	0.002
Kininogenase ng/ml	Infected	27.320	3.270	3.979*	0.000
	Control	23.342	3.559	-3.979*	0.000
PAF ng/ml	Infected	4.543	0.664	0.427*	0.001
	Control	4.116	0.538	-0.427*	0.001
Based on estimated marginal means *.The mean difference is significant at the .05 level.					

The results in Table (3) showed no significant differences at ($p>0.05$) for each of the immunological parameters D4, Kininogenase, and PAF concentration in different IgG titer level.

Table (3): The relationship between concentrations of some immunological parameters D4, Kininogenase, and PAF with infection titer (IgG)

Immune. parameters	Titer (IgG)	Descriptive Statistics				ANOVA Sig.
		N	%	Mean	S.D.	
D4 ng/ml	<100	36	72	3.18431	0.622604	0.802
	101-200	7	14	3.05557	0.553626	
	>200	7	14	3.25671	0.267188	
	Total	50	100	3.17642	0.571146	
Kininogenase ng/ml	<100	36	72	27.68678	3.390136	0.193
	101-200	7	14	26.34329	2.950046	
	>200	7	14	29.49986	2.391919	
	Total	50	100	27.75252	3.270866	
PAF ng/ml	<100	36	72	4.59217	0.714434	0.723
	101-200	7	14	4.45343	0.582901	
	>200	7	14	4.74457	0.494363	
	Total	50	100	4.59408	0.664964	

The results in Table (4) presented that there were noticeable differences in the concentrations of immunological parameters between age groups. Basically, the age group (20-30 years) recorded the highest concentrations than the other two age groups (older than 30 years) and (younger than 20 years).

Table (4): The concentration of D4, Kininogenase, and PAF in infected women with chronic toxoplasmosis and non-infected women according to age

Immunological parameters	Infection	Age	N	Mean	S D.
D4 ng/ml	Infected	<20	9	2.876	0.359
		20-30	26	3.473*	0.581
		>30	15	2.842	0.351
		Total	50	3.176	0.571
	Control	<20	6	2.987	0.440
		20-30	26	2.833	0.504
		>30	8	2.926	0.438
		Total	40	2.874	0.475
Kininogenase ng/ml	Infected	<20	9	26.337	2.387
		20-30	26	29.181*	3.078
		>30	15	26.124	3.056
		Total	50	27.752	3.270
	Control	<20	6	21.691	5.517
		20-30	26	24.047	3.031
		>30	8	25.486	2.988
		Total	40	23.981	3.559
PAF ng/ml	Infected	<20	9	4.270	0.513
		20-30	26	4.936	0.618
		>30	15	4.195	0.509
		Total	50	4.594	0.664
	Control	<20	6	4.291	0.697
		20-30	26	4.085	0.510
		>30	8	4.516	0.408
		Total	40	4.202	0.538

Based on estimated marginal means
*.The mean difference is significant at the .05 level.

IV. DISCUSSION

The examination results of 50(19.6%) sera out of 255 were positive for chronic toxoplasmosis.The current study result is compatible with those of the literature; a study by Al-kremy and Al-hassnawi (2020) conducted on 147 Babylon' women and the percentage of infection was (19%). Also, the results of the study conducted by Kadir *et al.* (2011) in Kirkuk province included cases of 319 patients with toxoplasmosis and showed 54 positives by using the ELISA technique (16.9%).Whilst, another study recorded a higher percentage of infection with toxoplasmosis, where a study in Diyala province included 100 women and the results showed that the percentage was (44%) of infection with toxoplasmosis (Shaker *et al.*, 2018). As well as a study by Al-Saeed *et al.*(2008)in Hilla city/Iraq recorded a higher infection percentage (41.66%) with toxoplasmosis, where 50 samples were infected out of 120.The different rates of infection with toxoplasmosis in various researches and in different cities all around the world, is a result of the difference in the method of nutrition, which has a major role in the variation in the infection incidence between different regions(AL-Awsi, 2020), as well as the lack of attention to health means increases the risk of contamination of water and food with oocysts, and eating them through the device digestive system (Lavine and Arrizabalaga, 2008).

Leukotrienes (LTs) have a key role in controlling the protozoa and helminth infection by modifying the immune system via direct parasites' cytotoxicity. However, it is worth mentioning that several proteins from the insect vectors saliva that transport protein and protozoans secreted from helminths can bind LTs, and thus may pathogenesis or modulate infection course (Rogerio and Anibal, 2012).

A significant differences increase ($p<0.05$)has been observedin D4 concentrations, where the concentration in infected women with chronic toxoplasmosis (3.124 ng/ml) is higher than control group (2.781 ng/ml), as shown

in Table (2). This result shows that D4 play a substantial role in the immune responses (Th1 and Th2) which are contributed in defense against helminth infections and protozoan (Peters-Golden and Henderson Jr, 2007; Rogerio and Anibal, 2012).

The current study results match with results that indicate the increases of LTs concentrates, which associated with the control of protozoandiseases, like cerebral malaria, and helminthic diseases over their ability to modulate inflammatory processes to promote direct protozoans cytotoxicity, also can limit the growth of protozoan (Peters-Golden *et al.*, 2005).

A significant differences increase ($p<0.05$) in Kininogenase concentrations has been observed, where the concentration in infected women with chronic toxoplasmosis (27.320 ng/ml) is higher than control group (23.342 ng/ml), as shown in Table (2).. The increase in the proportions of this immune parameter indicates an increase in the immune response in the resistance of parasites in terms of inhibiting the parasite and then eliminating it. Interestingly, the increasing levels of kininogen was related to infection and inflammations (Wong, 2016), where Kininogens has being involved in inflammatory regulation, blood coagulation, the regulation of the cardiovascular, renal systems, and vasodilation (Duchene and Ahluwalia, 2011).

The results of Del Nery *et al.* (1997) study, can demonstrate that the pathogenic parasite *T.cruzi* displays kinin-releasing activity as a response to the infection and that led to an increase and release of kininogenase.In addition, another study match with the increases of kininogenase levels, where the total kininogen levels were very low in control group, but increased by 75-fold during acute parasite infection (Griesbacher *et al.*, 2003).

PAF has several actions like decreased cardiac output, macrophages, monocytes, activation of platelets, stimulation of glycogenolysis in perfused liver, stimulation of uterine contraction, activation of polymorphonuclear leukocytes, hypotension, increased vascular permeability, and others. it looks like PAF act in both normal physiological events and to mediate pathological responses, specially allergy and inflammation (Prescott *et al.*, 1990).

A significant differences increase ($p<0.05$) in PAF concentrations has been observed, where the concentration in infected women with chronic toxoplasmosis (4.543 ng/ml) is higher than control group (4.116 ng/ml), as shown in Table (2). The increase in the proportions of this immune parameter indicates parasite growth inhibition has a better correlation with PAF existence (Lonardoni *et al.*, 2000). The present study finding illustrating that PAF has an essential role in controlling the infection of toxoplasmosis. Generally, PAF can be a possible curative target to various chronic diseases(Constantinos A Demopoulos *et al.*, 2003; A. Tsoupras *et al.*, 2018; Lordan *et al.*, 2019).

The results of the current study agreed with other studies' results, where a study by Lonardoni *et al.* (2000) investigated the role of PAF in the experimental *Leishmania* infection and the relationship between this mediator and nitric oxide (NO) production. the results of this study showed that the addition of PAF to C57BL/6 mouse macrophages significantly inhibited parasite growth and induced NO production for the infected mice with leishmaniasis.Also, the present study results match with Aliberti *et al.* (1999).

The results of no significant differences between titer of IgG and the concentrations of the immunological parameters (D4, Kininogenase, and PAF) may be because the largest number of titer concentrations were less than 100 with 36 titer IgG. Also, may be due to the means ratios of the immunological parameters are close to each other to three titer groups.

When calculating the distribution rate of titers IgG in immunological parameters (D4, Kininogenase, PAF), the results were showed that the highest percentage (72%) for titer IgG was for infected women with chronic toxoplasmosis with titer less than 100, while the lowest percentages (14%) for both 100-200 and more than 300 titer of IgG. The specified immunological response to parasites drives antibody production (Stewart, 2012). The high density of *anti-T.gondii* IgG antibody may be a response to a large number of parasites entering the host body. Moreover, the antibody number reflects infected host immune status, as well as the low concentrations of titer IgG (less than 100).The reason for low or highlevels of IgG antibodies concentration in infected women is due to the extent of their exposure to sources of pollution, as dietary habits have a role in the variation in the incidence of infection between different regions(Lavine and Arrizabalaga, 2008). Further, the low concentrations in the level of IgG antibodies may depend on the variance in the immune status of the women under study at the time of taking the sample. The role of the cellular and humoral immune response changes depending on the stage of infection and its location in the body(AL-Awsi, 2020).

One of the ways the parasite disappears from the host's immune system is its entry into cells, especially cells that have an immune role such as phagocytes and others, and this is what avoids the host's immunity, where the *T.gondii* parasite is an intracellular parasite. The parasite counteracting the cellular and humoral immune response remains the protozoan in a latent state and reactivated when the immune response is weakened (Da Silva and Langoni, 2009). The delicate balance between stimulation and escape from the immune response is key to create a chronic infection (Blader and Saeij, 2009). The *T.gondii* immune response of antibodies has a minor role but remains the primary method of personification toxoplasmosis in humans (Filisetti and Candolfi, 2004).A study results by Al-Sallami (2020) agreed with the present study results, it's recorded that the highest infection percentage was (75%) for infected women with toxoplasmosis titer of IgG (8-80), while toxoplasmosis infected women with titer IgG (≧ 300) got the lowest percentage of infection (6.25%).

Interestingly, the increase in the proportions of this immunological parameter (D4, Kininogenase, and PAF) for infected women with chronic toxoplasmosis and aged 20 to 30 years indicates that these immunological parameters have a better effect on *T.gondii* parasite, in terms of increasing the immune response to resistance parasites by inhibiting the parasite and then eliminating it(Al-Saeed *et al.*, 2008).

V. CONCLUSION

The current study revealed that the overall seroprevalence of chronic toxoplasmosis infection was (19.6%) for Babylon province women', and it was showed a significant increase in this immunological parameters concentrations (D4, Kininogenase, and PAF) in infected women compared with non-infected women, and this confirms the immunological criteria role in immune response against the *T.gondii* parasite, through its resistance and inhibition.

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