ejpmr, 2022,9(10), 15-19

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

STUDY OF FAS AND FAS LIGAND RESPONSE DURING TOXOPLASMOSIS INFECTION FOR ABORTED WOMEN

Reem A. Mansour*[1], Dina A.A Abdullah[2] and Doaa A. Abdulwahab[3]

Department of Medical Laboratory Techniques, Bilad Alrafidain University College, Diyala, Iraq.

***Corresponding Author: Reem A. Mansour**

Department of Medical Laboratory Techniques, Bilad Alrafidain University College, Diyala, Iraq.

Article Received on 25/07/2022 **Article Revised on 15/08/2022** **Article Accepted on 05/09/2022**

ABSTRACT

Background: The current study was conducted in Diyala governorate during the period from 10 October 2020 to February 2021 to investigate the levels of some immune variables among aborted women with *Toxoplasma gondii* infection by used ELISA test to detect IgM and IgG antibodies, also to evaluate the concentration of Fas and Fas-ligand in aborted women as well as to investigate the relation between *Toxoplasma gondii* infection and (Fas and Fas-ligand) and how they effects during the infection with *Toxoplasma gondi*. **Methodology**: One hundred twenty women were included in this study that suffering from previous abortion with close ages and apparently 30 healthy women were considered as a control group. All patients who shared in current study were attending Al-Batoul Maternity Teaching Hospital in Baquba city- Diyala governorate in the period from October 2020 to February 2021. Their ages ranged between (15 - 50) years. **Results**: This study showed that 60 women were positive by ELISA (IgG and IgM) which considered as confirmed toxoplasmosis cases, 19 (31.7%) of abortive women had IgM+, 32 (53.3%) had IgG+, and 9 (15.0%) had both IgM+ and IgG+, whereas the control women were 0% for all types of antibodies. The highest results of serological tests were observed in women of age group (25-34) years (30%). Also there was a relationship between the increasing in the seropositive of aborted women for anti-*T. gondii* in women who have single abortion 34 (56.7%), and women from rural areas 35 (58.3%). The present study observed that there was an increase of serum level of Fas and Fas-ligand among women with toxoplasmosis who have positive IgM and positive IgG or both positive IgM and positive IgG. **Conclusion:** There is an association between infection with *T. gondii* and occurrence of abortion and the age group (26-35) years was the most affected among the other age groups and the most likely to have abortion as well as *Toxoplasma gondii* has role in change of FAS, FAS-L

KEYWORDS: *Toxoplasma gondii*, pregnant women, fas, fas-ligand, ELISA test.

INTRODUCTION

Toxoplasma gondii is an opportunistic intracellular protozoan parasite that is capable of infecting nearly all warm-blooded creatures, including humans, and has a global distribution (Elmore, 2010). It is capable of producing serious and life-threatening disease, particularly in pregnant women and those who are immune-compromised (Opsteegh *et al*., 2016). Infection during pregnancy may lead to abortions, stillbirths and may cause spontaneous abortion due to congenital toxoplasmosis which is a risk factor and can cause repeated abortion (El-Sherbini *et al*., 2019). Human become infected with *Toxoplasma gondii* through coming into touch with the feces of infected animals, particularly domestic cats (Chefek, 2015). Also from consuming raw or undercooked meat that has live tissue cysts as well as drinking water that may contaminated with oocysts from cat feces that has been infected (Meireles *et al*., 2015). This parasite can induce both cell-mediated and humoral immune responses as antibody formation, including IgM and IgG antibody (Phuangphet, 2008). The immunological variable (fas and fas ligand) may play a role in the lethal pathology of parasite infection (Gavrilescu and Denkers 2003). The current study was conducted to investigate the levels of immune variables among women who had a history of abortions and have infection with *Toxoplasma gondii* by used ELISA test to detect IgM and IgG antibodies, also to evaluate the concentration of fas and fas ligand in abortion women among a group of women who had a history of abortions with toxoplasmosis in Diyala Governorate.

METHODOLOGY

Study Setting: A blood samples were collected from women who had previous miscarriages and attended Al-Batool Maternity Teaching Hospital in Baquba city - Diyala governorate during the period from October 2020 to February 2021. Based on the availability of complete clinical information about each patient, blood samples were taken from women at different ages ranged between (15-50) years, and additional thirty women with close

ages who had no spontaneous abortion or pregnancy and apparently healthy were considered as a control group. Sera were separated from each blood sample and tested for Toxo-IgM, Toxo-IgG, fas and fas ligand. Each collected sample was put in gel tube and left at room temperature then undergone 15 minutes of centrifugation at 10,000 rpm. The serum collected and stored at special Eppendorf tubes at -20 C until it was needed to determine the toxo IgM, the toxo IgG, fas and fas ligand.

Statistical Analysis

The data for the studied characteristics were analyzed according to the analysis of variance of a factor experiment that was applied according to the complete random design, and the significance of the relationship between the factors included in this study was tested using the chi-square test for independence, and the ready-made statistical program SPSS version 26 was used to complete the statistical analysis.

RESULTS

There is a substantial difference between the patient and control groups based on age groups, the results of this study showed that the highest percentage of patients in the age group (26-35) years which was (40.8%) and the lowest percentage in the age group (46-55) years which was (7.5%), While for the control group, the highest percentage was in the group (26-35) years which was (50%), while the age group (36-45) years recorded the lowest percentage which was (6.7%), as shown in table (4-1).

Table 1: The study groups distribution (patients and controls) according to age groups.

			Age groups				Total
			15 – 25	26 - 35	36 – 45	46 – 55	
Status	Patients	Count (%)	22 (18.3%)	49 (40.8%)	40 (33.3%)	9 (7.5%)	120 (100%)
	Control	Count (%)	10 (33.3%)	15 (50%)	2 (6.7%)	3 (10%)	30 (100%)
		P-value	0.026				

Table (2): The results of the present study showed that the Seroprevalence of positive IgM *toxoplasma gondii* was (31.7%) and for positive IgG was (53.3%) while for IgM+IgG (+ve) (15.0%)

Table 2: Overall seroprevalence of anti-*toxoplasma gondii* antibodies in patients and control groups.

Study groups	No.	IgM+ve	IgG+ve	IgM+IgG (+ve)	Total +ve
Patients	60	19 (31.7%)	32 (53.3%)	9 (15.0%)	60 (100%)
Controls	30	0	0	0	30 (100%)

Table (3): The results of the current study showed that in patients who had Positive IgM, Positive IgG and mix (positive IgM + positive IgG) the Mean ± SE of FAS (193.29 ± 10.92), (217.48 ± 12.88) and (201.05 ± 8.71), respectively, was higher from fas ligand which was (166.42 ± 11.64), (179.73 ± 8.05) and (168.93 ± 14.86), respectively, was the lowest.

Table 3: Comparison of immunological variables (Fas and Fas ligand) among patients.

Positive cases	Mean±SE(Conc. Pg/ml)	FAS	FAS-L
Positive IgM	N	19	19
	Mean ± SE	193.29 ± 10.92	166.42 ± 11.64
Positive IgG	N	32	32
	Mean ± SE	217.48 ± 12.88	179.73 ± 8.05
positive IgM + positive IgG	N	9	9
	Mean ± SE	201.05 ± 8.71	168.93 ± 14.86

Table (4): The results in this study showed that the patients who had Positive IgM the majority Mean ±SE (216.21 ± 10.57) of FAS was in age group (15-25) and FAS-L (182.96 ± 15.28) in age group(36-45) while the patients who had Positive IgM the majority Mean ±SE (230.33± 27.70) of FAS was in age group (36-45) and FAS-L (245.76±) in age group(46-55). Also the patients who had both Positive IgM+ Positive IgG the majority Mean ±SE (212.01±13.71) of FAS was in age group (15-25) and FAS-L (179.72±43.81) in age group (26-35).

Table (4): Comparation of immunological variable (FAS and FAS-ligand) in patients according to age groups.

Positive cases	classes of age	Mean ±SE(Conc.Pg/ml)	FAS	FAS-L
Positive IgM	15 – 25	N	4	4
		Mean ±SE	216.21 ± 10.57	142.45 ± 24.53
	26 – 35	N	9	9
		Mean ±SE	176.49 ± 20.68	166.18 ± 20.62
	36 – 45	N	5	5

		Mean ±SE	202.52± 13.10	182.96 ± 15.28
	46-55	N	1	1
		Mean ±SE	206.76 ± 0	181.71 ± 0
	Total	N	19	19
		Mean ±SE	193. 29± 10.92	166.42 ± 11.64
Positive IgG	15 – 25	N	4	4
		Mean ±SE	220.15± 12.66	179.14± 25.37
	26 – 35	N	15	15
		Mean ±SE	210.89± 16.11	178.73± 13.36
	36 – 45	N	12	12
		Mean ±SE	230.33± 27.70	175.68± 10.52
	46 – 55	N	1	1
		Mean ±SE	151.38±0	245.76±0
	Total	N	32	32
		Mean ±SE	217.48±12.88	179.73± 8.05
positive IgM + positive IgG	15 – 25	N	3	3
		Mean ±SE	212.01±13.71	160.22±23.91
	26 – 35	N	3	3
		Mean ±SE	207.73±20.13	179.72±43.81
	36 – 45	N	3	3
		Mean ±SE	183.42±8.94	166.84±7.74
	Total	N	9	9
		Mean ±SE	201.05±8.71	168.93±14.86

Table (5): The results of this study showed that the patients who are Positive toxo IgM the level of Fas (197.82±16.28) and FAS-L (194.75±11.94) was highest in patients with single abortion, double, multiple and double, respectively. While the patients who have positive toxo IgG the level of Fas (242.03±46.83) and FAS-L (233.15±11.94), was highest in patients with multiple abortion, multiple, single and multiple, respectively. Also the patients who are Positive for both toxo IgM + toxo IgG the level of Fas (217.34±7.39) and FAS-L (241.23±) was highest in patients with single abortion, multiple, multiple and double, respectively.

Table 5: Comparative of immunological variable (FAS and FAS-L) in patients according to abortion number.

IgM	Abortion no.	Mean ±SE (Conc. Pg/ml)	FAS	FAS-L
Positive	Single	N	11	11
		Mean ±SE	197.82±16.28	146.33±15.58
	Double	N	6	6
		Mean ±SE	184.05±17.62	194.75±11.94
	Multiple	N	2	2
		Mean ±SE	196.11± 28.07	191.91±49.31
	Total	N	19	19
		Mean ±SE	193.29±10.92	166.42±11.64
IgG Positive	Single	N	16	16
		Mean ±SE	231.05±21.60	156.34±10.90
	Double	N	12	12
		Mean ±SE	191.20±9.84	193.12±9.47
	Multiple	N	4	4
		Mean ±SE	242.03±46.83	233.15±11.94
	Total	N	32	32
		Mean ±SE	217.48±12.88	179.73±8.05
positive IgM + positive IgG	Single	N	4	4
		Mean ±SE	217.34±7.39	137.20±19.15
	Double	N	4	4
		Mean ±SE	193.01±14.14	182.58±11.16
	Multiple	N	1	1
		Mean ±SE	168.04±	241.23±
	Total	N	9	9
		Mean ±SE	201.05±8.71	168.93±14.86

DISCUSSION

The highest infection rate in patients with toxoplasmosis and who had previous abortion was shown in ages (26-35) years (40.8%) this result was in agree with a study done by shaker, in (2018) which was included the highest infection rates were (36.4%) in (25-34) years age group, followed by (34.1%) in (35-44) age group, (18.2%) in < 25 age group, and (11.4%) in 45 years and older group.[11] The explanation for this could be that those age groups are the most activate, and their chances of coming into contact with one of the various infection routes increases as they become older.[9] While another study gives an indicated that there was no association among age of women and the related abortion factors.[10] **as show in table (1)**

Table (2) showed the results of present study were in agreement with a study done by Aziz and Majida, in (2011) among women showed higher results, 25 (59.5%) women were positive for IgG, and 17 (40.5%) women were positive for IgM, while 9 (17.6%) women were positive for both.[11] As well as, agreement with Anwar, in (2018) recorded a rate of positive *T.gondii* IgG and IgM antibodies in sera of aborted women (38.15%) in Samara city.[12] The differences in the prevalence rates of *T. gondii* among studies can be attributed to the differences in the study populations and study areas. Also, the differences in climatic conditions of location and nutrition, various ages of patients study and immune status, hygienic habits, sanitary supplies and socioeconomic conditions in study regions.[13] **In table (3)** the results of this study showed that in general the level of immune variants (FAS and FAS-L) was high in patients who had positive IgM or positive IgG, also, in patients who had both IgMve+ and IgGve+. As well as agreement with juma, in (2011) which there study found the (fas and fas-L) have essential role in apoptosis[14] whereas the Caspase-8 (CASP8) is plays a role in the extrinsic apoptotic signaling pathway via death receptors.[15] The ligation of Fas with Fas-L results in the activation of a caspase cascade that initiates apoptosis.[16] Based on this studies conclude that the fas and fas-L become high by infected with toxoplasma. This finding goes with the fact that high levels of host cell apoptosis is associated with several protozoan infections including *T. gondii*, particularly among immune cells.[7] **In table (4, 5):** The present study showed variance results among the mean and SE of FAS and FAS-L, in patients. Some of this immune variables was high in younger age groups from older age groups and other was contrary, the causes of this variance might be attributed to a fact that the immunity varies from one person to others as well as immune variables increase with age, this meaning there is an increase in the patient's immunity as a result of forming more antibodies, this can be explained that when the human increase in age, the body's immunity increases and becomes its maximum at the age of 30-45 but the immunity start to decline in old age (aging), approximately from the age of 47 and over, Fas ligand increased from about 5% in cells from young animals to more than 50% in old counterparts.[17] Also in abortion number, there is a big difference in the value of mean and SE Perhaps this is due to the fact that when a woman undergoes repeated miscarriage, this may weaken her immune system, which leads to inducing the immune system to increase the concentration of these variables.

CONCLUSIONS AND RECOMMENDATIONS

In this research, there is an association between infection with *T. gondii* and occurrence of abortion. As well as the age group (26-35) years was the most affected among the other age groups and the most likely to have abortion.

Also, *Toxoplasma gondii* has role in change of fas andfas ligand during infection. So it is suggested that ELISA test for toxoplasmosis should be introduced as a routine test for pregnant women in Hospitals and health education for women of childbearing age should include information for improvement of sanitation.

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