The Impact of Toxoplasma Gondii Antibodies on Haematological Parameters Among Women in Zakho District/ Iraq

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Abstract
Background: The correlation between Toxoplasma gondii (T.gondii) infection and haematological parameters are poorly understood. Therefore, this investigation explores to determine the effects of toxoplasmosis on haematological alterations during infections. Methods: A total 125 women were contributed; each blood samples were evaluated for toxoplasmosis and haematological components. Results: The study presented the total rate of toxoplasma IgG antibodies (Abs) (n=16, 12.8%) and IgM Abs (n=6, 4.8%), and the maximum rate of enzyme linked immunosorbent assay (ELISA) IgG were noted among married, pregnant and female in 3rd trimester. For seropositive IgG cases significant association was obtained only among different levels of Haemoglobin (Hb) (P-value =0.052) while, for seropositive IgM cases significant differences was only verified among different levels of red blood cells (RBC) (P-value =0.013). Conclusion: T.gondii has vital influence on health, thus, screening mother's immunity, focusing on making T. gondii antibodies and CBC tests as preliminary examinations for all pregnant women. Besides, further investigation, improvement diagnostic methods are crucial.
Keyword: Toxoplasma gondii, Haematological Parameters, Women, Iraq
Preliminary examination of toxoplasmosis and haematological parameters are essential

1. Introduction

Toxoplasmosis is a zoonotic parasitic disease which infected both animals and humans; it belongs to the coccidian parasite, which has a two-generation life cycle [1]. Intermediate hosts are warm-blooded animals, while cats exist as definitive hosts [2, 3]. Eating of fresh (raw) meat, infected organ donation, drinking polluted water with oocysts, and transfusion of blood from infected person are the main mechanisms of T. gondii transmission [4]. Toxoplasmosis has an extensive clinical spectrum, ranging from asymptomatic to sever syndrome [5]. Moreover, causes birth defects in foetuses for instance: brain damage, stillbirth, and retinochoroiditis when females are infected throughout gestation [6]. Besides, causes a variety of immunological changes in the body [7]. Numerous serological tests uses for diagnosis of toxoplasmosis that rely on the existence of T.gondii IgM and IgG antibodies in serum [8]. Comprising, latex agglutination test, dye test, complement fixation test, immunofluorescent study test, and enzyme-linked immunosorbent assay (ELISA). Through using PCR (polymerase chain reaction) ribosomal DNA of parasite amplified for single organism detection in tissue samples recognized in latest years [9]. Seropositive (IgG) and (IgM) titters during pregnancy in women who had formerly negative to anti-toxoplasma IgG antibody titters designate a proliferative disease state which leads to serious birth defects [10]. Furthermore, hematologic findings obtained from blood screening assist in the calculation of medical distresses and serve as a baseline for upcoming health observations. The most common blood tests used in medicine is complete blood count (CBC) which evaluates all blood components [11]. T. gondii parasite found throughout the world, regarding to certain serological investigations, one over three of the world's inhabitants has been infected [12]. The influence of toxoplasmosis on the blood parameters have been discovered in a few studies. Some investigations revealed not any alterations in blood components, however others discovered increases in Hb and PCV, particularly throughout pregnancy [13]. Besides, some studies conducted in the Kurdistan region of Iraq that reveals the prevalence, diagnosis techniques and risk factors of T. gondii [1,2,14,15,16,17 and 18], but little was known about the haematological changes among women during infection. Therefore, the current study aims to determine consequence of toxoplasmosis on haematological changes among female in Zakho District/ Duhok City, Iraq.

2. Materials and Methods

2.1. Collection of Specimens

A total 125 patients were incorporated throughout the study (pregnant and non-pregnant women) the samples collected in Zakho General Hospital and some private clinics. The study was assumed from August 2021 to January 2022. A designed questionnaire form which was given to each contributor who subjected to the existing study.

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2.2. Haematological and Serological Testing

Five ml of intravenous blood was withdrawn for each participant. All blood samples separate to two different tubes one of them contain anticoagulant for (CBC, ABO blood group tests and rhesus (Rh) factors). The haematological parameters were measured by Blood Coulter machine (MISPA-i3/AGAPPE DIAGNOSTICS SWITZERLAND GmbH) such as WBC, Granulocytes, Lymphocytes, Hb, MID, RBC, HCT, MCV, MCH, MCHC and PLT. Also, blood groups and Rh factors identified by agglutination method (Biorex dignostics/ United Kingdom) using monoclonal blood grouping reagents. The other gel tube containing clot activator for serum separation then centrifuged at 4000rpm/ 5 min. The serum samples were preserved in research laboratory inside 2-ml Eppendorf tubes and stored at (−20 °C) until analysis. All samples tested for the detection of T. gondii antibodies (IgG, IgM) by using commercially ELISA kits (bioactiva diagnostica/ Germany). All tests performed regarding to the Manufacturer's information.

2.3. Statistical Analysis

The collected data were analysed performing (SPSS version 26). The data were designated by frequency and frequency percentage tables. The association between Toxoplasma positivity and other variables was tested using Chi-square, or when not applicable due to low cell frequency, Fisher's exact test. A p-value less than or equal (≤0.05) was measured significant statistically.

3. Results

Based on the results of (Table 1) displays the total rate of T.gondii IgG Abs 16 (12.8%) was higher than the rate of toxoplasma IgM Abs 6 (4.8%), also, there was no statically difference between both test by using ELISA technique (p>0.05). Conferring to ELISA IgG the rate was higher among married women than single women (n=14, 13.9%, n=4, 4.0%) respectively, while ELISA IgM shows the higher rate among single female than married one (n=2, 8.3%, n=2, 4.0%) respectively. Also, there was not statically difference between both groups (p>0.05).

Table 1. Seropositivity of Toxoplasma gondii IgG and IgM antibodies according to marital status

Marital Status	Total No.	IgG Positive		P-value	IgM Positive		P-value
		No.	%		No.	%	
Married	101	14	13.9	0.735	4	4.0	0.325
Single	24	2	8.3		2	8.3	
Total	125	16	12.8%	1.000	6	4.8%	1.000

Results in (Table 2) proved slight difference among pregnant and (non-pregnant and/or single women) concerning to ELISA IgG (n=4, 13.8%, n=12, 12.5%) and ELISA IgM (n=1, 3.4%, n=5, 5.2%) respectively and statistically there was no significance difference between them.

Table 2. Seropositivity of Toxoplasma gondii IgG and IgM antibodies according to gestational state

Gestational state	Total No.	IgG Positive		P-value	IgM Positive		*P*-value
		No.	%		No.	%	
Pregnant	29	4	13.8	1.000	1	3.4	1.000
Not pregnant and not married	96	12	12.5		5	5.2	
Total	125	16	12.8		6	4.8	

There were no significance differences noticed between different trimesters and the maximum rate of ELISA IgG were noted among female in 3rd trimester followed by 1st trimester (n=3, 20.0%, n=1, 14.3%) individually. Whereas, concerning to ELISA IgM only recorded among pregnant women in 3rd trimester (n=1, 6.7%) (Table 3).

Table 3. Seropositivity of Toxoplasma gondii IgG and IgM antibodies according to gestational stage

Gestational stage	Total No.	IgG Positive		P-value	IgM Positive		P-value
		No.	%		No.	%	
1st trimester	7	1	14.3	0.783	0	0.0	1.000
2nd trimester	7	0	0.0		0	0.0	
3rd trimester	15	3	20.0		1	6.7	
Total	29	4	13.8		1	3.4	

The outcomes of this study demonstrated that there was no significance difference statistically among different blood groups. Regarding to ELISA IgG the maximum rate recorded among female with (A- and B-) blood groups equally which were (n=1, 33.3%) and there is no infection recorded among blood group (O-) while, regarding to ELISA IgM only recorded among (AB+ and A+ blood groups) which were (n=3, 13.6%, n=3, 6.3%) individually (Table 4).

Table 4. Seropositivity of Toxoplasma gondii IgG and IgM antibodies according to Blood groups

Blood Groups	Total No.	IgG Positive		*P*-value	IgM Positive		*P*-value
		No.	%		No.	%	
A+	48	5	10.4	0.560	3	6.3	0.374
A-	3	1	33.3		0	0.0	
O+	31	4	12.9		0	0.0	
O-	6	0	0.0		0	0.0	
B+	12	2	16.7		0	0.0	
B-	3	1	33.3		0	0.0	
AB+	22	3	13.6		3	13.6	
Total	125	16	12.8		6	4.8	

T. gondii can infect almost nucleated cells, comprising erythroblasts. High level of (WBC, Hb and RBC) recorded among IgG seropositive women. While, regarding to seropositive IgM cases the maximum rate of low (WBC, granulocyte and lymphocyte) and the maximum rate of high RBC cells were existent. For seropositive IgG cases Significant association was obtained only among different levels of Hb (P-value =0.052) moreover, for seropositive IgM cases significant differences was only verified among different levels of RBC (P-value =0.013). However, there were no significant differences regarding to the other parameters. High rate of low level of (HCT, MCV, MCH and MCHC) regarding to seropositive IgG cases were estimated. Whereas, for seropositive IgM the level for all above parameters were normal. With the exception of platelet level, all seropositive cases for both tests (IgG, IgM) recorded normal level (Table 5).

Table 5. Anti- Toxoplasma gondii antibodies and haematological findings

Variables		Total No.	IgG Positive		P-value	IgM Positive		P-value
			No.	%		No.	%	
WBC	High	39	7	17.9	0.496	2	5.1	0.133
	Low	**4**	**0**	**0.0**		**1**	**25.0**	
	Normal	82	9	11.0		3	3.7	
Granulocytes	High	15	2	13.3	1.000	1	6.7	0.091
	Low	3	0	0.0		1	33.3	
	Normal	107	14	13.1		4	3.7	
Lymphocytes	High	8	1	12.5	0.825	1	12.5	0.135
	Low	6	1	16.7		1	16.7	
	Normal	111	14	12.6		4	3.6	
Hb	High	3	2	66.7	0.052	0	0.0	0.426
	Low	29	2	6.9		0	0.0	
	Normal	93	12	12.9		6	6.5	
MID	High	1	0	0.0	1.000	0	0.0	1.000
	Low	4	0	0.0		0	0.0	
	Normal	120	16	13.3		6	5.0	
RBC	High	4	1	25.0	0.511	2	50.0	0.013
	Low	12	2	16.7		0	0.0	
	Normal	109	13	11.9		4	3.7	
HCT	Low	34	5	14.7	0.765	1	2.9	1.000
	Normal	91	11	12.1		5	5.5	
MCV	Low	13	2	15.4	0.673	0	0.0	1.000
	Normal	112	14	12.5		6	5.4	
MCH	Low	13	3	23.1	0.372	0	0.0	1.000
	Normal	112	13	11.6		6	5.4	
MCHC	Low	4	1	25.0	0.426	0	0.0	1.000
	Normal	121	15	12.4		6	5.0	
PLT	Low	7	0	0.0	0.594	0	0.0	1.000
	Normal	118	16	13.6		6	5.1	
Total		125	16	12.8		6	4.8	

4. Discussion

The outcomes of the existing study displayed significant differences in some blood components, whereas no variation was identified in other components. The overall prevalence of toxoplasmosis among participants was 22/125 (17.6%) for both IgG and IgM. This result was higher than the previous studies conducted in Zakho and Kirkuk City/Iraq; 78/630 (12.38 %), 26/420 (6.19%) in turn [15,16]. This difference may be due to the samples sources because the samples were collected randomly among female in the existing study [16]. As regards to marital status, the frequency of T. gondii IgG Abs was higher among married when compared to unmarried women (n=14, 13.9%, n=2, 8.3%) respectively. Comparable outcomes identified in Zakho district by Mohammed and Mero [2] they stated the higher T.gondii IgG Abs among married women than unmarried one (n=67, 12.52%, n=6, 6.31%).This higher rate among married women could be caused by further responsibility of married women relating to, labour, gestation and child raising can affect their healthiness and leads to drop their immunity which let them more predisposed to infection [17]. Throughout existent study there were slight differences of T. gondii IgG and IgM Abs rates were recorded among pregnant and non-pregnant women. The current findings was agree with the study conducted in Erbil city [18]. The greater seropositivity rates concerning pregnant women may be due to increasing in the secretion progesterone and oestrogen sex hormones throughout pregnancy because these hormones can affect the women's immune system [19]. The study estimated highest seropositivity of anti-T.gondii IgG and IgM Abs amongst pregnant women at 3rd trimester, this outcome are comparable with studies achieved in Duhok and Ghana [20,21]. Whereas, reversed with the previous study achieved in Zakho district, they stated the higher rate among 1st, 2nd followed by

3rd trimester [2]. The variances between the studies might be due to the size and sources of the samples [20]. Concerning to different blood groups and Rh factors there were no significant differences detected, this consequence was disagreed with studies achieved in Kirkuk, Ghana and Thi-Qar/Iraq [15,21,22]. These differences could be as a result of different reasons for instance, ecological influences, nutrition and gender related hormones [23]. Furthermore, there are some cell antigens located on the surface of different blood groups that make natural resistance against infectious diseases [24]. In relation to the existing study, a significant difference was noticed only among different levels of Hb for ELISA IgG, while, for seropositive IgM cases significant differences was only recorded among different levels of RBC. Moreover, high level of (WBC, Hb and RBC) documented among IgG seropositive women. While, regarding to seropositive IgM cases the maximum rate of low (WBC, granulocyte and lymphocyte) and the maximum rate of high RBC cells were present. Concerning WBC the outcome in agreement with the study implemented in Makkah [6]. The value of Hb is statistically significant for seropositive IgG cases, likewise, Immunological and Physiological status of women can affect the level of Hb, In addition, toxoplasmosis can alter the level of WBCs [25].

5. Conclusion

Toxoplasmosis can cause harmful effects on healthy and non-healthy hosts which infects all nucleated cells and encourage the infected host's immune system that leads to alteration of blood components in response to the infections. It is necessary to improve health awareness programs to drop the severity of the disease among population, particularly pregnant women and intending mother's immunity. In addition, making toxoplasmosis and CBC tests as a routine examination for all pregnant women. Moreover, further investigations with larger sample size and follow up cases to estimate the hormonal and haematological variations are essential.

Ethics

The study was permitted by the ethics committee of University of Zakho and Zakho General Hospital, Zakho/Duhok, Iraq

Conflict of Interest

The authors stated that there is no conflict of interest

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