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The Importance of Detecting *Toxoplasma gondii* Antigens for Prognosis of Acute Toxoplasmosis

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Abstract

Background: The term toxoplasmosis is reserved to describe the clinical or pathological disease caused by *Toxoplasma gondii* and *T. gondii* infection for an asymptomatic primary infection or persistence of the parasite in tissues (chronic or latent infection). ***Objective:*** To evaluate the significance of detecting *Toxoplasma gondii* antigens in diagnosing and predicting the prognosis of acute toxoplasmosis, assessing its potential role in early intervention and treatment monitoring. ***Materials and Methods:*** The samples of the present study consisted of 39 women,31 of them infected with toxoplasmosis and 6 of them did not have toxoplasmosis, their age range was between (17-41) years, the blood samples were collected from Central Public health Laboratories (CPHL) in the period from (May 2022 to November 2022). Human *Toxoplasma gondii* ELISA kit is for the qualitative determination of *Toxo-gondii* in human serum. ***Results:*** The results of the present study showed in Table (1) since there were 26(96.3%) of housewife women had IgG Abs of *T.gondii*, while there was 1(3.7%) of employment women had IgG Abs of *T.gondii* , there were a significant differences between job categories and antibodies (IgG Abs) of *T.gondii* P=0.32. ***Conclusions:*** IgG Abs and IgM Abs of *T.gondii* presents more in housewife women than employment women. The Ag of *T.gondii* present in aborted women more than in non-aborted women. The Ag of *T.gondii* present more in women who had IgG Abs of *T.gondii* more than in women who had IgM Abs of *T.gondii*.

Keywords: Acute toxoplasmosis, Ag of *T.gondii*, IgM Abs of *T.gondii*, IgG Abs of *T. gondii,* abortion.

INTRODUCTION

Toxoplasmosis is a parasitic disease caused by the intercellular protozoan parasite *Toxoplasma gondii*, which can infect humans and warm-blooded animals [1]. In healthy individuals, a primary infection with *T. gondii* usually causes relatively mild flulike symptoms, whereas in immunocompromised patients, it can cause opportunistic life-threatening infections. Moreover, in pregnant women, toxoplasmosis may cause serious problems because transplacental transmission can occur and lead to abortion, stillbirth, or neonatal malformations. Overall, about a third of mothers with primary infection give birth to an infant with toxoplasmosis. These facts emphasize the importance of being able to make an accurate distinction between primary and chronic infection or reactivation, especially during pregnancy [2].

A diagnosis of toxoplasmosis can be established by the isolation of *T. gondii* from blood or body fluids, demonstration of the parasite in tissues, detection of specific nucleic acids with DNA probes, or by carrying out serologic tests in order to detect *T. gondii*-specific immunoglobulins synthesized by the host in response to infection. Currently, routine diagnosis of toxoplasmosis relies mainly on the use of various serological tests to detect specific antibodies in the serum samples of infected patients. The presence of a recent infection can be determined by detecting seroconversion of immunoglobulin M (IgM) or IgG antibodies, a substantial increase in IgG antibody titer, or a *Toxoplasma* serologic profile compatible with acute infection (using *Toxoplasma* serodiagnostic tests,

including an IgG avidity test) in sequential serum samples of infected individuals [3, 4]. However, this procedure bears limitations in estimating the time of *T. gondii* infection due to the fact that, in most cases, low IgM titers persist long after the acute phase of disease [5].

Most commercial serological kits use native antigens prepared from tachyzoites grown in mice and/or tissue culture. The methods of producing these antigens may also vary significantly between laboratories. In addition, selected antigens that are characteristic for the acute or chronic stages of the infection could serve as a tool to discriminate between the two stages. Moreover, obtaining homogenic recombinant specimens of antigenic proteins by molecular biology methods makes it possible to solve not only the biohazard problem but also the issue of time and labor consumption, all of which accompany the production of native antigen [6].

One problem in the field of serologic diagnosis of toxoplasmosis is the lack of a unique and easy-to-use reference test. Certainly, the use of recombinant proteins in the serodiagnosis of toxoplasmosis would be highly beneficial in improving standardization of the tests and reducing their production costs. The discovery of markers of new infections and the development of new diagnostic assays which can be used to confirm the acute phase of toxoplasmosis, especially in pregnant women, is also required. Nevertheless, although the diagnostic tests discussed are promising, therefore further work is needed before an immunoassay with recombinant products will be available for clinical purposes [7].

The diagnosis of *T. gondii* infection or toxoplasmosis established by serologic tests, amplification of specific nucleic acid sequences (i.e., polymerase chain reaction [PCR]), histologic demonstration of the parasite and/or its antigens (i.e., immunoperoxidase stain), or by isolation of the organism. Other rarely used methods include demonstration of antigenemia and antigen in serum and body fluids, a toxoplasma skin test, and antigen-specific lymphocyte transformation [8]. Recently, a number of tests for avidity of *Toxoplasma* IgG antibodies have been introduced to help discriminate between recently acquired and distant infection [9, 10, 11].

This study aims to assess the importance of detecting *Toxoplasma gondii* antigens in the diagnosis and prognosis of acute toxoplasmosis, evaluating its potential role in facilitating early intervention, improving treatment strategies, and monitoring disease progression for better clinical outcomes.

MATERIALS AND METHODS

The samples of the present study consisted of 39 women,31 of them infected with toxoplasmosis and 6 of them did not infect with toxoplasmosis, their age range was between (17-41)years , the blood samples were collected from Central Public health Laboratories(CPHL) in the period from (May 2022 to November 2022).Human *Toxoplasma gondii* ELISA kit is fot the qualitative determination of *Toxo-gondii* in human serum, from SUNLOG BIOTECH CO.LTD/China was utilized to detect the antigen of *T.gondii* catalog No. : SL1730Hu. The blood sample was collected from each women and the blood was centrifuged for 5 minutes at 1000g rpm to collect the serum to carry out the assay. ELISA test was used in the peresnt study, the *in vitro* qualitative determination of Antigen of *T.gondii* concentration in serum, according to manual procedure provided with kit.

Statistical Analysis

Statistical analyses were performed using SPSS statistical package for Social Sciences (version 20.0 for windows, SPSS, Chicago, IL, USA). Quantitative data were represented as mean, SD. Student's t-test was used to compare the mean of two groups and Chi-square test was used to find the relation between qualitative data. P value of <0.5 was considered as significant.

RESULTS

The results of the present study showed in Table (1) there were 26(96.3%) of housewife women had IgG Abs of *T.gondii*, while there were 1(3.7%) of employment women had IgG Abs of *T.gondii* , there were a significant differences between job categories and (IgG Abs) of *T.gondii,* P=0.032. No other significant relation or difference were detected, P>0.05.

Table 1: The correlation between demographic features of women and IgG Abs of *T.gondii*

		***T. gondii* IgG**			
		Yes		**No**	
		Count	**%**	**Count**	**%**
Job P=0.032	House wife	26	96.3%	8	72.7%
	Employment	1	3.7%	3	27.3%
Marital status P=Not detected	No	0	0.0%	0	0.0%
	Yes	27	100.0%	11	100.0%
Cycle P=Not detected	Regular	27	100.0%	11	100.0%
	Not regular	0	0.0%	0	0.0%
Menstrual cycle change P=0.196	Yes	1	3.7%	2	18.2%
	No	26	96.3%	9	81.8%
Uterus	Yes	27	100.0%	11	100.0%

P=Not detected	No	0	0.0%	0	0.0%
Abortion 0.729	Yes	12	44.4%	4	36.4%
	No	15	55.6%	7	63.6%
		Mean	**SD**	**Mean**	**SD**
Age P=0.302		27.81	6.65	25.36	6.39
Abortion no. P=0.734		0.63	0.63	0.55	0.82
Pregnancy no. P=0.703		1.15	0.86	1.27	1.01

Table (2) showed that there were 2(66.7%) of housewife women had IgM Abs of *T.gondii*, while there was only 1(33.3%) of employment women had IgM Abs of *T.gondii,* there were no significant differences between the two groups, P=0.291. The mean ±SD of age of women who had IgM Abs of *T.gondii* in the present study which was (21.33±1.53) ,the results showed there were a significant differences of age and the presence or absence of IgM Abs , P=0.001, also, mean ±SD of IgM Abs of *T.gondii* which was (2±0)and the number of abortion ,There was a significant difference of abortion number between the presence or absence of IgM groups (P<0.05).

Table 2: The correlation between Demograghic features of women and IgM Abs of *T.gondii*

		***T. gondii* IgM**			
		Yes		**No**	
		Count	**%**	**Count**	**%**
Job P=0.291	House wife	2	66.7%	32	91.4%
	Employment	1	33.3%	3	8.6%
Marital status P=Not detected	No	0	0.0%	0	0.0%
	Yes	3	100.0%	35	100.0%
Cycle P=Not detected	Regular	3	100.0%	35	100.0%
	Not regular	0	0.0%	0	0.0%
Menstrual cycle change P=0.224	Yes	1	33.3%	2	5.7%
	No	2	66.7%	33	94.3%
Uterus P=Not detected	Yes	3	100.0%	35	100.0%
	No	0	0.0%	0	0.0%
Abortion P=0.066	Yes	3	100.0%	13	37.1%
	No	0	0.0%	22	62.9%
		Mean	**SD**	**Mean**	**SD**
Age P=0.001		21.33	1.53	27.60	6.62
Abortion no. P=0.005		2.00	0.00	0.49	0.56
Pregnancy no. P=0.716		1.00	1.00	1.20	0.90

Table (3) showed that there were 15 (100%) of housewife women had Ag of *T.gondii*, while there was no of employment women (0%) had antigen of *T.gondii*, there was no significant difference detected P=0.318. Also, the antigen of *T.gondii* present in 9(60%) of aborted women more than in non aborted women which appear in 6(40%) of women, there was no significant differences between the two groups, P=0.099.

Table 3: The correlation between Ag of *T.gondii* and Demograghic features of women in the present study

		***T. gondii* Ag**			
		Yes		**No**	
		Count	**%**	**Count**	**%**
Job P=0.318	House wife	15	100.0%	19	82.6%
	Employment	0	0.0%	4	17.4%
Marital status P=Not detected	No	0	0.0%	0	0.0%
	Yes	15	100.0%	23	100.0%
Cycle P=Not detected	Regular	15	100.0%	23	100.0%
	Not regular	0	0.0%	0	0.0%

Menstrual cycle change P=0.999	Yes	1	6.7%	2	8.7%
	No	14	93.3%	21	91.3%
Uterus P=Not detected	Yes	15	100.0%	23	100.0%
	No	0	0.0%	0	0.0%
Abortion P=0.099	Yes	9	60.0%	7	30.4%
	No	6	40.0%	16	69.6%
		Mean	**SD**	**Mean**	**SD**
Age P=0.708		26.60	5.57	27.43	7.27
Abortion no. P=0.326		0.73	0.70	0.52	0.67
Pregnancy no. P=0.782		1.13	0.99	1.22	0.85

Table (4) showed that Ag of *T.gondii* present in 11(73.3%) of women who had IgG Abs of *T.gondii* also, while there were only 4 (26.7%) of women had Ag of *T.gondii* but did not have IgG Abs of *T.gondii*, there was no significant differences between the presence of Ag and IgG Abs of *T.gondii* , P=0.802.

Table 4: The correlation between Ag and IgG Abs of *T.gondii*

			***T. gondii* IgG**		**Total**
			Yes	**No**	
T. gondii Ag	Yes	Count	11	4	15
		%	73.3%	26.7%	100.0%
	No	Count	16	7	23
		%	69.6%	30.4%	100.0%
Total		**Count**	**27**	**11**	**38**
		%	**71.1%**	**28.9%**	**100.0%**

Table (5) showed that there was only 1(6.7%) of women had Ag of *T.gondii*and had IgM Abs of *T.gondii*, while there were 14(93.3%) of women had Ag of *T.gondii* and did not have IgM Abs of *T.gondii*, there was no significant relation between the presence of Ag and IgM Abs of *T.gondii*, P=0.801.

Table 5: The correlation between Ag and IgM Abs of *T.gondii*

			***T. gondii* IgM**		**Total**
			Yes	**No**	
T. gondii Ag	Yes	Count	1	14	15
		%	6.7%	93.3%	100.0%
	No	Count	2	21	23
		%	8.7%	91.3%	100.0%
Total		**Count**	**3**	**35**	**38**
		%	**7.9%**	**92.1%**	**100.0%**

DISCUSSION

The term toxoplasmosis is reserved to describe the clinical or pathological disease caused by *Toxoplasma gondii* and *T. gondii* infection for an asymptomatic primary infection or persistence of the parasite in tissues (chronic or latent infection). The use of serologic tests for demonstration of specific antibody to *T. gondii* is the initial and primary method of diagnosis. Different serologic tests often measure different antibodies that possess unique patterns of rise and fall with time after infection. A combination of serologic tests is usually required to establish whether an individual has been most likely infected in the distant past or has been recently infected. The clinician and clinical laboratories must be familiar with these problems and consult reference laboratories if the need arises [12, 13].

The results in Table (1) showed that IgG Abs levels of *T.gondii* were high in housewife women 26(96.3%) than employment women 1(3.7%), there were significant differences between the two groups, P= 0.032, These results in agreement with that reported by Nadia Velázquez-Hernández, *et al*., 2019 which is the first study regarding knowledge and practices about toxoplasmosis in housewives. that only 1.1% of women knew about the prevalence of *T. gondii* infection. Some (4.9%) women used to taste raw meat while cooking, and 7.6% used to undercook meat. In addition, 20% of women used to eat raw dried meat, and 13.5% consumed untreated water. Less than 90% of women always washed their hands before cooking, and washed fruits or vegetables. The majority (75.1%) of women never wore gloves when handling raw meat. About one quarter (27.6%) of women always froze meat, and 16.2% of

women cleaned cat feces. Poor knowledge regarding *T. gondii* infection, toxoplasmosis, and practices to avoid infection among the housewives studied was found [14].

In addition the results of the current study revealed that house wife women had high prevalence of IgM and IgG Abs of *T.gondii*, since there were 2(66.7%) and 26(96.3%) respectively, these results in agreement with that reported by (Salih *et al.*,2020) who reported that there were higher seroprevalence of IgM among housewives compared with student and employed females, in Duhok [15,16]. The findings of this investigation demonstrate the endemicity of toxoplasmosis among the female population in Zakho city, Iraq, since high seroprevalence of both anti-*Toxoplasma* IgG (32.46%) and IgM (8.68%) was reported [17]. Also, Bodaghi B. *et al* reported that contact with cats, marital status, a history of abortion, and the consumption of homemade food showed significant associations with anti-*Toxoplasma* IgM antibodies only. Serological and molecular investigations have been conducted globally, revealing that over 33% of the population carries antibodies against *T. gondii* [18].

Table (1) and Table (2) showed that mean±SD of the age of women who had IgG Abs of *T.gondii* was (27.81±6.65) which higher than mean±SD of age of women who had IgM Abs of *T.gondii* which was (21±1.53). This finding is somewhat consistent with two previous studies in this city; both studies reported the highest seroprevalence of anti-*Toxoplasma* IgG and IgM antibodies among those aged 33-45 and 31-35 years, respectively [19].

The results of the present study revealed that there were 27(100%) of married women had IgG Abs of *T.gondii,* while there were only 3(100%) of married females had IgM Abs of *T.gondii*, these results were in agreement with that reported by Milne and Webster,2020 that married women exhibited a significant (P<0.04) difference in IgM seroprevalence (49, 8.80%) compared to unmarried individuals (4, 3.81%), while non-significant differences (P=0.3) were found in IgG seroprevalence between married and unmarried females [20].

The results of the current study revealed that there were 12(44.4%) of aborted women had IgG Abs of *T.gondii*, while there were 3(100%)of aborted women had IgM Abs of *T.gondii*, there was no significant differences detected (P>0.05), these results were in agreement with that reported by(Mostafaal-Atroshi,2013). Moreover, the results of this study reveal a highly significant difference in IgM seropositivity between women with a history of abortions and those without. This finding is in line with studies that demonstrated the presence of high seroprevalence of anti-*Toxoplasma* IgM antibodies among women with a history of repeated abortions (21), while some studies didn't report any significant association between anti-*Toxoplasma* IgM antibodies and abortion (22, 23, 24). Also, the cause of high rate of IgG and IgM Abs of *T.gondii* in the present study since there were 26(96.3%) of IgG Abs, while there were only 2(66.7%) of IgM Abs, these results in agreement with that reported by(Abdullah *et al*,2022) that the rates of IgG Abs were 2.8% and of IgM Abs were 4.8% [25].

Table (4)that revealed that there 11(73.3%)of women had antigen and IgG Abs of *T.gondii* ,there was no significant relation between antigen and IgG Abs of *T.gondii* P=0.802.Whilethere where only 1(6.7%)of women had IgM Abs and had antigen of *T.gondii* (Table 5) and there were only 2(8.7%) of women had IgM Abs of *T.gondii* but did not have antigen of *T.gondii,* there was no significant relation between Ag and IgM Abs of *T.gondii,* P=0.801.These results in agreement with the that reported by James Hester *et al*., 2012) that it was examined whether tachyzoite proliferation in the brains of immunocompetent hosts during the chronic stage of infection with *Toxoplasma gondii* induces production of IgG antibodies that recognize parasite antigens different from those recognized by the antibodies of infected hosts that do not have tachyzoite growth [26]. Also, it was recently addressed what condition causes an increase in only the IgG antibody levels during chronic *T. gondii* infection using immunocompetent mice. The studies revealed that an occurrence of tachyzoite proliferation in the brain during the chronic stage of infection causes an increase in *Toxoplasma* IgG antibody titers but not in IgM antibody titers in the sera [27].

Finally, once the diagnosis of acute acquired infection during pregnancy has been presumptively established, diagnostic efforts should then focus on determining whether the fetus has been infected. Thus, it is recommended that a positive IgM test result should always undergo confirmatory testing at a reference laboratory [28, 29].

CONCLUSIONS

The presence of *Toxoplasma gondii* IgG and IgM antibodies is higher in housewife women compared to employed women. Additionally, the antigen of *T. gondii* is detected more frequently in women who have experienced abortion than in those who have not. Furthermore, the antigen is found in higher concentrations in women who test positive for IgG antibodies of *T. gondii* compared to those who test positive for IgM antibodies, indicating a potential correlation with chronic infection.

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