

PREVALENCE STUDY OF TOXOPLASMOSIS AMONG MALES

BLOOD DONORS IN THI-QAR PROVINCE –IRAQ

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ABSTRACT

Toxoplasma gondii is a unique intracellular parasite, which infects a large proportion of the world, population, but clinically uncommonly causes significant disease. The present study was performed for the first time in Thi-Qar province to estimate the prevalence of toxoplasmosis among males blood donors. Blood samples were collected from 184 healthy males they have ages between (18 – 60) years from main blood transfusion center in Nassaryia city during period from October 2014 to March 2015. The results indicated that 38.04 % of males were exposed to positive for anti-*Toxoplasma* antibodies, 2.7% of them had IgM, 35.3 % had IgG, statistically they were significant differences between them. The current study also showed that the highest positive percentage with *T. gondii* at age (18 – 27) years 38.5 % while the lowest percentage at age (48-60) 11.4 %. Seropositive toxoplasmosis was higher in males inhabited rural region 88.5% ,than in males inhabited urban region, 11.4 %. Chronic and acute toxoplasmosis infection in married males included in this study was significantly higher 55 (78.5%), 4(5.7%) respectively than unmarried males 10 (14.2%), 1(1.4%) respectively. However married males showed significant differences between fertile and infertile infected males they were 18 (30.5%), 3 (5.08%) and 37(62.7%), 1(0.1%) by ELISA IgG and IgM respectively. The present study revealed an association between blood group system and *Toxoplasma* infection with highest prevalence among samples of blood group A 37.1% and lowest prevalence in samples of blood group O (9.9%).

KEYWORDS: Age Group, Blood Group, ELISA, Toxoplasmosis, *Toxoplasma Gondii*

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite. The cat is the only definitive host but other animals can be infected incidentally (Beaver, 1984). Human can acquire infection by ingestion of raw or poorly cooked meat containing the *T. gondii* cysts or by ingestion of food or water contaminated with Oocysts (Kasper and Wilson, 1998). Although rare, *T. gondii* can also be transmitted via blood or leucocytes from immunocompetent and immunocompromised donors (Raisanen, 1978).

Human may remain infected for life will stay asymptomatic unless immune suppression may occur (Dubey ,2008), symptomatic infections usually cause low grade fever, malaise, headache and cervical lymphadenopathy. Severe manifestation such as encephalitis, myocarditis, hepatitis and pneumonia are rare but they can complication acute toxoplasmosis (Kravetz and Fedman, 2002).

T. gondii are recognized by IgG, IgM, IgE and IgA antibodies in patients with acute and chronic toxoplasmosis depending on the strain and stage of the parasite (Singh, 2003). Toxoplasmosis is ubiquitous infection affecting 500 million

person around the world, with rang incidence 12-90% increasing with age, low education, crowing, sanitary habits, socioeconomic, ethnic consideration, consumption of undercooked meat and animal contacts (Gaetano *et al.*, 2010). This study aim to evaluation and prevalence of male toxoplasmosis in Thi- Qar province from blood donors volunteers at main blood transfusion center by using ELISA (IgG and IgM) test.

MATERIAL & METHODS

184 blood samples were collected from healthy blood donors males age between (18 -60) years . Samples were collected from main blood transfusion center in Nassaryia city during period from October 2014 to March 2015 . Before the collection of samples an information sheet was prepared and designed according to questionnaire which covers different information.

Five ml of venous blood was collected from redial vein from each person , tested for blood groups and then the serum was dispensed in to two eppendrof tubes by using micropipette and stored at -20c until used for ELISA IgG and IgM test .

Blood Groups Phenotyping

Blood groups .anti- A and anti- B slide test (ABO blood grouping) were identified by using commercial monoclonal anti sera Anti – A and Anti – B , (Atlas Medical , UK)as recommended by the manufacture . The results were interpreted as positive if agglutination appears and negative if no agglutination observed (Issitt , 1985) .

Detection of Anti- *T. Gondii* Antibody Igg by ELISA

The bioCheck *Toxoplasma* IgG ELISA (BC-1085) kit (U.S.A.) was used . The *Toxoplasma* IgG ELISA is intended to evaluate a patient, serologic status to *T. gondii* infection.

Principle

Purified *T.gondii* antigen (Ag) is coated on the surface of micro wells. Diluted patient serum was added to the wells, and the *T.gondii* IgG- specific Ab if present, will bind to the Ag. All unbound materials were washed away. Horse radish peroxidase (HRP) conjugate is added, which binds to the Ab-Ag complex Excess HRP-conjugate is washed off and solution of tetra methyl benzidine(TMP) reagent was added. The enzyme conjugated catalytic reaction is stopped at a specific time. The color intensity generated is proportional to the amount of IgG-specific Ab in the sample.The results were read by ELISA reader .

Detection Anti-*T Gondii* Antibody Igm by ELISA

The bioCheck *Toxoplasma* IgM ELISA (BC-108 ELISA 7) kit (U.S.A.) was used. The *Toxoplasma* IgM ELISA is intended for using in detection of IgM status to *T.gondii* in human serum.

Principle

The same principle, reagents, assay procedure of ELISA-IgG technique was adopted in ELISA-IgM detection with few exception, the specific antibody IgM was used instead of IgG specific antibody.

Statistical analysis

Data was analyzed using statistical analysis system Statistical Package for Social Sciences (**SPSS**) to investigate the effect of different factors in *T. gondii* infection. Chi- square (X^2) test were used to compare the significant differences.

RESULTS & DISCUSSIONS

Out of a total 184 apparently healthy males blood donors included in this study , 70 sample revealed seropositive for toxoplasmosis giving an incidental rate of 38.04% by ELISA test compare with 114 (61.9%) seronegative for toxoplasmosis. The statistical analysis showed highly significant ($p<0.05$) differences in ELISA sero+ve and ELISA sero-ve. Our results agree with regional and universal trend for toxoplasmosis infection ratio , where aquarter to one third of various population showed immunity (Inci *et al .* , 2009 ;Hashemi and Saraei ,2010).The prevalence rate of our study agreement with study in different region in Iraq by AL-Ubadi(2011) ;AL-Saadii (2013) and AL-Abudy (2014) , but the results in present study was higher than that recorded by AL-Mosawi (2014) ; AL-Kaysi and Ali (2010) and Saleh (2011) . In the other hand the result of this study lower than these obtained by Saleh(2005) ; Karem (2007) and AL-Shikhly (2010) .The variation and similarities in results may be related to several factors including cultural patterns and climatic ,nutrition habits ,sample size ,target population ,sampling method , types of laboratory tests and tools (Etheredge and Frankal ,1995 and Dubey ,2010) or may due to different manufacture origin of the kits used (AL-Saadii ,2013) . Also the seroprevalence estimated for human population varies greatly among different countries ,among different geographical areas within country ,even within same city (Jones and Dubey ,2010) .

Toxoplasmosis Seroprevalence of Igm and Igg By ELISA

The current study was estimate the actual percentage of toxoplasmosis in males blood donors by using more specific test ELISA IgM and IgG , The acute toxoplasmosis characterized by the presence of positive IgM antibodies lowest than chronic toxoplasmosis characterized by the presence of positive IgG antibodies . Table (1) .There was high significant differences between them ($p<0.05$) . The present result agrees with AL-Ghezy (2012) ;AL-Abudy (2014) and AL-Mosawi (2014) in same province , AL-Saadii (2013) in Baghdad and Galvain –Ramirez *et al .* , (2010) in Maxico The results in current study could be explained by the fact that the group examined consisted only of healthy persons and IgG positive persons were infected with latent toxoplasmosis without a persistence of IgM antibodies after acute infection in the past(Carman *et al .* , 2006)

Table 1: The Percentage Distribution of Males Blood Donors According to ELISA Igg, Igm Test

Test	ELISA Test					
	Positive		Negative		Total	
	N	%	N	%	N	%
Subject						
IgG	65	35.3	119	64.6	184	100
IgM	5	2.7	179	97.28	184	100

$X^2 = 51.42$, $df=1$, $p=0.00$

Toxoplasmosis Percentage Distribution by ELISA Igg and Igm According to Age.

The present result showed high positive percentage samples, in ELISA IgG test at age group of (18 -27) years,

whereas the lowest one was at the age group of (48- 60) years. While, ELISA IgM showed presence of high percentage at age group of both (18 -27) and (28 -37) years and the lowest at age group of (38 -47) years. There was a significant difference between them ($p<0.05$) .Table (2). The result is coincided with other results obtained by Janna (2006) ;AL-Rawi(2009); AL-Saadii(2013) and AL-Mosawi (2014) in Iraq which reported that the main age group range of seropositive toxoplasmosis was between(20-30) years ,while there discrepantly with AL-Ubaydi (2004) and then by AL-Myahi (2011) who showed that the highest percentage of toxoplasmosis infection occurred at the age group (11-20) years and (16 -19) years respectively. Other results showed no significant difference with age factor in Iraq by AL-Zihiry *et al*, (2007);Kalil (2008) and AL-Azawi *et al*, (2013).

These differences between studies may be due to the differences in the specificity and sensitivity of method used for diagnosis of differences in the age of the studies groups and the response of each host to the strain of parasite ,the variation in parasite strains may play an important role in the stimulation of host immune response against the parasite (Suzuki and John ,1994) .

Table 2: Toxoplasmosis Percentage Distribution by Elisa Igg and Igm According to Age

Test Age Group	IgG		IgM		Total	
	N	%	N	%	N	%
18-27	25	38.4	2	40	27	38.5
28-37	22	33.8	2	40	24	34.2
38-47	11	15.7	0	0	11	15.7
48-60	7	10.7	1	20	8	11.4
Total	65	92.8	5	7.1	70	100

$$X^2 = 15.14, df=3, p=0.002$$

Toxoplasmosis Percentage Distribution by ELISA Igg and Igm According to Habitation

The high percentage of toxoplasmosis in males in habited rural area compared to urban resident . There was a significant difference between them ($p<0.05$) .Table (3) . This study agreed with previous study by AL-Saadii (2013) who found significantly higher ($p= 0.01$)seropositive rural than urban in Baghdad and Kawashima *et al.*, (2000) in Philippines who found significantly higher ($p=0.001$) seropositive in rural than urban setting , another study by Sroka *et al.*, (2010) in Poland they also showed that human living in farms had significantly greater percentage of anti- Toxoplasma antibodies with (59 %) compared to urban dawdlers(41%) . Other studies in Iraq by AL-Jubori (2005) regarding the residency of the patients and its relation with seropositive Toxoplasma Abs showed no significant difference between Toxoplasma Abs distribution and both urban and rural areas .

This result may be due to increasing trend of raw vegetable consumption and high usage of water that might have been contaminated with *T. gondii* Oocyst .

Table 3: Toxoplasmosis Percentage Distribution by ELISA Igg and Igm According to Habitation

Test Subject	ELISA test	
	N	%
Urban	8	11.2
Rural	62	88.5
Total	70	100

$X^2 = 41.65, df= 1, p =0.00$

Toxoplasmosis Percentage Distribution by ELISA Igg and Igm According to Marital Status

The present result showed that there were a significant differences ($p < 0.05$) between married and unmarried males blood donors in the presence of IgG and IgM antibodies Table (4) . this result was concerned by a previous study done in Iraq by Hamza (2006) who revealed that marital status was significantly associated with total Toxoplasma antibodies , but not with IgM Toxoplasma antibodies although the rate of seropositivity was higher in married than un married patient. However, AL-Saadii (2013) showed that there were a significant differences ($p = 0.01$) between married and un married males blood donors in the presence of IgG and IgM antibodies . However ,Davarpanah *et al .*, (2007) and Mohraz (2011) in Iran demonstrated that seroprevalance of toxoplasmosis were higher in married patient than single. These differences between the current study and pervious gender factor may play a role especially since this study was planned on males only. Another explanation for these differences in the present study where married patients were higher than single.

Table 4: Toxoplasmosis Percentage Distribution by ELISA Igg and Igm According to Marital Status

Test Subject	IgG		IgM		Total	
	N	%	N	%	N	%
Married	55	78.5	4	5.7	59	84.2
Unmarried	10	14.2	1	1.4	11	15.7
Total	65	92.8	5	7.1	70	100

$X^2 = 31.15, df= 1, p =0.00$

Toxoplasmosis Percentage Distribution by ELISA Igg and Igm According to Fertility

The present study showed that fertile males infected with chronic toxoplasmosis had a low percentage of anti *Toxoplasma* IgG antibodies while infertile males showed high percentage , there was a significant difference between them ($p < 0.05$). In contrast , fertile males infected with acute toxoplasmosis had a highest percentage of anti-Toxoplasma IgM antibodies in comparison to infertile . Table (5) . this result agreed with previous study by Zhou *et al.*, (2002) in china who found that Toxoplasma infection in infertile human couple was higher the fertile couple and he explained that may be related to the antisperm antibodies which were higher in Toxoplasma infected couple . As well as Ziping *et al .*, (2007) explored the effect of toxoplasmosis infection on male reproductive function on 140 infertile men which is evidently higher than the average infective rate of the normal. On the other hand Lus (2003) concluded that *T.gondii* infection may result in male sterility.

Table 5: Toxoplasmosis Percentage Distribution by Elisa Igg and Igm According to Fertility

Test Subject	IgG		IgM		Total	
	N	%	N	%	N	%
Fertile	18	30.5	3	5.08	21	35.5
In fertile	37	62.7	1	0.1	38	64.4
Total	55	93,2	4	6.7	59	100

$$X^2 = 4.89, df=1, p=0.02$$

Toxoplasmosis Percentage Distribution by Elisa Igg and Igm According to Blood Group

Blood group is of interest that the present study revealed an association between blood group system and *Toxoplasma* infection with highest prevalence among samples of blood group A and lowest prevalence in sample of blood group O. There was a significant between blood group ($p < 0.05$). Table (6). This result agrees with Al-Dujaily (1998), Al-Khaffaf (2001), Al-Khashab (2009) and AL-Mosawi (2014) who recorded that the highest prevalence among samples of blood group A+. This result disagrees with Al-Shikhly *et al.* (2013) and Al-Abudy (2014) who showed that the higher percentage result occurred in females with blood group O+ and AL-Saadii (2013) who showed that the higher percentage result occurred in blood group AB+. Other studies showed no significant relation of toxoplasmosis with ABO factors (Rodrigues *et al.*, 2011 and Mohamed *et al.*, 2013). The present result of this study is a possibility that the parasite utilized glycoconjugates, which characterize the blood phenotypes of the ABO blood group system, as a potential receptors (Midfvedt and Vaage, 1989; Lopez *et al.*, 1993; Kolbekova *et al.*, 2007). These glycosylated molecules are expressed in the gastrointestinal tract (GIT), which is also utilized as main route of *T.gondii* infection (Carruthers *et al.*, 2000). Additionally, there is biochemical evidence that several microorganisms utilize biochemical evidence that several microorganisms utilize glycoconjugates as receptor (Hooper and Gordon, 2001)

Table 6: Toxoplasmosis Percentage Distribution by ELISA Igg and Igm According to Blood Group

Test Blood group	ELISA test	
	N	%
A	26	37.1
B	18	25.6
AB	19	27.1
O	7	9.9
Total	70	100

$$X^2 = 10.57, df=3, p=0.014.$$

CONCLUSIONS

Our results showed a high seroprevalence of *T. gondii* in healthy voluntary donors in Thi-Qar province -Iraq. Such studies need to be done on a larger sample in other part of the country.

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