



Prevalence of Toxoplasmosis of Males Blood Donors in Baghdad

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Abstract

Toxoplasma gondii is intracellular parasites, which infect a large proportion of the world's population, but uncommonly causes clinically significant disease. The present study was performed to estimate the prevalence of toxoplasmosis in Iraqi male. Venous blood samples were collected from healthy male age between (18-57) years attended the National blood transfusion centre in Baghdad from Oct, 2011 to Jan. 2012. Latex agglutination test (LAT) and Enzyme linked Immunosorbant Assay (ELISA), were used to detect anti-*Toxoplasmosis* IgM and IgG antibodies. The results showed significant differences between seropositive toxoplasmosis infections between LAT, 136/400 (34%) and ELISA - IgG, 121/400 (30.25%). The blood group phenotypes of the infected male blood donors high light the role in toxoplasmosis infection and the AB blood group characterized by the highest significant percentage of infection. Chronic and acute toxoplasmosis infection in married males included in this study was significantly higher, 101(83.47%) and 7(70%) respectively than unmarried males 20 (16.52%) and 3(30%) respectively. However married males showed significant difference between fertile and infertile infected males, they were 31(30.69%), 6(85.71%) and 70 (69.30%), 1 (14.28%) by ELISA IgG and IgM respectively. Private worker males revealed highly significant percentages of chronic and acute toxoplasmosis 91(75.20%), 2(20%) respectively compared with government worker, 30(24.79%), 8 (80%) respectively. Chronic seropositive toxoplasmosis was higher in males inhabited rural regions, 111(91.73%), than in males inhabited urban regions, 10(8.26%).

Keywords: *Toxoplasma gondii*, Toxoplasmosis, blood group, LAT., ELISA, age group, marital

انتشار داء المقوسات للرجال المتبرعين بالدم في بغداد

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الخلاصة:

المقوسات الكونديه من الطفيليات داخل خلوية والتي تصيب نسبة كبيرة من سكان العالم ، ولكن من غير المألوف اظهار الطفيلي علامات سريرية واضحة عند الخمج. اجريت هذه الدراسة لتقدير معدل انتشار داء المقوسات الكونديه في الرجال العراقيين. تم جمع عينات الدم من الرجال الاصحاء باعمار تتراوح بين (18-57) سنة من المراجعين للمركز الوطني لنقل الدم في بغداد خلال الفترة من تشرين الاول 2011 ولغاية كانون الثاني 2012. تم استخدام اختبار (LAT) وتقنية الامتزاز المناعي المرتبط بالانزيم (الايلازا ELISA) للتحري عن الاضداد المناعية المضادة للمقوسات الكونديه نوع IgM و IgG. اظهرت النتائج وجود فروق معنوية بين الاصابات الموجبة لداء المقوسات الكونديه بين اختبار LAT 400/136 (34%) وبين اختبار الايلازا للضد النوعي IgG 400/121 (30.25%). الطور المزمن والحاد لداء المقوسات الكونديه في الرجال المتزوجين

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اظهر نسبة معنوية اعلى 101(83.47%) و 7(70%) بالمقارنة بالرجال غير المتزوجين 20(16.52%) و3(30%) على التوالي من ناحية ثانية اظهر ذكور المتزوجين فرق معنوي في خصوبة الرجال المصابين بالمرض وقد كانت نسبة الرجال الخصيين 31(30.69%) و 6(85.71%) وغير الخصيين 70(69.30%) و1(14.28%) عند استخدام اختبار الاليزا IgM و IgG على التوالي. كما اظهر الرجال ذوي الاعمال الخاصة نسبة معنوية عالية للاصابة بداء المقوسات بطوريه الحاد والمزمن 91(75.20%) و2(20%) عند مقارنته بالرجال ذوي الوظائف الحكومية 30(24.79%) و 8(80%) على التوالي. اظهر داء المقوسات المزمن نسبة اصابة اعلى عند الرجال المقيمين في المناطق الريفية 111(91.73%) مقارنة مع الرجال الذين يقطنون المناطق الحضرية 10(8.26%).

1. Introduction:

Toxoplasmosis is a zoonotic disease caused by the protozoan parasite *Toxoplasma gondii*, human and other warm blooded animals acts its hosts [1]. The infection has a world wide distribution; approximately one-third of all human being have been exposed to this parasite, but the seroprevalence varies considerably between countries (from less than 10% to more than 90%) and population group [2]. Humans may remain infected for life and will stay asymptomatic unless immunosuppression may occur [3]. Primary infection of toxoplasmosis in immunocompetent patient is usually asymptomatic or associated with self limited symptoms such as fever, malaise, and during pregnancy is frequently associated with transmission of *T. gondii* to the fetus, resulting in congenital disease. In immunocompromised patients, *T. gondii* infection causes severe manifestation, including splenomegaly, chorioretinitis, pneumonitis, encephalitis, multisystem organs failure, and even death [4]. In patient infected with human immuno-deficiency virus (HIV), more than 90% of *Toxoplasma* encephalitis cases involve reactivation of a latent infection this variability is related to various factors such as, age, sociocultural and nutritional habits, and contact with animals [5]. *T. gondii* are recognized by IgG, IgM, IgA and IgE antibodies in patients with acute and chronic toxoplasmosis depending on the strain and the stage of the parasite [6]. It is therefore essential to estimate the time of infection as precisely as possible for properly manage the risk patients. Lower cellular immunity which is associated with high levels of steroid hormones contributes to the survival of the parasite in the body. Such people cause of steroid hormones increase with weak immune system, [7]. Other reports indicate that *Toxoplasma* can increase the number of son, height in infected male and change personality factors in male and women [8]. It is likely that

sexual hormone changes can play an important role in relation with *Toxoplasma* and a mentioned phenomena.

2. Aim of study:

Evaluation and prevalence of male toxoplasmosis in Baghdad province from blood donor volunteers at the National blood transfusion centre by using LAT and ELISA (IgM, IgG).

Materials and Methods:

400 blood samples were collected from healthy blood donor males, age between (18-57) years. Samples were collected from National blood transfusion centre in Baghdad. Five ml of venous blood was collected from radial vein from each person, tested for blood groups and then the serum was dispensed into 5 eppendorf tubes by using micropipette and stored at -20 °C for future work.

Blood groups

Anti-A, B Blend test:

According to the manufacturer's procedure.

Anti-D Blend:

A slide test based on agglutination method for Rhesus typing in serum using monoclonal/polyclonal blend reagent.

Latex agglutination test:

(Toxocell-Latex) the kit was used from (biokit company-Spain). The principle of the test is based on gen-antibody reaction directly. The sensitivity of the test is 10-15 IU/ml [10].

Detection of anti-*T.gondii* antibody (IgG) and (IgM) by (ELISA) technique: Measurement of IgG and IgM antibodies was performed and interpreted according to the direction of the manufacturer's procedure.

3. Principle:

Purified *T.gondii* antigen (Ag) is coated on the surface of micro wells. Diluted of 1:40 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 100 ml, which binds to the Ab-Ag

complex. Excess HRP-conjugate is washed off and solution of tetra methyl benzidine (TMP) reagent was added 100 ml. The enzyme conjugated catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG – specific Ab in the sample. The results were read by ELISA reader.

4. Statistical analysis:

The statistical analysis system-SAS, 2004 was used to detect the difference factors in study parameters (percentage and level). The chi-square test was used to the significant comparison between percentages (P value < 0.01) was considered statistically significant.

5. Results and discussion:

Latex agglutination test (LAT): Out of a total 400 male blood donors included in this study , 136 revealed seropositive for toxoplasmosis giving an incidental rate of 34% by LAT as shown in, table 1.

Table1- percentage distribution of male blood donors from national blood transfusion centre by Latex agglutination test (LAT)

Test Result	Latex test		
	Latex(+)	Latex (-)	Total
N	136	264	400
%	34	66	100
Chi-square - χ^2	9.511 **		---
** (P<0.01).			

The statistical analysis showed high significant (P<0.01) difference in LAT sero (+ve) and LAT sero (-ve), in Iraqi provinces, similar studies showed but the results in present study was higher than that recorded by [11] which is 19.7%, 26.8% in Al-Najaf province respectively, and [12], recorded 29.2% in Baghdad as well as [13] who showed the percentage of toxoplasmosis of 22% seroprevalence in blood donors. However the present percentage was lower than those recorded by [14] 49.65% in Diwanyia and by [15]100% in Duhok provinces. Other study in Kut-city showed that the rate of toxoplasmosis was 45.8% in women by LAT [16]. The explanation of prevlous percentage differences stated by different researcheres may related to the seroprevalance estimated for human population varies greatly among different geographical area within one country, and within the same city [17] These differences may be related to several other factors, including cultural level, nutritional habits, age and rural or

urban area [18]; or even to the use of different kit origins.

ELISA IgM and IgG tests for toxoplasmosis seroprevalence:

The current study was to estimate the actual percentage of toxoplasmosis in male blood donors in national blood transfusion centre by using more specific tests ELISA IgM and IgG. More then 400 samples only 10(2.5%) had acute toxoplasmosis characterized by the presence of positive IgM antibodies, table (2).

Table 2- percentage distribution of male blood donors from National blood transfusion centre infected with toxoplas- mosis according to ELISA IgG and IgM test

Test Sub	ELISA test						Chi-sq. χ^2
	Positive		Negative		Total		
	N	%	N	%	N	%	
IgG	121	30.25	279	69.75	400	100	10.363 **
IgM	10	2.50	390	97.50	400	100	12.826 **
** (P<0.01).							

While 121(30.25%) of samples had chronic toxoplasmosis characterized by the presence of positive IgG antibodies only. Their was high significant differences between them (p<0.01). The result was similar with the results obtained by [19] in Iraq who showed that chronic infection 49(49%) in male with toxoplasmosis was higher than those of acute infection 6 (6%). Another study using showed that 77 positive result 16 (22.5%) gave IgM positive and 55 (77.5%) gave IgG positive, and the other six patients gave positive result for both IgG and IgM antibodies [20].A previous study by [21] showed high incidence of IgM antibodies in female compared to male. However, [22] showed that male are more susceptible than female to many parasites. Seropositive rate was lower in this study than those of [23], who demonstarted that seropositivly rate was (37%) by using ELISA technique, and with [24], who showed that (37.5%) seropositivly rate among miscarriage woman by using VIDAS *Toxoplasma* competition kit. These variability may be due to different sample size, geographical location, type of kit used and the resistance of different strains of *T. gondii* that plays an important role in differences of infection rates. Also animal petting and exposure to contaminated soil is another reseons for infection [18]. ELISA IgG test showed in the current study was lower percentage (30.25%)of

toxoplasmosis infection in comparison to LAT percentage (34%). This reflects the accurate specificity of ELISA test in detection of the presence of *T. gondii* parasite, table (3).

Table3- The percentage distribution of male blood donors from National blood transfusion centre infected with toxoplasmosis by Latex agglutination test and ELISA IgG, test.

Test	Latex		ELISA- IgG	
	No	%	No	%
positive	136	34	121	30.25
Negative	264	66	279	69.75
Total	400	100	400	100
Chi-square - χ^2	--	9.511 **	--	10.363 **

** (P<0.01).

Toxoplasmosis percentage distribution by ELISA IgG and IgM according to:

First: age groups:

In relation to age group the results showed high positive percentage samples, 30 (24.79%) in ELISA IgG test at age group of both (18-25) and (26-33) years, whereas the lowest one was 7(5.78%) as noticed at the age group of (50-57) years .While, ELISA-IgM showed variable results characterized by the presence of high percentage 3(30%) at the age group of (18-25) years and the lowest 1(10%) at the age group of (42-49) years. There was a significant difference between them (p<0.01), table (4).

Table 4- The percentage distribution of male blood donors from National blood transfusion centre infected with toxoplasmosis according to age groups by ELISA test (IgG, IgM).

Test Age groups	IgG		IgM		Total	
	No	%	No	%	No	%
18-25	30	24.79	3	30	33	25.19
26-33	30	24.79	2	20	32	24.42
34-41	29	23.69	2	20	31	23.66
42-49	25	20.66	1	10	26	19.84
50-57	7	5.78	2	20	9	6.87
Total	121	92.36	10	7.63	131	100
Chi-square - χ^2	---	6.271 **	---	6.50 **	---	6.092 **

** (P<0.01).

This result is agreed with other results obtained by [25] in Iraq as they concluded that the main age group of seropositive toxoplasmosis were between (20-30) years. Another study by [26] showed that the highest percentage of toxoplasmosis infection occurred

at the age group (11-20) and (15-19) years respectively. Whereas a previous study by [27] indicated that the incidence of toxoplasmosis increases with age no more 50 years but [28] found that the incidence increase with age but to a peak of 34 years. Other results showed no significant difference with age but the highest infection rate occurred in age group (31-35) years [29] . Also our result with several results from different regions which that indicated the percentage of anti-*T.gondii* antibodies was increased with age [30],[31]. High prevalence values of infection with *T.gondii* were found in young adult women, this may due to more frequent *Toxoplasma* contact in childhood and adolescence, through cats contact, soil exposure [32]. These differences between previous results and current result may be due to the differences in the specificity and sensitivity of method used for diagnosis and response of each host to the strain of parasite. [33].

Second: blood group:

It is of interest that the present study revealed an association between blood group system and *Toxoplasma* infection with highest prevalence among samples by ELISA IgG with blood group AB⁺ 50 (41.32%) and the lowest prevalence in samples of group O⁺19(15.70%) where there was significant difference between them (p<0.01). table (5).

Table 5- The percentage distribution of male blood donors infected with toxoplasmosis according to blood groups by ELISA test (IgG , IgM)

Test Blood group	IgG+		IgM+		Total	
	No	%	No	%	No	%
A+	27	22.31	3	30	30	22.90
B+	25	20.66	2	20	27	20.61
AB+	50	41.32	2	20	52	39.69
O+	19	15.70	3	30	22	16.79
Total	121	92.36	10	7.63	131	100
Chi-square - χ^2	---	7.926 **	---	3.856 **	---	6.455 **

* (P<0.05) , ** (P<0.01).

On the other hand, ELISA IgM antibodies test showed highly toxoplasmosis percentage 3 (30%) with blood group O⁺ and lowest percentage 2 (20%) in AB blood group.

A study among blood donors in Russia has reported similar findings, with toxoplasmosis seroprevalence being twice as high among subjects with blood group AB than among

subjects with blood group O (54% vs. 27%) respectively [35,36]. However this result is agreed with [13], they showed that *Toxoplasma* infection was more prevalent among O⁺ and AB blood group with the seroprevalence 35.8% and 38% respectively. Another result showed no-significant relation of toxoplasmosis with ABO factor [37] but [38] revealed that infection was concentrated in blood group type O⁺ with percentage of (43.47%). It was known that natural resistance to many infections may depend, on certain extent, on the blood group of an individual [39]. The A, B and O blood group system are determined by the presence or absence of A and B carbohydrate antigens on the surface of red blood cells [40]. This determines natural resistance in human to many infection disease agents that have cell surface antigens similar to the antigens of different blood group types. This mechanism may, in part, explains the higher susceptibility of individual with blood type AB to several infections diseases, since the blood of those individuals does not contain the corresponding natural antibodies. Previous studies have investigated the possible relationship between the ABO group system and the presence of anti-*T.gondii* antibodies. Their conclusions are conflicting four studies which have reported an association between infection by this parasite and B and AB blood group [41]. These studies proposed that B antigen could be a potential receptor for *T. gondii*. However, two other similar investigations did not find any evidence of this association [42]. It is suggested that B antigen represents a receptor for *T. gondii* did not appear to be valid in different population [37]. The controversy in these results and other studies may resulted from several factors. It is possible that the molecular variability of strains in Iraqi patients, or using only male patients in this study gave findings that may differ from findings in other population, or it is possible that AB antigens has large influence on the adherence of *T. gondii* to the gastrointestinal mucosa and its contribution is an evident by high prevalence of infection of these parasites in the Iraqi population.

Third: marital status:

The present result showed that there were a significant differences (P<0.01) between married and unmarried male blood donors in the presence of IgG and IgM antibodies they recorded 101(83.47%), 20(16.52%) and 7(70%), 3(30%) respectively. Fig (6), table (6).

Table 6- The percentage distribution of male blood donors from National blood transfusion centre infected with toxoplasmosis by ELISA test according to the marital status

Test subject	IgG+		IgM+		Total	
	No	%	No	%	No	%
Married	101	83.47	7	70	108	82.4
Unmarried	20	16.52	3	30	23	17.5
Total	121	92.36	10	7.63	131	100
Chi-square - χ^2	---	11.745**	---	9.50**	---	11.2**

** (P<0.01).

This result was concerned by a previous study done in Iraq by [43] who showed 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 single patients (43.1%), (31.1%) respectively. However, [44] in Iran demonstrated that seroprevalance of toxoplasmosis was higher (20%) and (56.5%) in married patient than single (16.5%) and (42.4%) respectively. These differences between the current study and previous gender factor may play a role especially since this study was planned on male only.

Fourth: fertility:

The present study showed that fertile male infected with chronic toxoplasmosis had a low percentage of anti-*Toxoplasma* IgG antibodies 31 (30.69%) while infertile males showed high percentage 70 (69.30%), there was a significant difference between them (P<0.01). In contrast, fertile males infected with acute toxoplasmosis had a highest percentage of anti *Toxoplasma* IgM antibodies 6 (85.71%) in comparison to infertile 1 (14.2%). table (7).

Table 7- The percentage distribution of married male blood donors from National blood transfusion centre infected with toxoplasmosis by ELISA test according to the Fertility

Test subject	+IgG		IgM+		Total	
	No	%	No	%	No	%
Fertile	31	30.69	6	85.71	37	34.25
infertile	70	69.30	1	14.281	71	65.74
Total	101	93.51	7	6.48	108	100
Chi-square - χ^2	---	10.364**	---	11.874**	---	9.491**

** (P<0.01).

This result agreed with previous study by [45] in China who found that *Toxoplasma* infection

in infertile human couple was higher than fertile couples and it was explained that it may be related to the antisperm antibodies which was higher in *Toxoplasma* infected couples. Another investigation by [46] on *T. gondii* infection in 100 male with sterility revealed that 16% of them were IgM-positive and (13%) were positive in healthy male. As well as [47] explored the effect of toxoplasmosis infection on male reproductive function in 140 infertile male which is evidently higher than the average infective rate of normal. The present study showed higher percentage than previous studies in China that showed the seroprevalence of *Toxoplasma* infection in male sterility cases was (22.8%) and (19.8%) obtained by [48] respectively, on the other hand, [49] concluded that *T. gondii* infection may result in male sterility. These results may due to apoptosis of spermatogenic cells in males with toxoplasmosis, [50] claimed that infection occur in fertile and infertile males infected with toxoplasmosis rate was (4.28%) and (13.76%) respectively, or due to spermatogenic obstruction characterized by low spermatozoa density, living rate and activity in males infected with toxoplasmosis [51].

Fifth: Occupation:

Toxoplasmosis in private workers (gardeners, barbers, mechanicals, electricians, workers, drivers) which showed included in this study, higher percentage 91(75.20%) than those having governmental workers 30 (24.79%) in the presence of anti-*Toxoplasma* IgG antibodies. While the presence of anti-*Toxoplasma* IgM antibodies, this result revealed higher percentage 8 (80%) in those works and 2(20%) in private workers. Fig (8), table (8).

Table 8- The percentage distribution of male blood donors from National blood transfusion centre infected with toxoplasmosis by ELISA test according to the occupation.

Test subject	IgG+		IgM+		Total	
	No	%	No	%	No	%
Official work	30	24.7	8	80	38	29
Private work	91	75.2	2	20	93	70.99
Total	121	92.3	10	7.63	131	100
Chi-square - χ^2	---	10.2**	---	10.7**	---	9.649**
** (P<0.01).						

There was significant differences between them (P<0.01). In Iraq [52] found that all seropositive rate was (33.42%), high prevalence rate in barbers (72.73%) followed by food handlers (41.18%) housewives (38.9%) donated blood (24.30%) and medical staff (21.43%).[44] found

that farmers had highest anti-*Toxoplasma* antibodies rate (47.4%) compared with other occupations (clerks 31%, teachers 42.1%, students 21.2%, workers 37.5%). The explanation of these results may related to the fact that farmers were likely to be of low education or their exposure to soil and other risk factors are more than others.[53] found that contact with soil, contaminated vegetables and fruit were identified as a risk factor for *Toxoplasma* infection which was confirmed by [54] indicated that seroprevalence *Toxoplasma* antibodies was significantly higher amongst individuals who keep livestock (52.2%) and abattoir workers (46.3%) and he suggested exposure to *T. gondii* infection is present among residents of Tanga district in Tanzania whom consume raw meat or under cooked meat and petting especially cats that presents more risk factors than other occupational groups. He also emphasized on the necessity to create awareness of this disease, and advocate protection of risky group from exposure to infected meat and contaminated environment. In Iran [44] revealed the seroprevalence of toxoplasmosis was (48.8%) in males and (55.2%) in female and the demographic and baseline characteristics of occupation were clerks (20%) merchant (58.6%) students (33.3%) unemployed (50%) other jobs (48.91%).[55] in China showed that some occupations was from required people to have contact with animals and meats and these frequently posses higher risk of infection with the parasite. Such as dairy workers (45.0%) slaughter house workers (25.6%) veterinarians (12.5%), meat processing workers (13.7%) meat sellers and cooks (29.7%). Another investigators demonstrated that out of the 200 fruit and vegetable workers, 15(7.5%) of them were positive for anti-*Toxoplasma* IgG antibodies while, anti-*Toxoplasma* IgM, antibodies were found in 2(1%) of the fruit workers [56].

Sixth: habitation:

Data obtained from Figure 9 and Table 9, showed significantly high percentage (P<0.01) of toxoplasmosis in male inhabited rural area where 111(91.73%) compared to urban residents 10 (8.26%). The present study agreed with previous study by [57] who found significantly higher (p<0.001) seropositive in rural than urban settings and with [58] in Philippines, where they showed that the rate of toxoplasmosis was higher in suburban patients than urban residents. Another study by [59] in Romania who found that higher seropositivity of toxoplasmosis was

among rural environment (63.68%) compared to the urban one (55.12%), for the two genders male and women. [60] in Poland they showed that human living in farms had significantly greater percentage of anti-*Toxoplasma* antibodies with (59%) compared to urban dwellers (41.0%). [16] in his recent study has noticed the same results in the presence of higher anti-*Toxoplasma* antibodies in women lived in rural area in comparison to those lived in urban area. Our result is disagreed with several previous studies by [24] that they indicated no significant difference of infection by *T. gondii* according to habitation. Other studies in Iraq by [52] 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 area, in which the rate was (33.98%) and (32.08%) respectively. Also, [43] showed a non significant association between the residency and prevalence of total *Toxoplasma* antibody by ELISA IgM test urban has (32,8%), and rural has (40.3%). [19] Showed that among the positively rate of anti-*Toxoplasma* IgG Ab selected explanatory variable among schizophrenic group was non significant among rural (49.4%) compared to urban residence (47.1%). Since people in Iraq do not have the habit of consuming raw meat, it can be concluded that accidental ingestion of oocysts is the primary route of transmission in Iraq. Therefore the difference in seropositivity between rural and urban area may be due to the hygienic and socioeconomic status that relates to oocyst shedding by cats and peoples contact with the soil, specifically, the pavement widely seen in the urban area is considered to contribute to the reduced surviving period of oocysts shed by cats.

Table 9- The percentage distribution of married male blood donors from National blood transfusion centre infected with toxoplasmosis by ELISA test according to the habitation.

Test subject	IgG+		IgM+		Total	
	No	%	No	%	No	%
Urban	10	8.26	9	90	19	14.50
Rural	111	91.73	1	10	112	85.49
Total	121	92.36	10	7.63	131	100
Chi-square - χ^2	---	12.863**	---	12.50**	---	11.638**
** (P<0.01).						

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