Seroprevalence of Anti *Toxoplasma gondii* IgG and IgM in Healthy Blood Donors in Kirkuk City

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Abstract

Toxoplasma gondii is an obligate and intracellular, parasitic protozoan that causes toxoplasmosis. It has a worldwide distrebution, and its capable of infecting virtually all warm-blooded animals. In the current study ninety one serum samples from a healthy blood donors in a blood bank in Kirkuk General Hospital were collected (eighty six males and five females).The data which collected from each donor was involve age, sex, blood group and Rh. The serum samples were examined for *Toxoplasma gondii* antibodies (IgM and IgG) by enzyme linked immunosorbent assay. The overall prevalence of the parasite was 18.7%. From the ninety one serum samples examined 10.9 % was positive for IgG, and 5.5% was positive for IgM and the positive rate of both IgG and IgM was 2.2 % (significant differences between them were present). The blood group A^+ was the blood group which is the most significantly infected with *Toxplasma* with rate of 33.3%. The male age group which was most significantly infected with parasite was 43-46 years with rate of 50 %, and the most female age group which was infected was 35-38 years with rate of 100%. The conclusions of this study is that the most Toxoplasma infection more for chronic cases and the most individuals which are at risk for Toxoplasmosis are those peoples having A^+ blood group.

Key words: Seroprevalence, *Toxoplasma gondii*, Blood donors, Kirkuk.

الخلاصة

المقوسات الكوندية طفيلي أبتدائي داخل خلوي اجباري يسبب داء المقوسات .له انتشار عالمي وقادر على اصابة كل الحيوانات ذوات الدم الحار..في الدراسة الحالية جمعت احدى وتسعون (86ذكر,5انثى) عينة مصل من الاصحاء المتبرعين بالدم في مصرف الدم بمستشفى كركوك العام.البيانات التي جمعت شملت العمر,الجنس,فصائل الدم والعامل الريسي.فحصت عينات المصل بتقنية الاليزا للبحث عن الاجسام المضادة للطفيلي. وجد ان النسبة الكلية لانتشار الطفيلي كان 18,7%.من المجموع الكلي للعينات, وان10,9% كان موجبا لأضداد IgG و 5,5% كان موجبا لأضداد IgM. وان نسبة العينات الموجبة لكلا IgG,IgM كانت 2,2%. بينت الدراسة ان فصيلة الدم نوع +A كانت الاكثر اصابة بالطفيلي وبنسبة 33,3%.المرحلة العمرية الاكثر اصابة في الذكور كانت 43-46 سنة بنسبة 50% والمرحلة العمريةالاكثر اصبة في الاناث كانت 35-38 سنة بنسبة 100%. نستنتج من الدراسة الحالية بان اكثر حالات الاصابة بداء المقوسات الكوندية هو الحالات المزمنة وان اكثر المجاميع المعرضة لخطر الاصابة بالداء هم من حملة فصيلة الدم +A.

الكلمات المفتاحية: الانتشارالمصلي, المقوسة الكوندية, المتبرعين بالدم, كركوك.

Introduction

Toxoplasma gondii is an obligate, intracellular, parasitic protozoan that causes toxoplasmosis (Louis and Kami, 2010). It has a worldwide distribution and *T. gondii* is capable of infecting virtually all warm-blooded animals (Dubey and Beattie, 2008). In humans, it is one of the most common parasites (Dubey, 2009), serological studies estimate that up to a third of the global population has been exposed to and may be chronically infected with *T. gondii*. The infection rates differ significantly from country to country (Pappas *et al*., 2009). Infection in humans and other warm-

blooded animals can occur by ingesting water, soil, vegetables, and anything contaminated with oocysts shed in the feces of an infected cat, or by consuming raw or undercooked meat contining *T. gondii* tissue cysts, or through transmission from mother to fetus, also by blood transfusion and organ transplantation (Tenter *et al*., 2000). In Iraq and other countries the possibility of *Toxoplasma* transmission by blood transfusion had been studied. The IgM prevalence among blood donors in Mosul city were 3% (Al-Dabbagh, 2011). In the National blood transfusion centre in Baghdad, Latex agglutination test (LAT) and Enzyme linked Immunosorbant Assay (ELISA), were used to detect anti-*Toxoplasma* IgM and IgG antibodies. The seropositive infections was 136/400 (34%) by LAT, and 121/400 (30.25%) by ELISA – IgG (Mahmood, 2013). In another study in Baghdad the seropositive cases for toxoplasmosis were 22% (Al-Kaysi and Ali, 2010). From the total serum samples (90) collected from healthy persons donated in the central blood banks in Thi-Qar city, the IgM and IgG antibodies seropositive was 13 (14.4%),25 (27.8%) respectively (Hadi, 2010). Overall, 155 (67.4%) of 230 asymptomatic blood donors were positive for anti-*T. gondii* IgG antibodies and 24 (10.4%) of them were also positive for anti-*T. gondii* IgG avidity antibodies, which was high in Egypt compared to many countries (Azab *et al*.,2012). In Iran of 250 samples, 58 (23.2%) and one were positive for IgG anti-*T. T. gondii* (chronic) and IgM anti-*T. T. gondii* (acute) antibodies levels respectively (Shaddel *et al*.,2014). Serological studies indicate a widespread distribution of the parasite in the human population. 322 samples of sera from blood donors in four areas of Kenya were screened for *Toxoplasma gondii* antibodies by haemagglutination and 54% proved positive. 299 of these were also tested by dye test and 42% were positive, with 5·9% showing high titres indicating possible active infection (Griffin and Williams, 1983). Overall 20.3 % were positive for *T. gondii* IgG antibody, of which, 63 % had high and 7 % low avidity, 3.6 % IgM positive. IgG titre ranged from 18-362 IU/ml in urban Karnataka (Sundar *et al*., 2007). Three hundred and two blood donors (213 men and 89 women) were tested for specific immunity against *Toxoplasma*. The prevalence rates of *Toxoplasma* infection in men and women were 32.9% and 27.0%, respectively in Prague, and of the Zbraslav hospital (Flegr *et al*., 2011). Thirty two (7.4%) of 432 blood donors had IgG anti-*T. gondii* antibodies. Eight (1.9%) of them had also IgM anti-*T. gondii* antibodies in Durango, Mexico (Alvarado *et al*., 2007) in Brazil 57.5% were positive for toxoplasmosis by immune fluorescent method (Loges *et al*., 2012), A serological survey of *Toxoplasma gondii* infection in blood donors was carried out in order to identify seroprevalence in Recife, Brazil, the seropositive samples were (75.0%) (Coelho *et al*., 2003). in Malaya medical center in Kuala-Lumpur the *Toxoplasma* seroprevalence were 28.1% (Nissapatorn *et al*., 2002). Of the 1,783 participants, 166 (9.3%) testes were positive for anti- Toxoplasma IgG, while 5 (0.28%) testes were positive for anti-Toxoplasma IgM in a Taiwanian study (Chiang *et al*., 2012). In a study done during 2011-2013 involved 543 samples taken from blood donors in Khartoum state/Sudan 299 (59%) sample were positive for toxoplasma by ELISA (Mohamed, 2013). For the importance of the possibility of the parasite transmission during blood donation process. The aim of this study is to detect the seroprevalence of *Toxoplasma gondii* antibody in healthy blood donors in Kirkuk city because routine screening for *T. gondii* in blood and blood products is not mandatory in the city.

Materials and methods

Population study: From 5 July to 1 Septmber 2013, a total of 91 serum samples (86male,5 femal) were collected randomly from Azadi teaching Hospital from healthy blood donors whom attended the blood bank. The questionnaire form was given to each male and female included: name, age, blood group, Rh.

Sample collection: 2-3 ml of venous blood was drawn carefully and transferred into disposable tube, the specimen was left for (15-30min) or put in water bath at 37˚C then centrifuged at 300 rpm for (5min) to separate clear serum, then the sera were tested, if not the sera were kept at (-20˚C) till use.

Enzyme linked immunosorbent assay for the detection of IgG and IgM antibodies of *Toxoplasma gondii* in human serum: Principle of the test: Purified *Toxoplasma gondii* antigen is coated on the surface of microwells. Diluted serum is added to the wells, and the *Toxoplasma gondii* IgG and IgM antibodies, if present, bind to the antigen. All unbound materials are washed away. HRP-conjugate is added, which bind to the antibody-antigen complex. Excess HRP-conjugate is washed off and a solution of TMB Reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG or IgM-specific antibody in the sample. The result is read by a microwell reader and is compared in a parallel manner with calibrator and controls.

Interpretation of results: *Negative: Toxo antibody index less than 0.90 is negative.*Equivocal: Toxo antibody index between 0.91-0.99 is equivocal. sample should be retested. *Positive: Toxo antibody index of 1.00 or greater is positive and this indicates the probability of current or recent toxoplasmosis (Biocheck, Foster City).

Statistical analysis: χ^2 (chi-square) test in style of independent and in style of homogeneous was used. The significant level used was $P< 0.05$(manually and not computerized analysis).

Results

From healthy blood donors in Kirkuk city, Table(1) show that among 91 serum samples examined by ELISA, 17 samples were positive with a rate of 18.6% and 74 samples were negative with a rate of 81.3%. No significant differences were appeared in the parasite incidence between each of males and females by using XL testing at $P<0.05$.

Table 1: Number and percentage of samples examined.

Sex	No. of samples examined	+ ve samples	%	-ve samples	%
Male	86	16	18.6	70	81.4
Female	5	1	20	4	80
Total	91	17	18.7	74	81.3
χ^2 value	Evaluated χ^2 value = 0.07, χ^2 value of $P< 0.05$ =3.84 (none significant**)				

The result of the current showed that the most female age group which were infected with the parasite was (35-38 years) with a rate of 100%(table 2).

Table2 :*Toxoplasma* prevalence according to age group in female.

Age group	Total No. examined	%	+ve No.	%	-ve No.	%
18-22	2	40	0	0	2	100
23-26	0	0	0	0	0	0
27-30	0	0	0	0	0	0
31-34	2	40	0	0	2	100
35-38	1	20	1	100	0	0
39-42	0	0	0	0	0	0
Total	5	100	1	20	4	80
χ^2 value	Evaluated χ^2 value =3.4,χ^2 value of P< 0.05 =11.1(none significant**)					

Table 3 shows that the most male age group which was infected with parasite was 43-46, 35-38with rates of 50, 33.3 % respectively, and 23-26 with a rate of 25%, followed by 18-22, 27-30 each with a rate of 16.6 % with significant differences between them.

Table 3 :*Toxoplasma* prevalence according to age group in male.

Age group	Total No.e xamined	%	+ve No.	%	-ve No.	%
18-22	12	13.9	2	16.6	10	83.3
23-26	16	18.6	×	25	12	75
27-30	18	20.9	3	16.6	15	83.3
31-34	9	10.4	0	0	9	100
35-38	12	13.9	×	33.3	8	66.7
39-42	8	9.3	1	12.5	7	87.5
43-46	×	4.6	2	50	2	50
47-50	×	4.6	0	0	×	100
51-54	3	3.4	0	0	3	100
Total	86	100	16	18.6	70	81.4
χ^2 value	Evaluated χ^2 value =8.2,χ^2 value of P< 0.05 =5.99(significant*)					

The relationship between the blood group antigen with *T.gondii* prevalence shows in table (4)shows the blood group A+ was the most significant blood group infected with *Toxoplasma* with a rate of 33.3%, followed by blood group AB+ with a rate of 20 %. No *Toxoplasma* positive samples were detected in all negative blood groups.

Table 4: Relationship between the blood group antigens and *Toxoplasma* prevalence.

Blood group	Total No. examined	%	+ve No.	%	-ve No.	%
A^+	24	26.4	8	33.3	16	66.6
A-	6	6.6	0	0	6	100
B+	14	15.4	2	14.2	12	85.7
B-	2	2.1	0	0	2	100
AB+	5	5.4	1	20	×	80
AB-	6	6.6	1	16.6	5	83.3
O+	26	28.5	5	19.2	21	8.7
O-	8	8.8	0	0	8	100
Total	91	100	17	18.7	74	81.3
χ^2 value	Evaluated χ^2 value =9.5, χ^2 value of P< 0.05 =5.99(significant**)					

From the ninety one serum samples examined 10.9 % was positive for IgG, and 5.5% was positive for IgM and the positive rate of both IgG and IgM was 2.2% (table 5). One female sample was positive for IgM antibody with rate of 20%, while the most significant male positive samples was for IgG antibody with rate of 11.6%, followed by IgM positive samples with rate of 4.6%. The significantly lowest one was for both of IgG and IgM with rate of 2.3%.

Table 5: Seroprevalence of *Toxiplasma* IgG and IgM in males and females

Sex	Total No. examined	Type of antibody					
		IgG+ve %	IgG-ve %	IgM+ve %	IgM-ve %	IgG+gM +ve %	IgG+IgM-ve %
Male	86	10 11.6	76 88.3	4 4.6	82 95	2 2.3	84 97.6
Female	5	0 0	5 100	1 20	4 80	0 0	5 100
Total	91	10 10.9	81 89	5 5.5	86 94.5	2 2.2	89 98.8
χ^2 value	Evaluated χ^2 value =3.9, χ^2 value of P< 0.05 =3.84 (significant*)						

Discussion

The present study evaluates the prevalence of toxoplasmosis in healthy blood donors in Kirkuk city by ELISA test. Blood transfusion is a potential transmission route for *Toxoplasma* infection; some studies suggest that toxoplasmosis transmitted through blood transfusion could lead to serious clinical consequences in immunocompromised, immunosuppressed patients and multiple blood transfusion recipients (Montoya and Liesenfeld, 2004).

The prevalence of *Toxoplasma gondii* in blood donors examined was 18.7%, This prevalence is much lower than that reported in Baghdad, the seropositive toxoplasmosis by LAT and ELISA were 136/400 (34%),121/400 (30.25%) respectively (Mahmood *et al*., 2013) and were 22 % (Al-Kaysi and Ali, 2010) while it was 13 (14.4%),25 (27.8%) for each of IgM and IgG respectively in Thi-Qar (Hadi, 2010). *Toxoplasma* infection in men and women were 32.9% and 27.0%, respectively in Prague, and of the Zbraslav hospital (Flegr, 2010). In the central

Mexican State of Jalisco (Galvan *et al*., 2005) the researchers found that 29% of blood donors were positive for anti-*T. gondii* antibodies, Compared to other studies, our overall seroprevalence was similar to the seroprevalence reported in Thailand (Pinlaor *et al*., 2000) but lower than that reported in blood donors from countries including Brazil, Chile (Coelho *et al*., 2003), Malaysia (Nissapatorn *et al*., 2002) and Egypt (Azab *et al*., 2012),where seroprevalences varied from 20.3% to 75.0%. but very lower frequency (3%) was found among Mosul blood donors (Al-Dabbagh, 2011). It is possible that differences in the characteristics of the blood donors and differences in the environments might contribute to explain the lower prevalence of *T. gondii* infection found in our blood donor population than those reported in blood donors from other areas.

The results of the current study refer to the possibility of *T. gondii* transfusion during blood donation process in both males and females, and that the seropositive cases are higher in men than they are in women, this may be due to the fact that a high number of blood donors are men. The number (5) of female donors in this study is much lower than that of male donors (86). Therefore, further studies are needed to elucidate risk factors associated with seropositivity in female donors. The prevalence of *T. gondii* according to the sex of the blood donors in present study is 18.6% for males and 20 % for females, no significant difference are
. detected on the incidence of toxoplasmosis between the two sexes, this is in agreement with (Nissapatorn, 2002; Coelho *et al*., 2003) who indicated that the seroprevalence of *T. gondii* infection in man rises with age and it does not vary greatly between sexes, but there are also studies that pointed either to higher incidence for males or for females (Nissapatorn *et al*., 2002, Al-Kaysi and Ali, 2010).
The most age groupsinfected with the parasite in this study are 43-46, 35-38 and 23-26 years old for men and 35-38 years old for women, this result agrees with that found in Mexico (Alvarado *et al*.,2007) they indicated that the infection will increase with age, and the age group of 45–60 years showed a significantly higher frequency of *T. gondii* infection. This also agree with the findings of others (Al-Dabbagh *et al*., 2011;Sundar *et al*., 2007; Loges, 2012; Coelho *et al*., 2003; Nissapatorn *at al*., 2002) whom confirmed that the parasite prevalence is concentrated in 18-50 years old among blood donors. This perhaps because the blood donors are limited to this age group, the younger and older ages are not allowed for blood donation.

For blood donors its they are more serious when they have IgM antibodies against *T. gondii* therefore, cases with an acute phase of infection should be excluded. Five of our blood donors had anti-*T. gondii*-specific IgM antibodies. IgM antibodies are detectable early after infection and can persist for prolonged times after infection (Montoya and Liesenfeld, 2004; Liesenfeld *et al*., 1997). IgM-positive donors with parasitemia may hold a potential for parasite transmission by blood transfusion. We were unable to judge whether a fraction of our blood donors might represent a risk group for parasite transmission by blood transfusion as reported previously. The rate of *T. gondii* IgM positive samples were much lower than that of IgG in this study, which agrees with others' results (Hadi, 2010; Chiang *et al*., 2012).
The frequency of *Toxoplasma* among Rh+ blood donors were higher, which agrees with (Al-Kaysi and Ali,2010; Novonta *et al*.,2008; Al-Shikhly *et al*., 2013). No *Toxoplasma* positive sample were detected in all negative blood groups, this may be due to the fact that the positive blood group (85%) is more common in human population than negative blood groups (15%) (Novonta *et al*., 2008). The A, B and O blood group phenotypes are determined by the presence or absence of A and/ or B carbohydrate antigens on the surface of red blood cells (Hakomori, 1999). There is a

possibility that parasite utilizes glycoconjugates, which characterize the blood phenotypes of the ABO blood group system, as potential receptors (Midfvedt and Vagge, 1989; Lopez *et al*., 1993; Kolbekova *et al*., 2007). The results of *Toxoplasma* prevalence in relation to blood group antigens had differed from a study to another. It is of a great interest that the current study scored an association between *Toxoplasma* infection and ABO blood group phenotypes with the highest prevalence of toxoplasmosis being exist among A^+ blood group (33.3%), AB+ (20%) and O+ (19.2%). In a study of Baghdad the highest seroprevalence was for O+ (28.8%) , A+, B+ and the lowest was for AB+ (10.3) (Alvarado *et al*.,2007).The result of current study nearly compatible with another study (Obid, 2014), which show that the highest prevalence of toxoplasmosis is found among A^+ blood group(66.6%). In an identical study of Baghdad, the rate of O+ blood donor toxoplasmasmosis was 64% followed by A+, B+ and the lowest was for AB+ (8%) (Ai-Kaysi and Ali, 2010). In Caucasian (Kolbekova *et al*., 2007) and Taiwan (Chiang *et al*., 2012) populations the carriers of AB group are the most susceptible to *T. gondii* infection. This discordant in the results may be attributed to the differences in the susceptibly of population races included in these studies, to the dissimilarities in sample sizes used, or to the limitation of studies regions.

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