Determination of Antibodies (IgG, IgM) against *Toxoplasma gondii* in Some Iraqi individuals by using ELISA technique

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Abstract:

A total of 258 voluntary blood donors (males 101; females 157) in the age range of 18-52 yr among males and 18-55 yr among females were examined for Toxoplasma gondii antibodies (IgG), and (IgM) by immunological technique (Enzyme linked Immunosorbant Assay) during the period from March 2009 to April 2010. This study covered a wide range of factors including immunological, age ,sex , place of residence and symptoms that may have a possible relationship with toxoplasmosis. Results presented in this study showed clearly that 38 (14.7%) of individuals participated in this study having IgG Toxoplasma Ab, among those 10 samples (9.9%) were males and 28 samples (17.8%) were females. Moreover, we found the prevalence of IgM seropositivity in the study population to be 5.8% ,as well as, the prevalence of IgM was 1.98 % in males and 8.3% in females. In addition to , the results of current study indicated that the seroprevalence of IgG Toxoplasma antibodies are more than IgM antibodies ,besides , the peak period range of toxoplasma gondii antibodies IgG among males donors was 30 to 39 years, while among female donors, the highest detection of *Toxoplasma gondii* antibodies IgG was between the ages of 40 to 49. What's more, the peak age range of *Toxoplasma gondii* antibodies IgM among males and females donors was 19 to 29 years. In conclusion , Our study showed a high prevalence of T. gondii antibodies in healthy voluntary blood donors. It may be appropriate to include screening test (ELISA) for T. gondii also in the pre transfusion blood testing schedule.

Key words : *Toxoplasma gondii*, antibodies (IgM voluntary blood donors . ,IgG) ,ELISA ,

Introduction:

Toxoplasma gondii is an obligate intracellular coccidian parasite with a worldwide distribution, the organism was first discovered in 1908 by Nicolle and Manceaux [1,2] . Serological data indicates that approximately 30% of the population of most developed nations are chronically infected with the organism and the prevalence varies among different populations [3,4]. Infection with T. gondii is asymptomatic in the majority of cases and sometime may be accompanied by fever, malaise and lymphadenopathy, while the infection in immunocompromised patients may develop as disseminated toxoplasmosis or toxoplasmic encephalitis , or both and this infection is often fatal[5,6]. When a sero-negative women become infected with *T. gondii* during pregnancy, the organism is often transmitted across the placenta to the fetus[7]. Acute infection during the first trimester lead to a severe disease that results in a spontaneous abortion, still birth , or death in utero , sometimes it leads to neurological

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lesions . Late trimester infections are more frequent but tend to be subclinical at birth[8]. However, Walker *et al.* [9] reported that most of the neonates (70% of cases) are asymptomatic at birth, but clinical symptoms may develop later in life.

A variety of serological tests for T.gondii antibodies have been used as an aid in diagnosis of acute infection and to assess previous exposure to the organism . The tests include the Sabin Feldman Dye Test (SFDT), Direct Agglutination Test(DT), Indirect Haemagglutination Test (IHAT), Enzyme Linked Fluorescent Assay (ELFA), Indirect Immuno Fluorescence Antibody Test(IFAT) and Enzyme Linked Immunosorbant Assay (ELISA) [10,11] . Screening for antibodies (IgM and IgG) to *Toxoplasma* in blood and blood product is not mandatory in Iraqi. This poses great risk to the recipients of blood and blood products, especially large volumes at multiple sittings. A study was therefore carried out to determine the antibodies (IgG, IgM) against *Toxoplasma gondii* in the blood collected from healthy voluntary donors by using ELISA technique and to update the available information on the serological prevalence of T. gondii infection in some Iraqi individuals .

Material and Methods:

Blood (5 ml) was collected from 258 voluntary blood donors at the blood donation in National Center for Blood Transfusion/Baghdad between March 2009 to April 2010 . There were 101 males and 157 females. Their age range was 18-52 yr for males and 18-55 yr for females. The donors were healthy adults, screened routinely by physical examination with no history of infections in the recent past. The demographic details noted were age, sex and residence. All collected serum samples were separated and preserved at -20^{o} C until being examined. The antibodies (IgG/IgM) in patients were measured by ELISA technique according to the manufacturer's instructions [12,13].

Enzyme linked immunosorbent assay for determination of IgM antibodies directed against T. gondii in humans serum .

Procedure

1. Diluting wash buffer and conjugate.
2. Diluting samples 1/100 using the Dilution buffer.
3. Adding 100µL of each control or diluted sample to each well.
4. Incubation for one hour at 37Co in a humid chamber.
5. Discarding well contents and wash 5 times.
6. Adding 100µL of diluted conjugate into each well.
7. Incubation for one hour at 37Co in a humid chamber.
8. Discarding well contents and washing 5 times.
9. Adding 100µL substrate solution to each well.
10. Incubate in the dark for 30 min at room temperature.
11. Adding 100µL of stop solution to each well.
12. Reading the optical densities with an EIA reader using a 450 nm [(12)] .

Enzyme linked immunosorbant assay for determination of IgG antibodies directed against T. gondii in human serum.

Procedure

1- Diluting calibrators and negative control 1:5 with sample diluent , diluting sample 1:505 with sample diluent .

2- Dispense reagents into the strip wells according to follow scheme, leaving an empty well for the blank .

3- Incubation for one hour at 37Co .

4- Aspiration the liquid . Repeatedly wash with wash buffer .

5- Dispense 100µL working enzyme tracer into all wells, except for blank well .

6- Incubation for one hour at 37C^{o} .

7- Aspiration the liquid. repeatedly wash with wash buffer .

8- Dispensed 100µL chromogen/ substrate into well except for blank well.

9- Incubation for 30min at room temperature, in the dark.

10- Dispense 200µL blocking reagent into all wells except for blank well.

11- Then read absorbance values with a photometer at 450 nm [13] .

Results and Discussion:

Results presented in this study showed clearly that 38 (14.72%) of individuals participated in this study having IgG anti-Toxoplasma antibody, among those 10 samples (9.1%) were male and 28 samples (17.83%) were female (Table 1). Moreover, we found the prevalence of IgM seropositivity in the study population to be 5.81% (15/258),as well as, the prevalence of IgM was 1.98 % in males and 8.28% in females. This result is in alliance with Carmen *etal.*, [14] who examined 508 serum samples of healthy blood donors IgG by IHAT and ELISA and toxoplasma IgM by ELISA and the results showed toxoplasma IgG ranging between 19.1. % (IHAT) and 18.2. % (ELISA), while IgM (ELISA) was 6.9% .

Table1 : Seroprevalence of specific immunoglobulin type G and M against *T .gondii* among research individuals .

Sex	No.	IgG Positive No.(%)	IgM Positive No.(%)
Male	101	10 (9.1 %)	2 (1.98 %)
Female	157	28 (17.83 %)	13 (8.28 %)
Total	258	38 (14.72%)	15 (5.81%)

The results of this study indicated that the overall prevalence of IgG Toxoplasma antibodies (14.72%) are more than IgM Toxoplasma antibodies (5.81 %) ,these findings 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.

The peak age range of toxoplasma gondii antibodies IgG among males donors was 30 to 39 years; followed 40 to 49 years. Among the female donors, the highest detection of Toxoplasma gondii antibodies IgG was between the ages of 40 to 49, followed by 30 to 39 years. The lowest seropositivity was in the age group between 19-29 years among males and > 50 among females (table 2). In accordance with other studies, we observed a correlation between increasing prevalence of antibodies against T. gondii and increasing age of blood donors [15,16, 17].

Table2: Seroprevalence of specific immunoglobulin type G to T.gondii according to age and sex.

Age groups (years)	Sex	No.	IgG-positive (No.)	IgG-positive (%)
19<	Male	9	---	---
	Female	4	---	---
19-29	Male	33	3	9
	Female	46	4	8.6
30-39	Male	45	6	13.3
	Female	63	14	22.2
40-49	Male	10	1	10
	Female	30	9	30
50>	Male	4	---	---
	Female	14	1	7.1

Additionally, The peak age range of *Toxoplasma gondii* antibodies IgM among males and females donors was 19 to 29 years (table 3), which mean that they have recently been exposed to the parasite. Similar finding for Toxoplasma IgM antibodies determination have been reported by some studies [15, 16, 17, 18].

Table3 : Seroprevalence of specific immunoglobulin type M to T.gondii according to age and sex .

Age groups (years)	Sex	No.	IgM-positive (No.)	gM-positive (%)
19<	Male	9	---	---
	Female	4	---	---
19-29	Male	33	1	3
	Female	46	5	10.8
30-39	Male	45	1	2.2
	Female	63	6	9.5
40-49	Male	10	---	---
	Female	30	2	6.6
50>	Male	4	---	---
	Female	14	---	---

The prevalence of toxoplasmosis can vary among different groups, and these differences have also been seen between rural and urban regions. Some studies determined a higher prevalence in rural regions[19,20,21], while others did not show any difference between urban and rural inhabitants [22,23] . In our study group, we found difference in the seroprevalence of toxoplasmosis between males and females among urban and rural residence (table 4). In general, the differences in the exposure rate to Toxoplasma infection between rural and urban population within a country may reflect the socio-economic differences and according to social and cultural habits, geographic factors, climate, and transmission route.

Table 4: IgG and IgM seropositivity in adult blood donor according to place of residence.

Place of residence	Sex	No. of individuals	IgG-Positive No.(%)	IgM-Positive No.(%)
Urban	Male	71	6(8.45 %)	----
	Female	102	15 (14.7%)	5 (4.9 %)
Rural	Male	30	4(13.3%)	2 (6.6 %)
	Female	55	13 (23.6 %)	8 (14.5%)

In clinical symptomatology of serologically IgG positive patients, lymphadenopathy was found in 27.8 % with or without other symptoms (influenza, arthralgia, fever, fatigue) ,on other hand the positive cases of IgG Ab in females had previous abortion was found in 25.0 % (Table 5).Additionally , prevalence of clinical symptoms in serologically IgM positive adult blood donors , lymphadenopathy was found in 15.38 % with or without other symptoms (influenza, arthralgia, fever, fatigue),moreover the positive cases of IgM Ab in females had previous abortion was detected in 7.69 % (Table 6).

Serological surveys indicate that about 80% of all primary infections are asymptomatic, due to the effectiveness of the immune system [24, 25]. The tissue parasitism during the proliferative phase may occur without symptoms. It may lead to a transient illness characterized by lymphadenopathy, fever, fatigue, arthralgia, dermatosis, malaise, headache, and myalgia. Latent toxoplasmosis, i.e., the lifelong presence of cysts and anamnestic concentrations of anti-T. gondii antibodies in immunocompetent subjects, is considered asymptomatic and harmless [26] .

Table 5: Prevalence of clinical symptoms in serologically IgG positive adult blood donors

Symptoms	Sex	No.	Percent (%)
Fever	Male (N= 10)	2	20
	Female (N=28)	4	14.28
lymphadenopathy	Male (N= 10)	1	10
	Female (N=28)	5	17.8
influenza	Male (N= 10)	4	40
	Female (N=28)	6	21.42
Previous abortion	Male (N= 10)	---	---
	Female (N=28)	7	25
Arthralgia	Male (N= 10)	2	20
	Female (N=28)	3	10.71
Fatigue	Male (N= 10)	1	10
	Female (N=28)	3	10.71

Table6: Prevalence of clinical symptoms in serologically IgM positive adult blood donors

Symptoms	Sex	No.	Percent (%)
Fever	Male (N= 2)	1	50
	Female (N=13)	3	23.07
lymphadenopathy	Male (N= 2)	---	---
	Female (N=13)	2	15.38
influenza	Male (N= 2)	1	50
	Female (N=13)	2	15.38
Previous abortion	Male (N= 2)	---	---
	Female (N=13)	1	7.69
Arthralgia	Male (N= 2)	----	----
	Female (N=13)	3	23.07
Fatigue	Male (N= 2)	----	----
	Female (N=13)	5	15.38

In conclusion, our findings showed a high seroprevalence of *T. gondii* in healthy voluntary
donors in Iraqi. Routine screening of blood for *T. gondii* antibodies may be considered to be included in the battery of serological screening tests to detect endemic infectious diseases in our country, as well as, it may be suitable to include screening investigation (ELISA) for T. gondii also in the pre transfusion blood testing schedule.

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قياس الأجسام المضادة (IgM وIgG) ضد المقوسات القندية في بعض الأشخاص العراقيين باستخدام تقنية الأليزا

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الخلاصة:

ما مجموعه 258 متبرّع بالدم طوعيين (ذكور 101، إناث 157) في مدى عُمرَ 18-52 سنة للذكورِ و18-55 سنة للإناث ، خضعوا لقياس الأجسام المضادة (IgM وIgG) ضد المقوسات القندية باستخدام التقنية المناعية (الأليزا) في أثناء المدةِ مِنْ مارس/آذارِ 2009 إلى أبريل/نيسانِ 2010. هذه الدراسةِ غَطّتْ تشكيلة واسعة من العواملِ التي تضمنت المناعة والعُمر و الجنس و مكان السكنِ والأعراضِ التي لَرُبَّما لَهُا علاقة محتملة مَع Toxoplasmosis. أظهرت نَتائِج هذه الدراسةِ بشكل واضح بأنّ 38 (14.7 %) مِنْ الأفرادِ الذين شاركَوا في هذه الدراسةِ كان لديهم IgG Toxoplasma ، بين تلك العيناتِ كان نحو 10 عينات (9.9 %) من الذكورَ و28 عينةَ (17.8 %) من الإناث كان لديهم IgG Toxoplasma. فضلا عن ذلك، أوضحت الدراسة بأن انتشار IgM Toxoplasma في مجموعة الدراسة نحو 5.8 %، فضلا عن ذلك كان انتشار IgM 1.98 % في الذكورِ و8.3 % في الإناث. من جهة أخرى أشارتْ نَتائِج الدراسة الحاليةِ إلى إن الانتشار المصلي للأجسام المضادةِ نوع Toxoplasma IgG أكثر مِنْ أجسام IgM ، فضلا عن ذلك ، سجلت أعلى موجبيه للأجسام المضادةِ نوع Toxoplasma IgG بين الذكورِ المتبرعين في المدى العمري الممتد من 30 إلى 39 سنةِ، بينما كانت بين المتبرعات الإناث في المدى العمري الممتد من 40 إلى 49 سنةِ ، ومن جانب أخر سجلت أعلى موجبية للأجسام المضادةِ نوع Toxoplasma IgM بين الذكورِ والإناث المتبرعين في المدى العمري الممتد من 19 إلى 29 سنةِ. بوصفها نتيجة نهائية أظهرت نتائج الدراسة وجود انتشار عالي لأجسام gondii المضادة في الأشخاص الأصحاء المتبرعين بالدم ولذلك يكون من الملائم تضمين فحص التحري(الأليزا) عن المقوسات القندية في جدول الفحوصات التي تجري على دم المتبرع قبل عملية النقل.