SEROPREVALENCE OF ANTI - TOXOPLASMA ANTIBODIES AMONG WOMEN OF CHILD BEARING AGE IN DUHOK PROVINCE

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ABSTRACTS

A total number of 1146 blood samples taken randomly from single and married women at child bearing age of different ages, different socioeconomic and educational classes in Duhok city. They were examined to detect anti-toxoplasma IgG and IgM Abs by using LAT and ELISA tests. The rate of seroprevalence was 38.7 % by using LAT, while it was 27.7 % by using ELISA IgG. On the other hand, the rate of anti-toxoplasma IgM Abs was only 0.4 % by using ELISA. Significant differences were observed in rate of seropositivity between single and married women, and also between various age groups, the highest rate (45.3%) was observed among the age group 31-35 years.

Also significant seropositive rates were observed between all educational levels with the highest rates (36.4% and 34.2%) being among illiterate and house wives, respectively. Contact with cats or eating undercooked meat has no effect on seropositivity rate. Regarding married status, pregnant women showed highest seropositivity (49.7%) especially at first trimester of gestation.

KEYWORD: *Toxoplasmosis, Women childbearing age, IgG , IgM and LAT-ELISA.*

INTRODUCTION

Toxoplasma gondii is an obligatory intracellular protozoan parasite which appears to have broad host specificity. Cats and wild Felines are the only definitive hosts, while humans and all other warm-blooded animals are intermediate hosts (Remington *et al*., 2001). It has a heteroxenous life cycle with two infectious stages, namely the tissue cysts and oocysts. Cats play a major role in spreading the oocysts in the environment (Dubey *et al*., 1998 and Tenter *et al*., 2000).

Infection is acquired by ingestion of viable tissue cysts in meat or sporulated oocysts excreted by cats that contaminate food or water (Montoya and Liesenfeld, 2004).Toxoplasmosis also can be acquired congenitally (transplacental transfer of tachyzoites), primarily are acquired if the pregnant mother becomes infected throughout the pregnancy or within six months prior to gestation (Jenum *et al*., 1998 and Jones *et al*., 2003). Horizontal transmission of toxoplasmosis occurs after ingestion of undercooked meat containing bradyzoites (tissue cysts), unwashed vegetables, fruits or drinking water contaminated with oocysts (Tenter *et al*., 2000).

In Duhok there are limited studies regarding the prevalence of toxoplasmosis such as Al-Doski (2000) which involved seroprevalence of toxoplasma antibodies among two groups of population, and the second study was performed by Razzak *et al*.,(2005) in this study they indicated that the contribution of toxoplasmosis to fetal loss was greatly overestimated .These studies did not give a full evaluation of the situation of toxoplasmosis in this part of Kurdistan, therefore the present study was undertaken to know : The prevalence of anti – toxoplasma Antibodies in women of child bearing age by using both latex agglutination test and ELISA for detecting both toxoplasma antibodies (IgG and IgM), and their evaluation.Its relationship with age, marital status, contact with cat, history of abortion and other miscarriages related with gestation. Follow up of some seropositive cases of anti – toxoplasma Abs among pregnant women till the end of third trimester of gestation.

MATERIALS AND METHODS

1. Materials

During this study, 1146 blood samples were collected from women of child bearing age randomly for screening anti-toxoplasma specific antibodies from various parts of Duhok city, some nearby villages and compilers in Zakho, Semil, Sharia, Domis as indicated in Table bellow from November 2009 to August 2010. Data about age, marital status, contact with cat, history of abortion and other miscarriages related with gestation were obtained on special questionnaire form along with each blood sample.

Place	No. of sample	Single	Married
Duhok quarters	739	283	456
Nearby villages	83	20	63
Zakho,Semil,Domis,Sharia	324	30	294
Total	1146	333	813

2. Methods

Serological tests

Firstly, the collected samples were checked for both immunoglobulin types IgG, IgM by using latex agglutination test, and then followed by ELISA IgG and IgM tests to increase the accuracy of the diagnosis.

The results were analyzed using (Graph Pad Prism program) for Chi-square test (and Fisher's exact) for comparison between positive and negative results as mentioned by Milton and Tsokos (1983).

RESULTS AND DISCUSSION

It is obvious from Table (1) that the prevalence rate of toxoplasmosis by using LAT was 38.7% and by using ELISA was 27.7%. On the other hand, the prevalence rate of IgM Abs was only 0.4%. These differences were statistically highly significant ($p<0.001$).

Table (1): The prevalence of anti-toxoplasma Abs among the studied samples by using both LAT and ELISA tests (n= 1146).

Test	No. of seropositive	% of seropositive
LAT	444	38.7
ELISA IgG	317	27.7
ELISA IgM	5	0.4

x^2= 514.3* df = 2 p< 0.001* Significant

The high rate of prevalence of anti-toxoplasma Abs recorded in this study by using LAT is somewhat similar to what have been observed by: Al-Doski, (2000) in Duhok, also he recorded a rate of 41.9 %. Much higher rates of anti-toxoplasma Ab by using the same test which were 84 % and 54.46 % in Erbil have been reported by Akreyi, (2008) and Hamad, (2009) respectively.

During present study only five samples (0.4%) were positive for IgM Ab by ELISA method, this result agree with those of Razzak *et al.*, (2005) who also reported low prevalence rate (0.97%) of anti-toxoplasma IgM antibody in Duhok Province.

The prevalence rate of seropositive among married women was 33.3% which was higher than that of single females (13.8%)(Table.2). Statistically this difference was highly significant ($p<0.001$). Similarly higher rates of anti-toxoplasma Abs in married women and lower rates in single have been reported in Mosul by Al- Khaffaf (2001) and Al- Delamy (2002).

Table (2): Prevalence of anti-toxoplasma IgG and IgM Abs among single and married women by using ELISA test (n = 1146).

Marital status	No. of samples	No. of seropositive		% of seropositive	
		IgG	IgM	IgG	IgM
Single	333	46	1	13.8	0.3
Married	813	271	4	33.3	0.5
Total	1146	317	5	27.7	0.4

X^2 = 44.98 * df = 1 p< 0.001 * Significant

The high rate of seroprevalence of IgG Ab in married women may be due to more responsibility of married women about, gestation, birth and child rearing which affect their health and lower their immunological status which make them more susceptible to infection (Al- Delamy, 2002).

The distribution of seropositive according to age is shown in Table (3). It is clear from the results that the age group 31 - 35 years showed the highest rate of seropositive which was 45.3%, while the lowest rate 14.6 % was among the ages 16 - 20 years .Statistical analysis revealed the presence of highly significant difference (P< 0.001) between them.

Table (3): The distribution of anti-toxoplasma IgG and IgM Abs among various age groups by using ELISA test (n= 1146)

Age group	Samples number	No. of seropositive		% of seropositive	
		IgG	IgM	IgG	IgM
(16 - 20)	313	46	2	14.6	0.6
(21 - 25)	244	63	0	25.8	0
(26 - 30)	253	81	3	32	1.2
(31 - 35)	128	58	0	45.3	0
(36 - 40)	108	36	0	33.3	0
(41 - 45)	100	33	0	33	0
Total	1146	317	5	27.7	0.4

$X^2 = 52.19^*$ df= 5 $p<0.001^*$ Significant

These results contradict with those obtained by Al-Doski,(2000) in Duhok; Hamad, (2009) in Erbil; Al-Najjar, (2005) and Al-Harbi, (2009) in Mosul city, they reported that the prevalence rate of anti-toxoplasma Abs increased with the age, as older people were more exposed to the sources of infection than younger age groups, therefore, their chance of infection will be increased. On the other hand some researchers found the higher rate of infection among the ages 20-24 years (Al-Khaffaf, 2001; Al-Delamy, 2002) in Mosul.

Table(4) illustrate the relationship between contact with cats and seropositive. It is obvious from the table that the prevalence among women with direct contact with cats was 30.8 % which was slightly higher than those without direct contact with cats (25.7 %) and this difference was statistically non significant.

Table (4): The distribution of anti-toxoplasma IgG and IgM Abs according to contact with cats by using ELISA test (n= 1146)

contact with cat	No. of samples	No. of seropositive		% of seropositive	
		IgG	IgM	IgG	IgM
Yes	428	132	3	30.8	0.7
No	718	185	2	25.7	0.2
Total	1146	317	5	27.7	0.4

$X^2 = 3.452$ df = 1 Non significant

The results of the present study are similar to that have been observed by Al-Doski (2000) in Duhok; Al-Najjar (2005) and Al-Harbi (2009) in Mosul. All of these researchers also did not reported any significant difference in prevalence of anti-toxoplasma Abs between women with direct contact with cats and others. This could be attributed to the fact that now cats are not used for hunting mice, and they spend most of their time outside houses and are not allowed to enter kitchens (Al-Doski, 2000).

The distribution of anti-toxoplasma IgG and IgM Abs according to the history of abortion is shown in Table (5), it is obvious from the Table that the percent of seropositive for anti-toxoplasma IgGAb among aborted women was 40 % but it' s rate from total number of seropositive married women is 45.02 %. This percent is relatively high as compared with women who did not have such history (29.3 %). Statistical analysis of the results showed the presence of significant difference ($p<0.01$) between abortion and seropositive.

Table (5): The distribution of anti-toxoplasma IgG and IgM Abs according to the history of abortion by using ELISA test (total number of married women = 813).

Status	No. of samples	No. of seropositive		% of seropositive	
		IgG	IgM	IgG	IgM
Aborted women	305	122	2	40	0.7
Non aborted	508	149	2	29.3	0.3
Total	813	271	4	33.3	0.5

$X^2 = 9.762^*$ df = 1 $p < 0.01$ * Significant

These results agree with those of Ali (2007) who reported that 40% of aborted women placenta were positive by mouse inoculation in Kalar. Akreyi (2008) in Erbil examined histological sections of aborted fetal placenta and found association between *T. gondii* and abortion, furthermore, Abdullah (2009) in Erbil also reported the presence of strong relation between abortion and toxoplasmosis in which the rate was 74.46 % and he further added that there is a little knowledge about the rate of infection with *T. gondii*, maternofetal transmission and abortion due to lack of molecular diagnose in Iraq.

The distribution of clinical cases associated with toxoplasmosis in married women (Table 6) showed a seropositive rate of 45.02 % among women aborted their babies, 3.32% among those delivered dead babies, and 51.7 % among those delivered normal babies. Statistical analysis showed the presence of highly significant difference ($p < 0.001$) among these cases.

Table (6): The distribution of clinical cases associated with toxoplasmosis among married women

Clinical cases associated with married women	No. of samples	No. of seropositive		% (from total positive)	
		IgG	IgM	IgG	IgM
Aborted women	305	122	2	45.02	50
Women with dead baby (Still birth)	13	9	0	3.32	0
women with normal pregnancy	495	140	2	51.7	50
Total	813	271	4	100	100

$X^2 = 19.32$ df = 2 $p < 0.001$* Significant

Since, there is no such study in Kurdistan region it is not possible to compare the results. Pratlong *et al*. (1996) reported that most of fetal infection which result in stillbirth, miscarriage is due to the fetus infection with toxoplasmosis at firsttrimester which causes severe neurologic diseaseespecially in brain lead to the damage of its cells or the damage may happen in another organs as heart . But there is no clinical signs (Sub clinical) may observe in neonate when infection occurs at second and third trimesters of gestation.

Table (7) show the follow up of seropositive cases of toxoplasmosis until the end of gestation, as it is clear from table that one case had positive IgG and IgM at first trimester, after taking a course of spiramycin, she recovered with normal baby at birth .Another case which was positive for IgG Ab and negative for IgM Ab at all trimesters, she delivered a dead baby. Four positive cases of IgG Ab and negative IgM ended with abortion, three of them at first trimester and one at second trimester. Four positive cases of IgG Ab and negative IgM completed their gestation with normal neonates. These miscarriages may be due to other reasons like bacterial or viral infection. It is not possible to compare the results, since there are no previous studies in this direction.

Table (7) Follow up of some seropositive cases of Toxoplasmosis

Cases	First trimester					Second trimester					Third trimester					Note
	1	2	3	IgG	IgM	4	5	6	IgG	IgM	7	8	9	IgG	IgM	
Case one			***	4.3	1	***			2.8	0.2	***			3	0.4	Normal baby
Case two	***			3	0.4	***			1.4	0.3						Dead baby
Case three	***			3.5	0.4											Abortion in third month
Case four	***			3.4	0.1											Abortion in third month
Case five		***		4.1	0.1											Abortion in second month
Case six	***			3.2	0.2											Abortion in fifth month
Case seven	***			3.6	0.8						***			1.8	0.6	Normal baby
Case eight		***		3.9	0.4							***		1.5	0.2	Normal baby
Case nine								***	2.5	0.2			***	2	0.1	Normal baby
Case ten		***		1.6	0.2						***			2	0.3	Normal baby

Note : *** refers to which month that test was done

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الخلاصة

تم جمع (1146) عينة دم بصورة عشوائية من الاناث الباكرات والنساء المتزوجات في سن الانجاب ومن مستويات عمرية واجتماية واقتصادية وثقافية مختلفة في مدينة دهوك وبعض القرى والمجمعات السكنية القريبة .تم التحري عن الأضداد (IgG) و(IgM) باستخدام اختبار تلازن اللاتكس(LAT) واختبار ارتباط الأنزيم للأدمصاص المناعي (ELISA). بلغت نسبة الأصابة الكلية عند استخدام تلازن اللاتكس (38.7 %)بينما بلغت نسبة الأصابة عند استخدام (ELISA - IgG) (27.7%) وبلغت نسبة الأصابة باستخدام (ELISA - IgM) (0.4 %). وجدت فروقات معنوية واضحة جداً في نسبة الأصابة بين الاناث الباكرات والمتزوجات وكذلك بين المجاميع العمرية المختلفة ، وجدت اعلى نسبة اصابة (45.3 %) في الفئة العمرية 31-35 سنة.

وجدت فروقات معنوية واضحة ايضا في نسبة الاصابة بين النساء من مختلف المستويات الثقافية والوظيفية حيث سجلت اعلى نسبة اصابة عند النساء الأميات وربات البيوت والتي بلغت 36.4 % و 34.2 %على التوالي. وجدت فروقات غير معنوية في نسبة الأصابة بين النساء اللواتي يكنَّ في تماس مباشر مع القطط واللواتى تتناولن اللحوم غير المطهية بصورة جيدة مقارنة بغيرهن .بالنسبة للنساء المتزوجات سجلت النساء الحوامل اعلى نسبة اصابة (49.7 %) وخاصة عند الأشهر الثلاثة الأولى.