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Serological and molecular diagnosis of toxoplasmosis in pregnant women in Omdurman, Khartoum state

Satti, A.B.[1]; Suliman, H.[2]; Suad, B.M.[3]

1 Department of Parasitology, Omdurman Islamic University, Khartoum-Sudan
2 Department of Parasitology, Uof K, Khartoum-Sudan
3 Department of Obstetrics and Gynecology, Al zaeem Al Azhari University, Khartoum-Sudan

Abstract:

This study was conducted to determine the sero-diagnosis and risk factors of *toxoplasmaosis* among pregnant women in Omdurman, Khartoume state, during the period of June 2006 to July 2008. The study included 455 pregnant women between 25 – 35 years of ages.

The blood samples were collected and examined by direct latex agglutination, ELISA IgG and IgM and ten by PCR test. All serological investigations were carried out in the center for research and laboratory sciences, Faculty of Medicine, Ain Shams University, Egypt. Data were collected from the pregnant women by interview using modified questionnaire.

The results showed that the prevalence of *Toxoplasma gondii* was 17.8% by direct latex agglutination, 38.9% by ELISA IgG (indicating chronic infections) and 12.9% by IgMindicating acute infections. The results of PCR test showed 16.7%. Although of questionable accuracy, the results of the present study revealed a relatively high sero-prevalence of acute infection in the pregnant women, with *Toxoplasma*.

Introduction:

Frankel et al [(1)] reported that *Toxoplasma gondii* was first discovered by Nicolle and Manceaux in (1908), Hutchison in (1965) isolated *Toxoplasma* oocysts from cats.

Toxoplasma gondii is a worldwide polyxenous intracellular coccidian parasite in which cats are known to be the definitive hosts and probably all warm blooded animals and man are intermediate hosts. Human toxoplasmosis may occur as an acquired infection or as a congenital infection in infants of infected mothers. Intra-uterine infection with *Toxoplasma gondii* can cause severe and often fatal cerebral damage to the fetus. *Toxoplasma gondii* is serving as source of infection to humans and animals and usually leads to economic losses due to miscarriage. Although, occasionally, *Toxoplasma* can be isolated in smears of lymph nodes, bone marrow and other tissues, diagnosis of toxoplasmosis in man and also in animals relies entirely on detection of circulating antibodies by serological tests.

The risk of infection with *Toxoplasma gondii* can be reduced by avoiding ingestion of contaminated food and by avoiding contact with infected and / or stray cats.

Toxoplasmosis in Sudan:

The first report of human toxoplasmosis dates back to 1966 when Carter and Fleck [(2)] studied four types of population from different parts of Sudan, using the Dye test. They reported a seroprevalence rate of 22% in Kordofan and Darfur and southern provinces, whereas in the

northern provinces, the seroprevalence rate was as high as 70%. The difference in the prevalence rates is thought to be due to racial habits which may affect the transmission of toxoplasmosis [(2)].

An overall prevalence rate of 41.7% was reported by Abd Elhameed [(3)] among the residents of Gezira province aged 10 years and above using IgG ELISA, ISAGA and latex agglutination test. In the study made by Carter and Fleck [(2)] among the residents of Khartoum and Gezira, excluding children under 10 years, the overall *Toxoplasma gondii* seroprevalence rate was reported to be as high as 72.8%. The prevalence rate reported by Abd Elhameed [(3)] among females aged 20-49 years was significantly higher than that of males of the same age; a percentage of 44.8 and 39.1, respectively. Exposure of females to *Toxoplasma gondii* cysts in uncooked meat during food preparation, and consumption of raw liver, and Mararra (raw viscera of herbivores) are suggested to be the attributable factors for the high prevalence rate of *Toxoplasma gondii* infection among females.

Another *Toxoplasma gondii* seroepidemiological study among pregnant women was made by Elnahas [(4)]. He detected an obvious increase in IgG seroprevalence status with low levels of education and socioeconomic status. The socio cultural habit of eating raw liver and marrara was found to be an important risk factor for acquiring *Toxoplasma gondii* infection. As cats are not popular pets among Sudanese populations, the study has shown no significant correlation between cat contact and transmission of *Toxoplasma gondii* infection. *Toxoplasma gondii* IgG seroprevalence was found to be not significantly correlated with women with history of miscarriage, preterm labor, low birth weight and congenital anomalies. He concluded that, serologically, toxoplasmosis is not uncommon among Sudanese pregnant women; and recommended screening and follow up programmes as well as health education.

Materials and methods:

Study area: Samples collection was carried out during the period June 2006-July 2007 at different obstetric and gynecology department in different hospitals in Khartoum state. A total of 455 out patients, were screened. Of this number, 230 were checked at Omdurman center which in located in Wad Nubawi, Omdurman and 120 were examined at the friendships hospital and 105 at Omdurman Saudi hospital.

Study population: The target group comprised women presented to the hospital with history of miscarriage and pregnant women at various stages of pregnancy who were attending the antennal clinic. Total of 455 samples were examined for presence of anti-*Toxoplasma* antibodies using direct latex agglutination test and 180 samples were examined by ELISA IgG, IgM and PCR test.

Ethical clearance: Ethical approval of the study taken from the Research Committees, Omdurman Islamic University.

Samples collection and preservation:

* Blood samples were collected from both aborted and normally delivered women using direct latex agglutination to screen *Toxoplasma*.

* 5ml of venous blood was collected from all the study and control individuals. Samples were

allowed to clot at room temperature and centrifuged of 3000 rpm to separate the sera. The sera were kept frozen at -20C° for ELISA test.

* Blood samples were also collected from aborted women for molecular studies.

All the tubes were labeled with the name of the patient and the date of collection.

All tests including ELISA test, DNA extraction, and genetic analysis were performed at the serology and molecular biology laboratory/ medical research center –faculty of medicine –El Neelin university, and medical research center – faculty of medicine- Ain Shams University. Cairo-Egypt.

Methodology: Three techniques were used in this study; these are:

1- Screening assays which was carried firstly as a direct latex agglutination test for detection of *antitoxoplasma* antibodies. The commercial kits produced by Spin-react, (Girona) Spain were used.

2- Enzyme linked Immuno Sorbent Assay (ELISA) was used for detection of IgG and IgM.

3- Polymerase chain reaction (PCR) was used for detection of affecting of toxoplasmosis among pregnant women. The primers used were B1F1 (5'GGAACTGCATCCGTTCAT GAG 3') B1R1 (5'TCTTTAAAGCGTTCGTGGTC3')(TIB-MOLBIOL -Berlin). The primers correspond to the *Toxoplasma gondii*. All PCRs were performed in Gene Amp ® PCR System 9700.

Statistical analysis: SPSS program was used to analyze the demographic and clinical data.

Results

Total population: The results showed that out of 455 women screened by latex agglutination test 72 (17.8%) were found to be sero-positive (table1).

Table 1: The prevalence rate of anti – Toxoplasma antibodies, in all study groups as examined by latex agglutination test:

Group tested	No. examined	No.+ ve	Percentage
Study	405	72	17.8%
Control	50	8	16.0%
Total	455	80	16.9%

Seropositivity in age groups: The percentage of seropositive cases detected by ELISA test ranged between 22 – 27% in women when ages were 15 to 35 years ,the percentage of seropositive cases in 35- 39 years women reached 15.4% .The rate in over 40 years was low as it did not exceed 10.0% (table 2).

Table 2: Percentage of seropositivity (S.P) and sero prevalence (S.R) rate by age group:

Age group in years	No .study groups	S.P (+ve)	S.R
15-25	43	9	21.1%
25-35	168	41	22.9%
35-39	13	2	15.4%
>40	10	1	10.0%
Total	230	53	26.1%

S.P=Seropositivity
S.R=Seroprevalence rate

Association between sero – prevalence status and obstetrical history:

Out of 104 recently aborted women, 22 (21.2%) showed positive anti-*Toxoplasma* antibodies by ELISA test. Out of 50 controls, the sero-positivity was 8 (16%) (Table3.12-Fig3.17). There was no significant difference between them (x 2= 9.43, P. value 0.0021)

Table 3: Comparison between prevalence of anti- *Toxoplasma* antibodies in women with history of miscarrage and control examined by ELISA test:

Group tested	**No. examined**	**No. +ve**	**Percentage**
miscarriage	104	22	21.2%
controls	50	8	16.0%
Total	154	30	19.5%

Association between Toxoplasmosis and pregnancy:

The prevalence rate of anti- *Toxaplama* antibodies was determined by ELISA test in pregnant women who had no previous history of miscarriage. Out of 51 pregnant women 24 (47.1%) were positive .In controls (non-pregnant women), 8(16%) out of 50 were found positive. There was no significant difference between the two groups (x = 2.25, p value 1.339) (table 4).

Table 4: The rate of anti- *Toxaplama* antibodies in pregnant women and controls obtained by ELISA test:

Group tested	**No. examined**	**No.+ve**	**Percentage**
Pregnant women	51	24	47.1%
Controls	50	8	16.0%
Total	101	32	31.7%

Previous history of congenital toxoplasmosis:

Women whose babies were born with congenital malformations in the study groups had sero-prevalence rate of 16 (32%) compared to others with no past history of malformation 3(6%) There is significant difference between them (x = 5.98, P. value = 0.1226) (table 5)**.**

Table 5: The rate of Anti-Toxoplasma antibodies in pervious history of congenital toxoplasmosis examined by ELISA test:

Previous history of congenital toxoplasmosis	No. examined	No.+ve	Percentage
Yes	50	16	32.0 %
No	50	3	6.0 %
total	100	19	19.0 %

Blood transfusion:

Table 6 shows the rate in women who were given blood transfusion. There was no significant difference between the study and control groups in relation to blood transfusion (x = 1.46, P. value = 0.69).

Table 6: Women with previous history of blood transfusion:

Blood transfusion	No. examined	No. +ve	Percentage
Yes	7	1	14.3%
controls	50	6	12.03%
total	57	7	12.3%

Direct latex agglutination test in pregnant women:

When pregnant women examined for anti-*Toxoplasma* antibodies by latex agglutination test, The results showed antibodies titers between 1/8 iu/l to 1/32 iu/l. The number of women who were positive at this range amount to 12 (16%) out of 51 examined (table 7). (X 2:3.533, P. Value: 0.171).

Table 7: Titer of anti-*Toxoplasma* antibodies by latex agglutination test in pregnant women:

Titers	No. examined	No. +ve	Percentage
1\8	4	1	10.0 %
1\16	12	3	44.0 %
1\32	19	8	11.0 %
1\64	16	0	0.0 %
Total	51	12	16.0 %

Table 8: Seropositive of anti-*Toxoplasma* antibodies by latex agglutination test in pregnant women and control:

Group test	No. examined	No. +ve	Percentage
Pregnant women	76	12	15.8%
Control	40	8	20.0 %
Total	116	20	17.3%

Antibody titers by latex agglutination test in aborted women:

Out of 104 examined women 18(17.3%) were with history of miscarriage. The antibodies titers ranged between 1/8 to 1/64. In non–aborted controls, the percentage was 8% showing no significant difference between titers in aborted and non-aborted women (X 2: 6.62, P. Value: 0.0850) (table 9).

Table 9: Titer of anti- *Toxaplama* antibodies examined by latex agglutination test in women with history of miscarriage:

Titers	No. examined	No. +ve	Percentage
1\8	22	8	44.0 %
1\16	37	6	77.0 %
1\32	29	3	3.8 %
1\64	16	1	1.3 %
total	104	18	17.3 %

IgG level determined by ELISA:

The results showed that out of 180 women tested by ELISA, 70 (39.0%) were sero-positive .As for controls, out of 50 women, 16(32%) were found positive (table 10). There is no significant difference in sero-positivity rate between the two groups (X 2: 0.793, P. value: 0.373).

Table 10: IgG level by ELISA among study and control groups:

Group	No. examined	No. +ve	Percentage
Study group	180	70	39.0%
Controls	50	16	32.0%
Total	230	86	37.4%

IgM antibodies by ELISA in examined women:

Out of 70 examined women, 9 (12.9%) were found positive compared to 4(10.0%) out of 40 controls (table 11). There was no significant difference between the two groups (X 2: 0.190, P. value: 0.656).

Table 11: Sero- positive status and ELISA IgM among study and control groups:

Group	No. examined	No. +ve	Percentage
Study group	70	9	12.9%
Controls	40	4	10.0 %
Total	110	13	11.8 %

Polymerase chain reaction (PCR):

Application of PCR test in the study group revealed 30 (16.7%) positive cases, while 50 (18%) women were positive in the control group (table 12). No significant difference was found between the examined and controls (x2= .049.P.value = 0.825).

Table 12: Positive cases by PCR among examined women.

Group	No. examined	No. +ve	Percentage
Study group	180	30	16.7%
Controls	50	9	18.0%
Total	230	39	16.7%

Association between direct latex agglutination and PCR test in the study groups:

In study group (table 13), out of 26 women positive examined by direct latex agglutination test 21 (80.8%) were positive whereas (19.2) were negative by PCR test .Out 18(100%) were negative by latex agglutination test were negative by PCR the difference statistically was highly significant (X 2: 3.905 P. value: 0.049).

Table 13: Association between direct latex agglutination and PCR test in study

Test	**No. examined**	**No. +ve**	**Percentage**
DLA	26	21	80.8%
PCR	18	18	100.0%

DLA=Direct latex agglutination

Association between ELISA IgG in aborted and PCR test in study group:

59.1% (13 out of 22 aborted women positive by ELISA IgM) were PCR positive whereas 9 (41%) were negative by PCR test all the 82 aborted women negative by ELISA. IgG test were negative by PCR test statistically there was highly difference significant between ELISA and PCR test (X 2: 36.723, P. Value: 0.001) (table 14).

Table 14: Association between ELISA IgG in women with history of miscarrage and PCR test in study group :

	ELISA I gG Ser (+Ve)		ELISA I gG Ser(-Ve)	
	N0	%	N0	%
positive	13	59.1%	82	100.0%
negative	9	41.0%	0	0.0%
Total	22	100.0%	82	100.0%

Association between ELISA IgG test and PCR test examined in pregnant women:

Table 15 shows the association between ELISA IgG as examined in pregnant women and PCR test, 18(64.3%) were positive, whereas 10 (35.7%) were negative by PCR test .All the 37 women negative by PCR test, were negative by ELISA test. Statistically there was significant difference between ELISA and PCR test. (X 2 : 7.12,P. Value: 0.0676).

Table 15: Association between ELISA IgG test and PCR test as examined in pregnant women:

	ELISA I gG. Ser +Ve		ELISA I Gg. Ser –Ve	
	N0	%	N0	%
positive	18	64.3%	37	100.0%
negative	10	35.7%	0	0.0%
Total	28	100.0%	37	100.0%

Association between ELISA IgM and PCR test in study group:

In study group (Table 16), 18 out of the 61 women negative by ELISA IgM test were positive by PCR test (29.5), and 70.5% were negative (43 out of 61) .All the 9 positive women by ELISA IgM were positive by PCR test. Statistically there was significant different between them. (X 2: 16.448-P. Value: 0.001).

Table 16: Association between ELISA IgM and PCR test in study group:

	I gM sero- positive		I gM sero- negative	
	N0	%	N0	%
positive	9	100.0 %	18	29.5%
negative	0	0.0 %	43	70.5%
Total	9	100.0 %	61	100.0%

Association between ELISA and PCR tests:

When the rate of anti-*Toxoplasma* antibodies by IgG were tested in all study groups, 38.9% were positive for IgG by ELISA test, and 16.6% were positive by PCR test (table 17). There was statistically significant difference between ELISA and PCR (X 2: 22.15, P. value: 0.001).

Table 17: Comparison of the prevalence rate of anti-Toxoplasma antibodies examined by ELISA and PCR .Tests in all study groups.

Group tested	**No. examined**	**No. + ve**	**Percentage**
ELISA	180	70	38.9%
PCR	180	30	16.6%
Total	360	100	27.8%

Discussion:

The proportion of women at risk of acquiring the infection during pregnancy in many countries, including Sudan, is not well known. Primary infection with *Toxoplasma* during pregnancy may lead to severe complications, if not fatal infection of the foetus [(5)].Therefore, emphasis is placed on preventive measures and early diagnosis of the infection to prevent these severe complications. The ideal situation for the diagnosis of *Toxoplasma gondii* infection in pregnancy of having antibody –negative serum sample collected at the very begging of pregnancy or preferably before conception is usually not possible [(6)]. In Sudan, as in some other countries such as United States, testing for antibodies to *Toxoplasma* in pregnancy is performed in only suspected cases.

In the present study, although the prevalence rate was higher by latex agglutination test and ELISA in the aborted women compared to normally pregnant women, there was no significant difference between the two groups, who also found that women with history of miscarriage was not correlated to *Toxoplasma gondii* seroprevelance in Sudan. Elnhas [(4)] found in a study carried out in Khartoum, Sudan prevalence rates of 35.4% and 34% among women with past history of miscarriage and others with no past history, with no significant difference between them. Compared to some countries, in Saudi Arabia, the prevalence of *Toxoplasma gondii* antibodies was not found to be associated with women with history of miscarriage [(7)]. El.Ridi et al [(8)] reported that in Egypt there was no correlation between *Toxoplasma gondii* seropositivity rate and women with history of miscarriage. The present finding was also in accordance with those of Abdel-Hafez [(9)] and Qublan et al [(10)] who found no correlation between *Toxoplasma gondii* sero-prevalence and women with history of miscarriage in Amman-Jordan. The present results were also similar to

those of Ashfunnessa [11] who reported that *Toxoplasma gondii* seropositivity was not correlated to recurrent fatal loss among pregnant women from Bangladesh.

Despite the variations in percentages of infections examined by the foemtioned authors which may be attributed to some epidemiological factors, sample sizes and different techniques, our results point out to the fact that the diseases in aimilocaly common in susceptible individuals including pregnant ladies and that pregnancy does not initiate nor trigger infections.

The results of the present study agree with those Elnahas [4] who found that *Toxoplasma gondii* sero-prevalence gradually increased by age but he did find not statistically significant correlation between the under 30 versus the over 30 years old.

In contrast, Sahawi et al [12] showed that there was correlation between seropositivity in pregnancy.

The role of toxoplasmosis in women with history of miscarriage is still unsettled [13]. There are conflicting data concerning its relationship to habitual or sporadic women with history of miscarriage. Women with previous history of miscarriage in the study group showed *Toxoplasma gondii* sero- prevalence rate of 21.2% compared to 27.4% among others with no past history of miscarriage with no significant difference between them (P value=0.21).

In the control group, 17% were the sero-prevalence rates found in women with previous history of miscarriage. Statistically, the difference between them was also not significant.

Several studies found no association between *Toxoplasma gondii* sero-prevalence and history of miscarriage [10, 12, and 13]. Similarly result of Al Hindy [14] and Elnahas [4] in Khartoum showed no strong association between toxoplasmosis and women with history of miscarriage. In contrast, a study involving 5000 patients, made by Kimble et al [15], concluded that toxoplasmosis may be the cause for women with history of miscarriage and will vary in cultural habits and geographical areas. Their results suggested but did not prove a causative relationship with infection.

The highest *Toxoplasma gondii* sero- prevalence rate in the study group was shown by women past history of having babies with congenital malformations than others with no past history of malformations (30% and 6%) respectively. Statistically, the difference between them was insignificant (P=0.1226). Significant correlation between *Toxoplasma gondii* antibodies sero-prevalence and past history of congenital malformations was observed in the control group as well. Similar findings were reported by Maha [16] who found significant correlation between *Toxoplasma gondii* antibodies and past history of congenital malformations. These results agreed with those of Elfakahani et al [17] who found 60% among mothers who delivered congenitally abnormal babies using PCR test. Many studies showed that congenital disease can be caused by acquired as well as congenital *toxoplasmosis* [18, 19].

The present results showed that 14.3% of women were positive for the blood transfusion compared to 12% of the control with no significant difference between them.

It is well know that *Toxoplasma gondii* may cause serious toxoplasmosis with clinical manifestations when the host in immune - compromised. The infection can be transmitted through blood and some cases of post – transfusion. Similar study in china showed that 4.86% should be expected in the population of blood donors [(20)].

Application of PCR test on all study groups, showed similar positivists in pregnant women and control groups. No statistical significant difference was found between the groups examined.

Compared with direct latex agglutination test in the study group, percentage of positive women was 80.7%, whereas, 5% (19.23%) were negative by PCR test and out of 43 negative by direct latex agglutination, 34.8% were positive by PCR test and 65.2% were negative by PCR test. Statistically, the difference was highly significant (p value= 0.049).

When immunoglobulis were titrated by ELISA, the results revealed that the level of IgG in the study and controls groups was similar and there were no statistical differences between them. Similarly, it also indicated that the level of IgM was similar in both groups. Since there is no increase in antibody titers, it seems reasonable to conclude that there appears to be no recent infections among the studied pregnant women or that the infections may be sub patient to an extent that antibody level is maintained in low titers.

When these IgM results were related to the PCR findings it was found that all the samples (100%) positive by IgM in the study group were positive by PCR (9 out of 9), compared with none in the control group. Previous studies confirmed that the PCR could actually detect *Toxoplasma gondii* in blood samples of women before or during pregnancy [(21)]. This may be due to the fact that *Toxoplasma* DNA may not be cleared soon from the blood of patients with acute *Toxoplasmic*. Based on this, the presence of *Toxoplasma* DNA in the maternal blood most probably indicated a recent infection or an indicator of apparent parasitaemia, which likely to be clinically significant. Other explanation was that, serological test may have limitation; they may fail to detected specific *Toxoplasma* IgG or IgM antibodies during the active please of *Toxoplasma gondii* infection [(22)].

When IgG was negative, the IgM could be positive become IgM antibodies appear earlier than IgG and they stay for a short time [(23)]. Comparing the IgM results with others should be taken with caution because generally; positive IgM is either an indication of recent infection or might be a false positive result [(24, 25)]. This might also be due to generation of false positive PCR results which commonly occur during DNA processing and PCR reaction. The laboratory contamination of the samples can not be completely present even when different stages of the PCR reaction procedure are carried out in separate rooms and the carry over contamination is controlled [(26)].

However, a negative PCR result dose not exclude recent infections because the sensitivity of the PCR, in which a single trophozoite can be detected in clinical sample has potential problems for some types of specimen and become the exact kinetics of parasitaemia in infected people are not well known .The short duration of parasitaemia, or if the numbers of the trophozites circulating in peripheral blood are low, could lead to a sampling error that will produce false negative result in such cases. The sensitivity of the PCR was found to be significantly higher for maternal infections that infections that occurred between 17 and 21 weeks gestation ($p<0.02$) when the amniotic fluid was tested [(27)]. If the some applies to blood samples, further studies are needed.

A pervious study of serial blood samples from acutely infected pregnant women indicated that in the presence of *toxoplasmosis* specific IgG and IgM antibodies, and additional presence of high direct latex agglutination test titer were insufficient criteria for identifying *Toxoplasma* infection in early pregnancy because some acute infections will not be detected [(28)].

Conversely, some women will be falsely identified was being infected [(25)], and undergo unnecessary diagnostic amniocentesis and anti-parasitic treatment [(6)].

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