



SERO-PREVALENCE AND RISK FACTORS OF *TOXOPLASMA GONDII* IN PREGNANT WOMEN IN KOLKATA, INDIA ”

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ABSTRACT

Aims

The aim of the study was to assess the seroprevalence of Toxoplasmosis in Indian pregnant women with bad obstetrics history and among primigravid women. The seropositivity was analysed according to parity, bad obstetric history, socioeconomic level, and food habits.

Settings and Design

Serum samples were obtained from fifty pregnant women with bad obstetric history and gestation period upto 20 weeks (Group I) and forty primigravid women with gestation period upto 20 weeks (Group II Control), who were attending the antenatal clinics in the Department of Gynaecology Calcutta Medical College and Hospital.

Material and Methods

The serum samples were collected and tested for IgG and IgM antibodies using a commercial ELISA kit. The different epidemiological features were characterized depending on a pretested structured questionnaire.

Statistical analysis used:

The descriptive data were assessed as mean + standard deviation (SD). The χ^2 - test and Z - test was used for the analytic assessment .

Results

The seroprevalence (IgG) was 13[26%] and IgM was 09[18%] in Group I. In Group II 18[45%] were positive for IgG and (IgM) was positive in 7[17.5%]. The prevalence of IgG positive was less in Group I than in Group II and the difference was statistically significant in 1 tail z-test between the groups. A statistical significance between frequency of meat consumption and IgG seroprevalence and consumption of milk with IgM seroprevalence was found in Group II.

Conclusions

The findings of this study gives support to a Toxoplasma screening programme and health education in women during child bearing age .

KEY WORDS: Antenatal , ELISA, India , Seroprevalence , T.gondii,

INTRODUCTION

Toxoplasmosis is zoonosis caused by Toxoplasma gondii, (T.gondii) an obligate intracellular parasite.⁽¹⁾ Infections by the protozoan parasite Toxoplasma gondii are widely prevalent in humans and animals worldwide.^(2,3,4) Cats are important in the natural life cycle of T.gondii because they are the only host that can directly spread T.gondii in the environment. Cats can recycle and amplify the infection by releasing millions of infective units (oocysts) in the environment. Toxoplasma gondii infection in humans especially woman of child bearing age, varies a great deal, depending on climate, cultural habits and animal fauna.

Toxoplasmosis is generally asymptomatic except in immunocompromised adults and congenitally infected children.⁽²⁾ Toxoplasmosis is one of the diseases comprising the 'TORCH' infections. It is known to cause perinatal death if the organism is acquired during pregnancy. Toxoplasmosis during pregnancy can cause congenital infection and manifest as mental retardation and blindness in the infant. The severity of fetal disease varies

inversely with the gestational age at which maternal infection occurs.⁽⁵⁾

As contrary to earlier reports of low prevalence, recently high seroprevalence rate of this protozoan infection has been found in community – based studies carried out in geographically separate area of India. An Indian study with primigravid women has shown that (41.75%) were seropositive for Toxoplasma gondii infection . These results indicate that a large number of the study subjects were vulnerable to toxoplasma infection.⁽⁶⁾

Objectives of the study

The aims of the present study were to assess the seroprevalence of Toxoplasmosis in Indian pregnant women with bad obstetrics history and among primigravid women attending antenatal clinic in the city of Kolkata . The seropositivity was analysed according to parity, bad obstetric history, socioeconomic level, eating raw or poorly cooked meat. Toxoplasmosis has been identified as a serious disease in Europe and compulsory screening for pregnant women has been practiced in many countries.⁷ This study was designed to

examine the seroprevalence of toxoplasmosis in pregnant women with bad obstetric history and primigravid women attending antenatal clinics.

Subjects and Methods

Subject selection

Ninety antenatal mothers were selected who were attending the antenatal clinics in the Department of Gynaecology Calcutta Medical College and Hospital, Kolkata in the year 2007. Fifty were pregnant women with bad obstetric history and gestation period upto twenty weeks and forty women were primigravid women and with gestation period upto twenty weeks attending the antenatal clinic. The different epidemiological features were characterized depending on a pretested structured questionnaire. The serum samples were collected and tested for IgG and IgM antibodies. The project had been approved by suitably constituted Ethics Committee of the Institution within which the work was undertaken. The subjects gave informed consent and patient anonymity was preserved.

Serum samples

Approximately 5 ml of a venous blood was drawn from a qualified subject who fulfills the specified criteria using disposable syringe.

The separated serum was tested for IgG and IgM antibodies for *Toxoplasma gondii* in the laboratory of Department of Microbiology of All India Institute of Hygiene and Public Health Kolkata.

Serum: Detection of IgG & IgM antibodies to *Toxoplasma gondii* was done by a standard ELISA commercial kit.

IgG antibodies to *Toxoplasma gondii* was done by Elisa Commercial Kit by CALBIOTECH INC. (CBI)

IgM antibodies to *Toxoplasma gondii* was done by Elisa Commercial Kit by United Biotech (UBI MAGIWEL).

The test was done according to instructions in the kit and the test run was considered valid provided the following criteria were met:

The O.D. of the calibrator should be greater than 0.250.

The Ab index for negative control should be less than 0.9

The Ab Index for Positive control should be greater than 1.2.

The Ab (Antibody) Index of each determination was calculated by dividing the mean values of each sample by cut-off value.(Callibrator OD X Callibrator Factor)

INTERPRETATION

The following was intended as a guide to interpretation of Toxoplasma IgG test result.

Antibody Index Interpretation

<0.9 No detectable IgG antibody to Toxoplasma by ELISA

0.9 – 1.1 Boderline positive. Follow-up testing is recommended if clinically indicated

> 1.1 Detectable IgG antibody to Toxoplasma by ELISA

The test for Toxo-IgM was done as per instructions provided in the kit and the results were calculated as follows

EU/mL of Sample= O.D. of Samples X EU/mL of Calibrator O.D. of Calibrator.

EXPECTED VALUES AND INTERPRETATION OF RESULT

Negative: less than 80EU/mL.

Equivocal: between 80-100 EU/mL . If an equivocal result is obtained, the sample should be repeated with this or another method. If a primary infection is suspected, a second sample should be obtained 7 to 14 days after the first sample and test to look for seroconversion that is indicative of a primary infection.

Positive: Equal or greater than 100 EU/mL.

This indicates the possibility of current or recent Toxoplasmosis.

Results

The study population is divided in two groups.

Group I - Pregnant women with bad obstetrics history (BOH)

Group II – Primigravid pregnant women

The seroprevalence (IgG) of Toxoplasmosis (latent infection) was 13[26%] (95% CI 25.88- 26.12) in Group I . The IgG borderline cases were 3[6%] (95% CI 5.93- 6.07). Where as in Group II 18[45%] (95% CI 44.85- 45.15) were positive for IgG. There were no borderline cases. The seroprevalence (IgM) of Toxoplasmosis (recently acquired infection) was 09[18%] (95% CI 17.89- 18.11) in Group I. The IgM equivocal cases were 7[14%] (95% CI 13.90- 14.10). Where as in Group II 7[17.5%] (95% CI 17.38- 17.62) were positive for IgM. A high percentage of subjects were equivocal in this group 11[27.5%] (95% CI 27.36- 27.64). 5[10%] (95% CI 9.92- 10.08) subjects were positive for both IgG and IgM infection in Group I and 5[12.5%] (95% CI 12.40- 12.60) were positive for both IgG and IgM infection in Group II. (Table1). The prevalence of IgG positive is less in Group I than in Group II and the difference is statistically significant in 1 tail z-test between the groups.

The association between seroprevalence of IgG and trimester of pregnancy was statistically significant ($p < 0.05$) showing that seroprevalence is more in first trimester of pregnancy. In the association between possible risk factors and Toxoplasma seroprevalence (IgG) in pregnant women was studied and the study found statistical

Table 1 : The seroprevalence of *Toxoplasma gondii* in pregnant women as assessed by the ELISA test

	Group I (BOH)		Group II (Primigravid)		
	Number	95% CI	Number	95% CI	p
Seroprevalence					
IgG positive	13 [26%]	[25.88 – 26.12]	18 [45%]	[44.85 – 45.15]	<0.05
IgG borderline	3 [6%]	[5.93 – 6.07]			
IgG negative	34 [68%]	[67.87 – 68.13]	22 [55%]	[54.85 – 55.15]	>0.05
IgM positive	9 [18%]	[17.89 – 18.11]	7 [17.5%]	[17.38 – 17.62]	>0.05
IgM equivocal	7 [14%]	[13.90 - 14.10]	11 [27.5%]	[27.36 – 27.64]	>0.05
IgM negative	34 [68%]	[67.87 – 68.13]	22 [55%]	[54.85 -55.15]	>0.05
IgG & IgM positive	5 [10%]	[9.92 – 10.08]	5 [12.5%]	[12.40 – 12.60]	>0.05

Table 2 The association between possible risk factors and *Toxoplasma gondii* seroprevalence (IgG) in pregnant women

Variables		Group I (BOH)			Group II (Primigravid)			No. 3
		Total	Present[%]	P value	Total	Present[%]	P value	
Trimester of pregnancy	First	27 [54]	11[40.7]	<.05+	25[62.5]	10[40.0]	0.518	
	Second	23 [46]	02 [8.7]		15[37.5]	08[53.3]		
Parity	None	32 [64]	10[31.3]	0.328				
	Having children	18[36]	03 [27.3]					
Contact with cat	Yes	42[84]	10[23.8]	>0.05	34[85]	15[44.1]	>0.05	
	No	08[16]	3[38]		06[15]	03[50]		
Owner of cat	Yes	06[12]			04[10]	02[50]		
	No	36[72]			30[75]	0		
Close Contact with cat	Yes	36[72]	10[27.8]	>0.05	30[75]	13[43.3]	>0.05	
	No	06[12]	03[50]		04[10]	03[75]		
Contact with other animal	Yes	17[34]	04[23.5]	>0.05	16[40]	07[43.8]	>0.05	
	No	33[66]	09[27.3]		24[60]	11[45.8]		
Consumption of meat	Yes	48[96]	13[27.1]		38[95]	18[47.4]		
	No	02[4]	0		02[5]	0		
Frequency of meat consumption	Less than once a week	15[30]	3[20]	>0.05	8[20]	1[12.5]	<0.05	
	Once a week	33[66]	10[30.3]		30[75]	17[56.7]		
Type of meat	Yes	45[93.7]	13[28.9]		32[84.2]	16[50]	>0.05	
	No	03[7.9]	0		06[15.8]	02[33.3]		
Chicken	Yes	45[93.7]	12[26.7]	>0.05	31[81.6]	15[48.4]	>0.05	
	No	03[7.9]	01[33.3]		07[18.4]	03[42.9]		
Mutton	Yes	11[22.9]	01[9.1]	>0.05	09[23.7]	05[55.6]	>0.05	
	No	37[77.1]	12[32.4]		29[76.3]	13[44.4]		
Beef	Yes	49[98]	12[24.5]	>0.05	40[100]	18[45]	>0.05	
	No	01[2]	01[100]					
Eating raw vegetables	Yes	28[56]	08[28.6]	>0.05	29[72.5]	14[48.3]	>0.05	
	No	23[44]	05[22.7]		11[27.5]	4[36.4]		
Source of drinking water	Boiled	28[56]	10[35.7]	>0.05	20[50]	10[50]	>0.05	
	Pasteurized	22[44]	03[13.6]		20[50]	8[40]		

association between possible risk factors and *Toxoplasma gondii* seroprevalence (IgM) in pregnant women

Variables		Group I (BOH)			Group II (Primigravid)		
		Total	Present[%]	P value	Total	Present[%]	P value
Trimester of pregnancy	First	27 [54]	06[22.2]	>0.05	25[62.5]	04[16.0]	>0.05
	Second	23 [46]	03 [13.0]		15[37.5]	03[20.0]	
Parity	None	32 [64]	07[21.9]	>0.05	34[85]	06[17.6]	>0.05
	One & above	18[36]	02 [11.1]		06[15]	01[16.7]	
Contact with cat	Yes	42[84]	07[16.7]	>0.05	34[85]	06[17.6]	>0.05
	No	08[16]	2[25]		06[15]	01[16.7]	
Owner of cat	Yes	06[12]		>0.05	04[10]		>0.05
	No	36[72]	07[19]		30[75]	06[20]	
Close Contact with cat	Yes	36[72]	07[19.4]	>0.05	30[75]	06[20]	>0.05
	No	06[12]	02[33.3]		04[10]	01[25]	
Contact with other animal	Yes	17[34]	03[17.6]	>0.05	16[40]	02[12.5]	>0.05
	No	33[66]	06[18.2]		24[60]	05[20.8]	
Consumption of meat	Yes	48[96]	09[18.8]	>0.05	38[95]	7[18.4]	>0.05
	No	02[4]	0		02[5]	0	
Frequency of meat consumption	Less than once a week	15[31.3]	4[26.6]	>0.05	8[21]	3[37.5]	>0.05
	Once a week	33[66]	05[15.15]		30[75]	04[13.3]	
Type of meat Chicken	Yes	45[93.7]	09[20]	>0.05	32[84.2]	06[18.8]	>0.05
	No	03[7.9]	0		06[15.8]	01[16.7]	
Mutton	Yes	45[93.7]	09[20]	>0.05	31[81.6]	06[19.4]	>0.05
	No	03[7.9]	0		07[18.4]	01[14.3]	
Beef	Yes	11[22.9]	01[9.1]	>0.05	09[23.7]	03[33.3]	>0.05
	No	37[77.1]	08[21.6]		29[76.3]	04[13.8]	
Eating raw vegetables	Yes	49[98]	09[18.4]	>0.05	40[100]	07[17.5]	>0.05
	No	01[2]					
Source of drinking water	Tap water	28[56]	05[17.9]	>0.05	29[72.5]	05[17.2]	>0.05
	Tube well	23[44]	04[18.2]		11[27.5]	2[18.2]	
Drinking milk	Boiled	28[56]	05[17.9]	>0.05	20[50]	06[30.0]	<0.05
	Pasteurised	22[44]	04[18.2]		20[50]	01[5.0]	

significance between frequency of meat consumption and seroprevalence (IgG) in Group II. The seroprevalence in subjects with meat consumption less than once a week was less 1 [12.5 %] as compared with consumption of meat once a week. 17[56.7%] ($p < 0.05$). There was

association between other parameters but they were not quite statistically significant.(Table 2)

In the association between possible risk factors and *Toxoplasma* seroprevalence (IgM) in pregnant women it was found that only consumption of milk

is associated with seroprevalence (IgM) in Group II. The seroprevalence of subjects consuming boiled milk [06(30.0%)] was more than subjects consuming pasteurized milk [01 (5.0%)] ($p < 0.05$). There was association between other parameters but they were not quite statistically significant. (Table 3)

Discussion:

In this study it was determined that the seroprevalence of latent Toxoplasma infection (IgG) among pregnant women with bad obstetrics history was 26%, which indicates that primary infection appears to be subclinical and prevalent throughout life. The incidence of anti Toxoplasma antibodies in women with abnormal pregnancies and abortions ranges from 17.5% to 52.3%⁽¹⁾. Nowakowska D et al showed specific IgG antibody in 41.3% (95% CI 39.9-42.7) of pregnant women,⁽⁸⁾ while Morris et al showed that 33% women had IgG antibody to *T. gondii*⁽⁹⁾. Whereas in Brazil the study resulted in 74.5% seroprevalence in pregnant women. Contact with soil was found to be the major factor for infection⁽¹⁰⁾.

The Indian studies showed varied result ranging from 11% to 55%.⁽¹¹⁾ Their data demonstrated high frequency of primary infections during pregnancy and supported the conclusion that routine prenatal screening is justified. Joshi et al showed seropositivity rates of one or both classes of antibodies as 17.2%⁽¹²⁾. Singh et al documented the incidence and prevalence of toxoplasmosis IgG seroprevalence as 45%⁽¹³⁾. The incidences in other studies were 41.7% and 55% respectively.^(14,15)

However the seroprevalence in primigravid women for latent infection (IgG) was 45%. It was shown in a study among primigravid women attending a secondary level hospital in a district of North India. Of these 41.75% were seropositive for Toxoplasma gondii infection⁶. In worldwide studies the seropositivity had varying result for e.g. 17.2% in Singapore. In this study there seemed to be a trend of increasing seropositivity with age. It also showed the incidence of all three infections were higher among the Malays, Indians and other races compared to the Chinese⁽²⁾. In Nepal when sera was studied from randomly selected 345 pregnant Nepalese women aged 16-36 years for the presence of Toxoplasma antibodies using microlatex agglutination (MLA) and ELISA methods. The overall prevalence was 55.4%¹⁶. Turkey reported 52.1% seropositive pregnant women⁽¹⁷⁾. In Saudi Arabia the seroprevalence was reported as 35.6%⁽¹⁸⁾. The possible explanations may be due to different diagnostic methods and the possible risk factors contributing to the development of Toxoplasma infection.

The study showed the seroprevalence of Toxoplasma (IgM) antibodies as 18% in women

with bad obstetrics history, which is more or less similar to seropositivity shown in other studies for eg. In one study 13 women with bad obstetric history (BOH) were tested for the presence of Toxoplasma antibodies using microlatex agglutination (MLA) and ELISA methods. Of the total 13 women with BOH, 38.5% had Toxoplasma antibodies of which 40.0% were positive for Toxoplasma -IgM antibodies⁽¹⁹⁾. In another study it was observed ELISA IgG, IgM antibodies and PCR for toxoplasmosis on 55 women with complicated gestation and their babies. Besides, ELISA IgG and IgM were applied on 27 uncomplicated gestation (mothers & babies) and 152 randomly selected individuals. Seropositivity to specific IgG antibodies was 36.4%, 59.2% and 57.9% and for IgM was 27.3%, 7.4% and 10.5% in complicated gestation, uncomplicated gestation and random population respectively⁽²⁰⁾. In a study 380 serum samples were evaluated from pregnant women having bad obstetric history, attending antenatal clinic. The prevalence of Toxoplasma infection in pregnant women was demonstrated by the presence of IgM by ELISA test. It was found that, IgM antibodies were positive in 10.52% for Toxoplasma⁽¹⁴⁾.

In primigravida 14% were seropositive for IgM, which is similar to as observed in other study where 13.1% were seropositive for IgM⁽²¹⁾. No clinically active toxoplasmosis was seen among these subjects. These indicate that the subjects were asymptomatic despite having a serological status indicating the recent infection.

On analyzing the comparison of seroprevalence between women with bad obstetric history and primigravid women it was found that the prevalence of IgG positive is less in women with bad obstetric history than in primigravid women ($p < 0.05$). With the present sample size we could not come to any definite conclusions. The other possible explanations may be that the latter are more likely to have association with the possible risk factors.

This study showed in women with bad obstetric history, that there is association between trimester of pregnancy and seroprevalence (IgG). Statistical significance was found showing seroprevalence as more in the first trimester of the pregnancy. 40.7% as compared to 8.7%. No statistical significance was found between other variables. The possible reason contributing to this might be small sample size of the study.

In the present study a significant association was not found between possible risk factors except in primigravid women where there was association between frequency of meat consumption and seroprevalence IgG. Seroprevalence was less in subjects who consumed meat less than once a week as compared to meat consumption once a week ($p < 0.05$). Though all the subjects had well-

cooked meat the subjects may be were exposed to other risk factors. The association between cats and *Toxoplasma* seroprevalence was not found to be significantly different in previous reported study. One of the study reported the risk factors most strongly predictive of acute infection in pregnant women who were eating undercooked lamb, beef, or game and contact with soil. Contact with cats was not a risk factor⁽²²⁾. Between 30% and 63% of infections in different centres were attributed to consumption of undercooked or cured meat products and 6% to 17% to soil contact⁽²²⁾. The Malaysian study showed a varying prevalence of specific *Toxoplasma* antibodies among the Malaysian population. In general, the route of transmission, such as contact with a cat, were shown to have no significant association with *Toxoplasma* seropositivity ($p > 0.05$)⁽⁷⁾. The increased seroprevalence of *Toxoplasma* was with consumption of municipal and uncontrolled water (well/spring water)⁽²³⁾. This data may give an idea that the contact with cats might not pose a major risk for *Toxoplasma* infection but contamination of ingested food with the oocyst of the *Toxoplasma gondii* might pose a major risk.

The IgM seroprevalence in primigravid women was more in subjects consuming boiled milk 30% than pasteurized milk 5%. ($p < 0.05$). In other studies it has been shown that human acquire *T.gondii* infection post-natally mainly by ingesting food and water contaminated with oocysts passed in the faeces of infected cats⁽²⁾. Infection with *Toxoplasma gondii* is transmitted to man by infected meat or meat products and by contact with soil or surface water⁽²⁴⁾.

Toxoplasmosis is a zoonotic infection which has immense public health importance. Contrary to earlier reports of low prevalence recently high seroprevalence rate of this protozoan infection has been found in community based studies. This high rate of infection suggests significant contamination of soil, water and food with *T. gondii* oocysts. In conclusion the findings of this study gives support to a *Toxoplasma* screening programme and health education including promotion of healthy lifestyle exclusively in seronegative women during child bearing age in order to prevent the frequent occurrence of this zoonosis.

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