

Original Research Article

Sero-epidemiology of toxoplasmosis and associated risk factors among antenatal women in Ranchi, Jharkhand, India

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ABSTRACT

Background: Toxoplasmosis seroprevalence in antenatal women has remained a contentious issue in Indian subcontinent. Bad obstetric history (BOH) is specific to women of childbearing age and can be caused by infection with *Toxoplasma gondii*. In Jharkhand, scarce data exist for the roles of toxoplasmosis in the aetiology of adverse pregnancy outcome. Aim: To study the sero-epidemiology of Toxoplasmosis and associated risk factors among antenatal women attending antenatal clinic of a tertiary care hospital in Ranchi, Jharkhand, India.

Methods: A case-control study was conducted on antenatal women attending antenatal clinic from the June 2010 to May 2011. The study group comprised 102 antenatal women with BOH while the control group comprised 102 multiparous, age-matched antenatal women without BOH. Latex Slide agglutination test for antibodies to *T.gondii* was utilized. The research was approved by institutional ethical committee. Descriptive and inferential statistics was applied.

Results: The overall seroprevalence of *T.gondii* among antenatal women was 15.19%, with significantly more in women with BOH (24.50%) as compared to women without BOH (5.88%). The seroprevalence in women with BOH gradually increased with advancing age: 8.33% in age less than 20 years to 50% in women older than 30 years. The seroprevalence increased steadily with number of BOH: 9.67% in women with one BOH to 37.50% in women with three or more BOH. Risk factors like Illiteracy, rural residence, owning a cat and non-veg dietary habit showed significant association.

Conclusions: The seroprevalence of toxoplasmosis is high among antenatal women with BOH. Routine serological test is recommended for all pregnant women with BOH.

Keywords: Bad obstetric history, India, Latex agglutination test, Seroprevalence, *Toxoplasma gondii*

INTRODUCTION

Toxoplasma gondii, an obligate intracellular tissue protozoan parasite infects nearly one-third of world's population although often unrecognized, as most patient do not exhibit symptoms.¹ *Toxoplasma gondii* infection, if acquired in pregnancy, can be transmitted vertically across the placenta to the fetus, resulting in congenital toxoplasmosis, which can cause fetal anomalies. Also, pregnant women having toxoplasmosis may have spontaneous abortions, stillbirths, intrauterine growth

retardation (IUGR) and preterm deliveries. These adverse fetal outcomes are designated as Bad obstetric history (BOH).^{2,3}

Humans can also acquire *T.gondii* infection through oral ingestion of food or water contaminated with oocyst from cat feces and cysts contained in raw or undercooked meat.⁴ Toxoplasmosis is often described as an environmental disease because transmission has been shown to be promoted by overcrowding, poor sanitation, poverty, certain eating habits and poor hygiene.^{5,6}

The detection of *T.gondii* is based on serological detection of antibodies using latex agglutination (LA) testing, enzyme-linked immunosorbent assay (ELISA), and/or indirect fluorescent antibody testing (IFAT). LA testing is better adapted for developing countries where rural areas often lack the equipment needed for ELISA. Moreover, LA is simpler and easy to perform, and it also shows high qualitative agreement with the Sabin-Feldman dye test, which is considered *gold standard*.⁷⁻⁹

Against this background, we attempted to study the sero-epidemiology of Toxoplasmosis and associated risk factors among antenatal women attending antenatal clinic of a tertiary care hospital in Ranchi, Jharkhand, India. We will also determine the extent of seropositivity of toxoplasmosis in antenatal women with BOH and to compare it with that in antenatal women having previous normal obstetric history.

METHODS

A descriptive case-control study was conducted on patients attending the Department of Microbiology and the Department of Obstetrics and Gynaecology, RIMS, Ranchi from the June 2010 to May 2011. The study group comprised antenatal women of reproductive age (15-45 years) with previous unfavourable foetal outcome in terms of two or more consecutive spontaneous abortions, premature delivery, history of intrauterine foetal death, intrauterine growth retardation, still births, early neonatal and congenital anomalies.

The control group comprised multiparous, age-matched antenatal women without BOH attending the same clinic. Subjects with known causes like hypertension, Diabetes Mellitus, Syphilis, Rh (rhesus) incompatibility, physical causes of abortion, and consanguinity of fetal wastage were excluded from study.

To work out the required sample-size for the determination of seropositivity in antenatal women with bad BOH, the equation suggested by Vaughan and Morrow was used.¹⁰ Considering 49.47% seropositivity of toxoplasmosis in women with BOH, 10% margin of sampling error, and 1% non-response rate, the desired sample size was 105.³ About 1,510 pregnant women

attended antenatal clinic in the previous year, of these women, 110 presented with BOH. On the basis of admission trend, the investigators expected to obtain the desired sample within a year. In total, 204 women were included in the study, comprising of 102 with BOH (study group) and 102 without BOH (control group). The investigators visited outpatient department on alternate days, selected the study subjects, and screened them according to inclusion and exclusion criteria with a predesigned and pretested questionnaire. First study subjects having BOH was selected, and then the next available age-matched multiparous antenatal without BOH was included in the control group subjects. This control group comprised of attendants accompanying patients or those women who are married and do not have history of BOH. Prior to interview, the purpose and procedures of the study were explained to all the subjects, and informed consent was obtained from them. All of them were interviewed to ascertain age, medical and obstetric information.

For serological analysis, about 5ml of patient's blood was collected by venepuncture in a sterile dry vial with full aseptic and antiseptic precaution. Clear serum was separated to carry out the tests. Latex Slide agglutination test (kit of Toxogen manufactured by TULIP diagnostics) for antibodies to *T.gondii* was utilized. The reagent and samples were brought to room temperature before use. The sample to be tested was diluted to 1:16 with normal saline (0.1 ml of serum + 1.5 ml of 0.9% saline). The Latex agglutination test employed in the present study has shown high sensitivity and specificity, which proves its validity for the detection of antibodies against *T.gondii*.¹¹⁻¹³ The research was approved by institutional ethical committee. Data was collected and compiled in Microsoft Excel spread sheet. Descriptive and inferential statistics was applied using the Epi Info software (version 3.5.1).

RESULTS

The total seroprevalence of toxoplasmosis in this study is 15.19% (31/204). Table 1 show that 24.50% were seropositive cases in study group while only 5.88% were positive in control group and this difference is statistically significant (p=0.0002).

Table 1: Seropositivity among study and control groups.

Group	No. of cases	Positive (%)	Negative (%)	OR (95% CI)	χ^2 (p)
Study	102	25 (24.50)	77 (75.50)	5.19 (2.02-13.30)	13.73
Control	102	6 (5.88)	96 (94.12)	1	(0.0002)

The analysis of seropositivity according to age of the study subjects is shown in Table 2. The seropositivity increased with advancing age in both the groups. The

seropositivity rate in different age groups ranged from 8.33% to 50.00% in study subjects and from 0% to 13.04% in controls. The difference in the proportions of

seropositivity between the study and control groups was analysed by using z-test. The difference was significant

for the age-groups of above 25 years although it was not significant for younger age-groups.

Table 2: Seropositivity in relation to age of subjects.

Age (Years)	Study Group			Control Group			z-Score	p value
	Sera Tested	Positive sera		Sera Tested	Positive sera			
		No.	%		No.	%		
≤20	12	1	8.33	10	0	0.00	0.93	0.352#
21-25	27	4	14.81	30	1	3.33	1.53	0.126#
26-30	41	9	21.95	39	2	5.13	2.18	0.029**
>30	22	11	50.00	23	3	13.04	2.67	0.007**

#Non-Significant; **Significant

Table 3: Distribution of seropositivity in relation to risk factors for women with BOH.

Risk Factor	Variable	Seropositivity		Total (204) N (%)	χ ² (p)
		Positive	Negative		
		N (%)	N (%)		
Number of BOH	0	6 (5.88)	96 (94.12)	102 (50)	24.4 ($<0.0001^{**}$)
	1	3 (9.67)	28 (90.33)	31 (15.20)	
	2	7 (22.58)	24 (77.42)	31 (15.20)	
	≥ 3	15 (37.50)	25 (62.50)	40 (19.60)	
Education	Illiterate	20 (25.00)	60 (75.00)	80 (39.21)	9.82 (0.002*)
	Literate	11 (8.87)	113 (91.13)	124 (60.79)	
Occupation	Working	19 (21.11)	90 (78.89)	109 (53.43)	0.91 (0.341#)
	Housewife	12 (12.63)	83 (87.37)	95 (46.57)	
Residence	Rural	28 (17.94)	128 (82.06)	156 (76.47)	3.90 (0.048*)
	Semi-Urban	3 (6.25)	45 (93.75)	48 (23.53)	
Overcrowding	Yes	25 (19.68)	102 (80.32)	127 (62.25)	1.94 (0.164#)
	No	6 (7.79)	71 (92.21)	77 (37.75)	
Owns cat	Yes	2 (22.22)	7 (77.78)	9 (4.41)	5.26 (0.022*)
	No	29 (14.87)	166 (85.13)	195 (95.59)	
Dietary Habits	Non-Veg	19 (22.89)	64 (77.11)	83 (40.68)	6.43 (0.011*)
	Veg	12 (9.91)	109 (90.09)	121 (59.32)	
Eat Vegetables	Raw Yes	3 (13.04)	20 (86.96)	23 (11.27)	0.93 (0.760#)
	No	28 (15.47)	153 (84.53)	181 (88.73)	

Out of 102 women with BOH, equal (30.39%; 31/102) percentages of women had one or two BOH while 39.21% of women had three or more BOH. Table 3 shows the seropositivity of Toxoplasma in relation to the number of times the antenatal women experienced BOH. The highest percentages (37.50%) of positivity were noted among women with three or more BOH, and lowest (9.67%) among women having one BOH. The difference was highly significant ($p<0.00001$).

Table 3 shows some socio-demographic and other risk factors in relation to T.gondii infection. Illiteracy, rural residence, keeping cats as pet animal and non-vegetarians were having more chances of having Toxoplasma infection and the difference was also significant. Also, seropositivity was found more in working women indulged

in any kind of activity as compared to housewives, but difference was non-significant.

DISCUSSION

Toxoplasma gondii is one of the few protozoan parasites that cross the placenta and lead to various complications. Antenatal screening of toxoplasma infection is important in endemic areas. However, cost-effectiveness of such screening programmes in developing countries like India is still not known despite having high prevalence of pregnancy wastage in this countries.¹⁴

Different studies done in India shows prevalence of toxoplasmosis among pregnant women as low as 5% to as high as 80%.¹⁵⁻¹⁹ The prevalence in this study is

15.19%, which is in accordance to a study done by Malarvizhi et al.²⁰ The seroprevalence of toxoplasmosis in study group was 24.50% , a little higher than studies done on women with BOH by other authors.^{21,22} In this study, a statistically significant higher (24.50%) prevalence of seropositive cases were seen in study group as compared to women in control group (5.88%). This finding is in accordance with studies conducted by Sentahmarai S et al. (13.09% vs 8%), Ebadi et al (17.5% vs 14%), and Suryamani et al.²³ (45% vs 8.33%).

Toxoplasma has a predilection for placenta and its infection can induce foetal loss in women. It can be congenitally acquired by transfer through the placenta, if contracted during pregnancy and may lead to abortions, stillbirths and congenital malformations.^{24,25} It was observed that seropositivity of toxoplasmosis increased with increasing age. This is similar to many earlier results.^{26,27} With increasing age, risk of acquiring toxoplasmosis as well its harmful pathological complications become higher and more possible. Also, the probability of an individual coming into contact with the transmission routes increases as her age increases.

The seropositivity of toxoplasmosis was highest among women with three or more BOH and lowest among women having one BOH. There is evidence that active *Toxoplasma* infection can occasionally persist from one pregnancy to the next and such infections may lead to repeated loss of pregnancy. It appears that in these cases, *T.gondii* may encyst in the uterine endometrium and can persist there for longer duration. This may cause low grade endometritis.²⁸

This study indicates that socio-economic and environmental factors like illiteracy, overcrowding, occupation, rural residence, non-vegetarian food habits and owning a cat play a crucial role for transmission and spread of toxoplasmosis, a finding also seen by Yasodhara P et al. It is important to avoid undercooked, raw, or cured meat, and raw, unwashed fruits or vegetables.²⁹

Most of the women in our study belonged to low socio-economic group which can lead to repeated infections attributable to the unhygienic environment in which they reside. Proper hand hygiene is essential for decreasing the risk of toxoplasmosis infection. Presence of cats plays an important role in transmission of toxoplasmosis in many areas of world. This study also observed an association between positive seroprevalence and contact with cats. These cats may obtain their food from remains of raw meat thrown in open by families in neighbourhood. Cats may play an indirect role in transmission of infection through contaminated meat, vegetables and fruits.³⁰

CONCLUSION

An important challenge for health care professionals is the detection of toxoplasmosis infection in antenatal

women. From our study, we conclude that the prevalence of toxoplasmosis is high in antenatal women. The seroprevalence is significantly higher in women with BOH as compared to those in control group. There was a gradual increase in seroprevalence with advancing age.

Present study, although a small one is very important considering the fact that it was done among antenatal women from poor socioeconomic condition in Jharkhand. We recommend routine serological testing for all antenatal women as a regular screening procedure, similar to other countries.³¹ The initiation of treatment in time can, thus, be provided to prevent complications.

Limitations

Due to some monetary constrains, we could not perform ELISA test which is more sensitive test compared to Latex Slide agglutination test. Moreover, this is the first study on toxoplasmosis from this area. No previous data were available for reference. So, this study can provide an important database for current situation, magnitude of risk and may help in future for further studies to enhance the knowledge on toxoplasmosis among antenatal mothers.

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