<div style="writing-mode: vertical">ORIGINAL ARTICLE</div>

Seroprevalence of Toxoplasmosis in Pregnant Females Attending a Tertiary Care Hospital in Uttar Pradesh, India and its Effect on Perinatal Morbidity and Mortality

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ABSTRACT

Objective: To document the seroprevalence of toxoplasma in pregnant females attending a tertiary care hospital and to study its effect on perinatal morbidity and mortality. **Design:** Prospective follow-up study. **Setting:** Queen Mary's Hospital, a tertiary level hospital affiliated to King George Medical University (earlier known as KGMC), Lucknow, Uttar Pradesh, India. **Sample:** All pregnant women coming to this hospital for antenatal care in the Obstetrics and Gynaecology Dept. **Methods:** Pregnant females were selected through systematic random sampling. A pre-tested semi-structured questionnaire was administered. Blood sample was drawn to detect IgM and IgG antibodies against toxoplasma. Delivery outcomes were recorded with reference to abortions, still births, delivery of congenitally malformed baby and gestational age at delivery. **Main outcome measures:** Perinatal outcomes among those positive for toxoplasma antibodies. **Results:** Out of 260 subjects screened, 23 (8.8%) were IgM positive and 40 were IgG positive (15.4%). IgM sero-positivity was observed more in females aged >30 years, those ≥3 gravida, belonging to low socio-economic status, Muslims, those predominantly non-vegetarian and those exposed to raw meat. Still births (17.4% vs. 3.8%; p=0.006), congenital abnormalities (8.7% vs. 0.5%; p=0.002) and abortions (17.4% vs. 2.7%; p=0.001) were more common in those positive for IgM antibodies compared to sero-negatives. **Conclusions:** Toxoplasma infection plays a role in adverse foetal outcome. Socio-epidemiological aspects constitute an important contributing factor for the spread of the disease. All pregnant women should be educated and counselled for Routine serological testing for toxoplasma-specific antibodies.

INTRODUCTION

Toxoplasma along with infections such as parvovirus, herpes zoster, rubella, cytomegalovirus, syphilis and herpes simplex virus (also known as TORCH group of infections) are the most serious infectious disease during pregnancy that could lead to embryo-fetal lesions.[1-3] Toxoplasmosis is present in every country

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and seropositivity rates range from less than 10% to over 90%.[4]

Globally the count of people infected with *T. gondii* is over 6 billion.[5] There is variation in IgG based Seroprevalence globally against *T. gondii*. IgG based seroprevalence of toxoplasma was found to be in the range of 6 to 46%(Korea(6.7%), China(12.3%), Nigeria(23.9%), Tanzania(46%)) and can be as high as 98% in some regions.[5] The global estimated incidence of congenital toxoplasmosis is 1,90,100 annual cases (95% CI: 1,79,300–2,06,300).[6] This amounts to an incidence rate of approximately 1.5 cases of per 1000 live births.[6] The regions with the highest incidence of congenital toxoplasmosis include parts of the Middle East and some low-income countries in Africa.[6] The seroprevalence of

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toxoplasma in pregnancy in India is 3.3% for IgM and 45% for IgG antibodies.[7]

Infection with *Toxoplasma gondii* an obligate, intra-cellular coccidian parasite, causes Toxoplasmosis.[8] Transmission of infection usually occurs by oral route and can be attributed to ingestion of undercooked meat is most common reason. The undercooked meat may contain sporulated oocysts or bradyzoites.[8] Its infection in fetus usually occurs via transplacental transmission of the parasite following maternal parasitemia.[8,9] Toxoplasmosis, a zoonotic disease is usually asymptomatic in pregnant women but its effects on the fetus may be life-threatening.[10] An acute infection in pregnant woman is associated with a range of outcomes from subclinical infection to intrauterine death. Foetal infection may result in cerebral calcification, hydrocephalus, microcephaly, choroidoretinitis.[10] The newborn often has a low birth weight, hepatosplenomegaly, jaundice, anemia, petechiae and eye damage. About 10% of the affected fetuses have the severe form of congenital toxoplasmosis which can include blindness, mental retardation or even death.[10]

The social and reproductive maladjustment because of repeated pregnancy wastage, cost of treatment and morbidity caused to the infant makes toxoplasma infection, a major cause of concern. This becomes even more worrisome against the background that maternal toxoplasmosis and consequently congenital toxoplasmosis is largely preventable. In India, there is a lack of adequate literature on the burden of toxoplasma infection in pregnant females and the effect it has on perinatal morbidity and mortality. Thus, the current study was planned with the objective(s) to document the seroprevalence of toxoplasma in pregnant females attending a tertiary care hospital and to study the effect on perinatal morbidity and mortality.

MATERIALS AND METHODS

Study Setting and Participants
The study was conducted in Queen Mary's Hospital, King George Medical University (earlier known as KGMC), Lucknow, Uttar Pradesh, India. It is a tertiary level hospital that serves predominantly lower and middle class population. All pregnant women coming to hospital for antenatal care at Queen Mary's Hospital were contacted, explained and asked to participate in the study. Inclusion criteria consisted of pregnant women in any month or trimester of pregnancy. Antenatal women who have given written consent for voluntary participation in the study and consent was also taken from the patient for affording the cost of the serological test were included in the study.

Sample Size and Sampling Framework
Assuming a prevalence of 15%, 95% confidence interval and a power of 80%, the required sample size came to be

236.[11] Usually 10% of the participants don't respond, taking this rate in the account; the total sample was rounded off to 260 finally. Systematic random sampling was adopted with every third pregnant female that attended the antenatal clinic being included in the study. Enrolment of the pregnant females continued until the required sample size was achieved.

Data Collection
The study participants were contacted by the investigator personally and a written consent was taken. Ethical clearance was obtained from institutional ethics review committee of King George Medical University (earlier known as KGMC), Lucknow, Uttar Pradesh, India. Pregnant females in any of the three trimesters were enrolled in the study. Proper antenatal care was provided in the form of obstetrical examination and antenatal advice(s). They were invited to participate in the present study after counselling and informing them about the effects of maternal toxoplasma infection on pregnancy outcome. The willing subjects were exposed to a set of questions from a pre-tested questionnaire. The information obtained from the subjects included socio-demographic data such as age, parity, residence, religion, dietary habits and socio-economic status. Clinical data registered in questionnaire included obstetric history (past and present), gestational age and contact of the mothers with cat, exposure to raw meat and source of water.

Participants were also enquired about the occurrence of any comorbidity during pregnancy and were asked specifically about any episode of fever or night sweats, rashes, malaise or flu like illness, headache and lymphadenopathy. Relevant investigations (haemoglobin, blood group, VDRL, HBsAg and ultrasound) were conducted and medical history was recorded. After an informed consent, blood sample was drawn to detect IgM and IgG antibodies against toxoplasma. Delivery outcomes were recorded with reference to abortions, still births, delivery of congenitally malformed baby and gestational age at delivery.

Laboratory Investigations
For the purpose of serological investigation, 4 ml of blood sample was drawn from each case and after separation of sera, was stored at 4 degree centigrade until analysis. The samples were then transported to Department of Microbiology, King George Medical University, Lucknow for analysis. Toxoplasma IgM and IgG titres were measured using ENZYWELL (ELISA kit) using appropriate techniques. Those with IgM positive were retested for IgG after 6 weeks and labelled Toxoplasma positive if there was either seroconversion from negative to positive or atleast a four fold rise in IgG titres.

Statistical Analysis

Data was analysed using SPSS version 18.0 for statistical analysis. Proportion and mean(Std. Dev) were calculated wherever applicable. Chi-square test was used to compare significance of difference between two proportions. Value of $p < 0.05$ was taken as significant statistically.

RESULTS

Majority of the 260 total pregnant females (participants), i.e. 114 (43.9%) were in less than 25 years age group (Table 1). Majorities (80.4%) of the subjects had urban residence and were Hindu by religion (221/260; 85%). The subjects were classified according to the Kuppuswami socio-economic classification. Maximum number of subjects were from middle socio-economic class (i.e. 47.3%) followed by lower class (33.5%). In 260 pregnant women, 100 (38.5%) were gravida 1, 67 (25.77%) were gravida 2 and 93 (35.77%) were gravida 3 or more (Table 1). Majority (47.3%) of the participants were enrolled in the second trimester followed by third trimester (31.54%) while only one-fifth (21.1%) were enrolled in first trimester.

IgM and IgG Seroprevalence in Study Participants

A total of 260 subjects were screened for IgM Toxo antibodies, among which 23 (8.8%) were IgM positive. Majority (20/23; 86.9%) of the cases seropositive for Toxoplasma IgM antibodies were asymptomatic. IgM seropositivity was found to be highest among females aged more than 30 years (19.7%), compared to other two age groups (p=0.007) (Table 2). Also, IgM seroprevalence was found to be highest in gravida more than or equal to 3 (15%) (p=0.009), pregnant females belonging to low socioeconomic group (14.9%) (p=0.01) and those who were Muslims (35.9%) by religion (p=0.001). IgM seropositivity was found to be higher in predominantly non-vegetarians (17.65%) than in vegetarians (6.7%) (p=0.01). Further, IgM seroprevalence in subjects with exposure to raw meat was 66.67% while it was 6.77% in those who had no exposure to raw meat (p=0.001) (Table 2).

Out of the 260 subjects screened for IgG toxo antibodies, 40 were IgG positive (15.4%). Further, of these 260 pregnant

Table 1: Distribution of socio-demographic and pregnancy related characteristics among the study participants (N=260)

Variable (s)	Number (n)	Percentage
Age (in completed years)		
≤25	114	43.8
26-30	85	32.7
>30	61	23.5
Socio-economic class		
Lower	87	33.5
Middle	123	47.3
Upper	50	19.2
Religion		
Hindu	221	85
Muslim	39	15
Place of residence		
Urban	209	80.4
Rural	51	19.6
Gravida		
1	100	38.5
2	67	25.8
≥3	93	35.7
Gestational age (weeks)		
Upto 12	55	21.2
12-24	123	47.3
>24	82	31.5

Table 2: Distribution of the socio-demographic, pregnancy related and other factors amongst the IgM seropositive and seronegative groups (*N*=260)

Variable	IgM (*n* (%))		p-value
	Present	Absent	
Age (in completed years)			
≤25	6 (5.3)	108 (94.7)	0.007
26-30	5 (5.8)	80 (94.2)	
>30	12 (19.7)	49 (80.3)	
Gravida*			
1	7 (7.0)	93 (93.0)	0.009
2	2 (3.0)	65 (97.0)	
≥3	14 (15.0)	79 (85.0)	
Gestational age (weeks)*			
Upto 12	8 (14.5)	47 (85.5)	0.95
>12-24	11 (16.3)	112 (83.7)	
>24	4 (4.9)	78 (95.1)	
Socio-economic status*			
Low	13 (14.9)	74 (85.1)	0.01
Middle	8 (6.5)	115 (93.5)	
High	2 (4.0)	48 (96.0)	
Religion			
Hindu	9 (4.1)	212 (95.9)	0.001
Muslim	14 (35.9)	25 (64.1)	
Place of residence*			
Urban	20 (9.8)	189 (90.2)	0.40
Rural	3 (5.9)	48 (94.1)	
Dietary habit			
Predominantly vegetarian	14 (6.7)	195 (93.3)	0.01
Predominantly non-vegetarian	9 (17.7)	42 (82.3)	
Source of drinking water*			
Tap water	20 (8.3)	222 (91.7)	0.80
Tubewell/bore well	3 (16.6)	15 (83.4)	
Exposure to cats*			
Yes	2 (14.3)	12 (85.7)	0.46
No	21 (8.5)	225 (91.5)	
Exposure to raw meat*			
Yes	6 (66.7)	3 (33.3)	0.001
No	17 (6.7)	234 (93.3)	

*Fischer exact test was used to compare proportions

females, 69 (26.5%) subjects had previous bad obstetrics history. IgG seroprevalence was significantly higher (17/69; 24.7%) in women with previous bad obstetrics history than in those without previous bad obstetrics history (23/191; 12.1%) (p=0.013).

Pregnancy Outcomes of the Study Participants

The study enrolled 260 antenatal women, out of which 207 (79.6%) could be followed for the pregnancy outcome. Among 207 subjects, 146 women had no pregnancy related complications and delivered normal babies at term either vaginally or by caesarian section. The rest 61 subjects had pregnancy complications, the important ones being pre-term delivery (34/61; 55.7%), unexplained still births (11/61; 18%), and abortions (9/61; 14.7%) (Figure 1). There were 23 IgM positive females and all of them could be followed for pregnancy outcome. Out of them, 5 had preterm delivery, 4 had unexplained stillbirth, 4 had abortions, 2 had congenitally malformed babies and there was 1 case of intra-uterine growth retardation (IUGR). There were 4 cases of unexplained stillbirths in seropositive group (4/23; 17.4%) compared to 7 within seronegative group (7/184; 3.8%) (p=0.006) (Table 3). Similarly, the proportion of congenitally malformed babies in the two groups varied significantly i.e. 8.7% (2/23) in seropositive and 0.54% (1/184) in seronegative group (p=0.002). There were 9 subjects who had abortions in present pregnancy in this study; 17.4% (4/23) were from IgM positive group and 2.7% (5/184) were from IgM negative group (p=0.001) (Table 3).

DISCUSSION

Toxoplasmosis is the most important infection among the TORCH group of infections. The transplacental
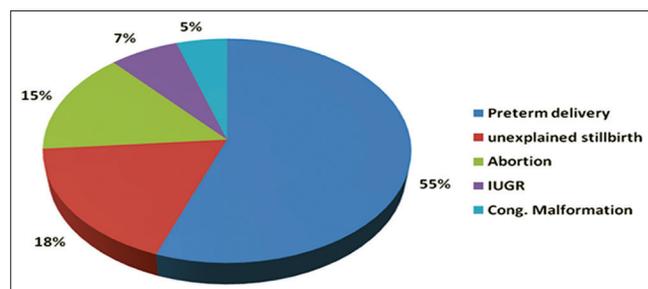

Figure 1. Birth outcomes of expecting females who had complications during pregnancy (N=61)

transmission of the infection to the fetus may lead to abortion, intrauterine growth retardation, neurological and ophthalmological complications, congenital malformation, still births and preterm deliveries.[1,2,10,12] It may present late in childhood as deafness, blindness and mental retardation. The infection is preventable and treatable, therefore early serological screening for toxoplasma antibodies in mothers can reduce the perinatal morbidity and mortality. The present study was conducted to find out the seroprevalence (burden) of toxoplasmosis in pregnant women. The various socio-demographic factors predisposing to this infection and the impact of the infection on the pregnancy outcome was also studied.

In the current study, around 9% of the pregnant females were positive for IgM and nearly 15% were positive for IgG. These findings corroborate with studies done in various parts of the country. Yasodhara P et al in their hospital based study in Andhra Pradesh found the prevalence of IgM in pregnant females to be 13%.[13] Similarly, Padmavathy M et al in Bangalore and Turbadkar D et al in Mumbai reported the prevalence of IgM positivity to be around 6% and 11% respectively.[14,15] Further, Padmavathy M et al also documented IgG seropositivity to be 8% whereas in Turbadkar D et al study, the prevalence came out to be 42%.[14,15] Also, Kaur R et al in Delhi found the seropositivity for toxoplasma to be 11.6%.[16] In contrary, there are few studies which report a higher prevalence than the outcome of this study. This observed difference could be due to the fact that majority of these studies had included pregnant females with BOH (bad obstetric history) and the socio demographic profile and morbidity pattern of the study participants would have differed from the present study population. Sarkar MD et al in their study at a tertiary care hospital in Andhra Pradesh reported the prevalence of IgM seropositivity for toxoplasma to be 49.5%.[17] Similarly, Chintapalli S et al in a hospital based study in Vishakhapatnam reported IgM and IgG toxoplasma seropositivity in pregnant females to be 20% and 45% respectively.[18] Chopra S et al in Amritsar documented IgM seropositivity in nearly 43% of the pregnant females with bad obstetric history.[19]

IgM seropositivity was highest in >30 years age group (19.7%) and the seroprevalence was found to be significantly increasing with increasing age. This increase trend with

Table 3: Correlation of adverse perinatal outcomes with IgM seropositivity (N=207)

IgM positivity	IUGR n (%)	Preterm birth n (%)	Still births n (%)*	Congenital abnormality n (%)*	Abortion n (%)*	No adverse perinatal outcome n (%)
Yes (N=23)	1 (4.3)	5 (21.7)	4 (17.4)	2 (8.7)	4 (17.4)	7 (30.4)
No (N=184)	3 (1.6)	29 (15.8)	7 (3.8)	1 (0.5)	5 (2.7)	139 (75.5)
P-value	0.372	0.466	0.006	0.002	0.001	0.004

*P<0.05; Fischer exact test used to compare proportions

age was similarly reported by Nowakowska D et al in their study in Poland.[20] They found that seroprevalence linearly and significantly increases with age(p<0.001). Also, Sarkar MD et al in Andhra Pradesh found that the maximum seroprevalence was observed in pregnant females aged >30 years.[17] In our study, multigravid females (gravida ≥3) had higher prevalence of IgM seropositivity (15%), similar to a seroepidemiological survey by Nissaportan V et al among Malaysian pregnant women.[21] They found an increase in prevalence with increasing parity (p<0.05). Ashrafunnesha et al also found that seroprevalence increased with age and parity.[22] In this study, the seroprevalence was significantly highest among mothers belonging to low socio-economic class (14.9%) (p=0.01). The same observation was made by Yashodhara P et al in their study on correlation between socio-economic status and prevalence of the toxoplasma infection.[23] Around one-third (33%) women of lower socio-economic status and 22% of high socio-economic status were seropositive (p<0.01).[23] Ashrafunnesha et al also found a higher seroprevalence in lower socio-economic group (53%) than in upper class (22%).[22]

The seroprevalence of IgM, in the present study, was significantly higher in Muslims (35.9%) than in Hindus (4.1%) (p<0.001). This might be because Muslims are generally non-vegetarians and engaged in meat business. Higher prevalence of infection is seen in mothers who were exposed to raw meat (66.67%) compared to those without exposure to raw meat (6.77%). The difference was found statistically significant (p<0.001). Akoijam BS et al found a statistically significant association with raw-meat consumption was found.[24]

Elnahas A et al conducted a sero-epidemiological survey in Sudan in pregnant females with toxoplasmosis, raw meat eating was found to be one of the important risk factors for toxoplasma specific seropositivity.[25] Cat possession appeared not to affect the prevalence of toxoplasmosis antibody, which is in agreement with the findings of Legnain M et al in Libiya and Cook AJ et al in European countries.[26,27] Presence of cats was found to be of primary reason in the transmission of infection in many areas of the world but in India, harboring cats is not a frequent practice and most cats are straying.

In the present study, preterm delivery (21.7%), abortion (17.4%) and still birth (17.4%) were the three common outcomes observed in the females who had tested IgM positive. Sarkar MD et al had also reported similar findings.[17] He found that the commonest form of pregnancy wastage was abortion (52%). The second most common reason was stillbirths (37%) followed by premature deliveries (8%). Padmavathy M et al in their study also found that maximum number of IgM positive cases of abortion (33.4%) were

associated with toxoplasma.[14] Surpam RB et al in Nagpur found that maximum number of cases of abortion (27%), intrauterine death (18%) and preterm labor (18%) was associated with toxoplasma infection.[28] Chopra S et found that the highest percentage of antibodies to toxoplasma was in cases of abortions.[19] Further, Elnahas A et al in their seroepidemiological study on toxoplasmosis in Sudanese women found that 33% of seropositive women and 9.7% of seronegative mothers had history of intrauterine deaths.[25] Also, Muna M et al in a case-control study in 200 pregnant women attending Basra Maternity Hospital in Iraq found that risk of developing abortion among women who were seropositive was 3.6 times higher than in seronegative women.[29]

This is amongst the few studies in North India that attempted to look into the burden of toxoplasma infection its risk factors.

The findings has provided a practical scenario of the current health situation in terms of toxoplasmosis, provide an actual morbidity and mortality pattern and hence, has given reliable estimate of the burden of the disease, its risk factors and may help to plan screening and prevention activities accordingly. However, the study has some limitations and these should be considered while interpreting the findings. Firstly, this is a study conducted in a hospital, which relatively has higher percentage of morbidity and its extrapolation can't be done. Secondly, around one-fifth of the pregnant females could not be followed up to document their birth outcomes. This might affect the result findings.

CONCLUSION

The seroprevalence of the anti- toxoplasma antibodies was notable amongst the pregnant women who attended antenatal care clinic. This study has supported the fact that toxoplasma infection plays a pivotal role in adverse foetal outcome. The study also found that the socio-epidemiological aspects constitute an important contributing factor for the spread of the disease. Considering that toxoplasmosis is amenable to treatment, early detection with repeated serological examination and treatment in all pregnancies could reduce the hazard substantially. Both toxoplasma-specific IgG and IgM antibodies based routine serological screening should be prescribed, counselled and advised to all pregnant women. Even for those females with negative results, targeted IEC activities and counselling related to preventive and protective measures are of importance.

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