Original Article

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Microbiology Section

Screening of Pregnant Women for Anti-*Toxoplasma* Antibodies and their Newborn for Vertical Transmission

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ABSTRACT

Introduction: Toxoplasmosis is a world-wide protozoan-zoonosis caused by *Toxoplasma gondii* (*T. gondii*). Primary infections during pregnancy may result in miscarriages, still births, and congenital malformations in the new born. Studies on vertical transmission of toxoplasmosis from India are lacking.

Aim: To estimate the seroprevalence of antibodies to *T. gondii* among pregnant women from the rural population of Kolar and to document vertical transmissions, if any.

Materials and Methods: Anti-*Toxoplasma* IgG levels were estimated among 251 women admitted for labour at a tertiary care hospital in Kolar, Karnataka, between December 2014 and October 2016, by Enzyme Linked Immunosorbent Assay (ELISA). Demographic, socio-economic, and obstetrical data along with exposure to risk factors among the participants were recorded. Two hundred and fifty one cord blood samples of the newborns of the above mothers were tested for anti-*Toxoplasma* IgM antibodies by μ capture ELISA. The validity of an IgM positive reaction was evaluated. The differences in proportions were analysed by the Chi-square test and the differences in means were analysed by the unpaired t-test. A p-value <0.05 was considered significant.

Results: IgG antibodies to *T. gondii* could be detected in 53 (21.1%) of the mothers tested; the titres ranged between 35 IU/ml – 350 IU/ml. Mothers from lower socio-economic strata had significantly higher prevalence as compared to mothers from middle classes. The seropositivity was not significantly associated with gravid status, literacy, occupation, exposure to cats, consumption of raw meat, salad, or drinking untreated water, gestational age, previous history of abortion or the mode of delivery. Cord blood samples from 5 (2 %) of the newborns gave positive IgM reactions, but they were interpreted as false positives as there was no evidence of infection in their respective mothers or the baby lacked antibodies on follow up.

Conclusion: About one fifth of the pregnant women in Kolar region, possess anti-*Toxoplasma* IgG antibodies and are immune to toxoplasmosis. The rest, constituting a large proportion, are susceptible and run the risk of infection during pregnancy. Routine screening of women for *Toxoplasma* infections during pregnancy and screening of newborns for congenital toxoplasmosis are recommended.

Keywords: Abortion, Cord blood serology, Maternal *Toxoplasma* infection, Risk factors for toxoplasmosis

INTRODUCTION

Toxoplasmosis is a protozoan-zoonosis caused by *Toxoplasma gondii (T. gondii)*. Domestic cats and wild felines act as definitive hosts by supporting the sexual development of the parasite in their small intestine and excreting oocysts. Many species of vertebrates such as cattle, sheep, goats, and mice get infected by consuming oocysts and act as intermediate hosts [1].

Human infections by *T. gondii* occur by consumption of food and water contaminated with oocysts found in the soil or by eating improperly cooked meat of infected animals. Human infections by *T. gondii* are usually asymptomatic, but primary infections during pregnancy may result in miscarriages, still births, and congenital malformations in the newborn [1].

The vertical transmission of *T. gondii* infection from mother to the baby in a particular geographical region is known to be associated with the seroprevalence rate [2]. Serological surveys from different parts of India have reported a wide variation in the prevalence of anti-*Toxoplasma* antibodies among women in the child bearing age [3,4]. There are a few case reports on congenital toxoplasmosis from India, but there is a need for studies involving mother baby pairs [5-8]. There is no data on the seroprevalence of antibodies to *T. gondii* among pregnant women from the rural population of Kolar region and the possible existence of vertical transmission in this region has not been explored. We have undertaken a serological study to estimate the prevalence of IgG antibodies to *T. gondii* among pregnant women admitted for labour at a tertiary care hospital in Kolar and looked for serological evidence for vertical transmission of *T. gondii* among newborns.

MATERIALS AND METHODS

Two hundred and fifty one mother's admitted for labour at a tertiary care hospital at Kolar, between December 2014 and October 2016, who consented to participate in the study along with their newborns, were included in this cross-sectional observation study. Pregnant women admitted as obstetric emergencies for labour were excluded from the study. Anti-*Toxoplasma* IgG antibody titres were estimated in the sera of mothers and anti-*Toxoplasma* IgM antibodies were determined in the cord blood of the newborns. The sample size was calculated taking into consideration the pan India sero-prevalence of 22.4% [9]. The study was approved by the institutional ethics committee and informed consent was obtained from the participating mothers.

Detection of maternal anti-*Toxoplasma* IgG antibodies

The antibody levels were estimated using a commercial anti-*Toxoplasma* IgG ELISA kit (NovaTec Immundiagnostica, Germany). A titre of ≥35 International Units/ millilitre (IU/ml) was considered as positive as per the manufacturer's criteria. The demographic and socio-economic data, educational status, obstetrical history

and exposure to risk factors of *toxoplasma* infection among the participants in the study were recorded on a predesigned proforma. The socio-economic classification was done on the basis of B.G. Prasad socioeconomic scale May 2016 [10].

Detection of anti-*Toxoplasma* IgM antibodies in cord blood samples

Two hundred and fifty one cord blood samples of the newborns of the above mothers were tested for anti-*Toxoplasma* IgM antibodies by the anti-*Toxoplasma* IgM μ capture ELISA (NovaTec Immundiagnostica kit, Germany) as per the manufacturer's instructions.

Establishing validity of IgM positive reactions

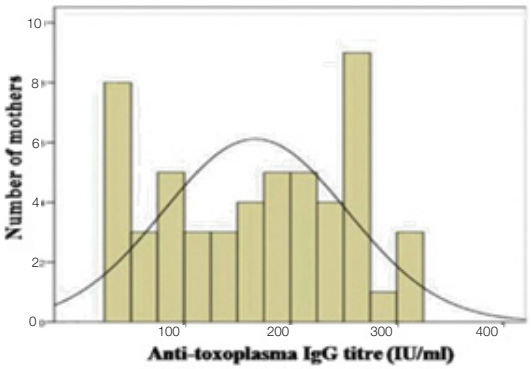
The validity of an IgM positive reaction in the cord blood was evaluated by: a) estimating the IgG antibody levels in the babies' cord blood, as done for serum samples from mothers, to look for any significant difference in titres between babies' and that of mothers; and b) by testing the respective mother's serum sample for anti-*Toxoplasma* IgM antibodies which could suggest recent infection in the mother. Wherever necessary, the children were followed up by doing a thorough clinical examination and specially looking for any of the clinical features of congenital infections such as hydrocephalus, chorioretinitis and delay in developmental mile stones. In such children, anti-*Toxoplasma* IgG levels and IgM antibodies were also determined after 6 months of age.

STATISTICAL ANALYSIS

The data is presented as frequencies in tables with percentages. The quantitative measures of central tendency have been expressed as mean±standard deviation. The IgG titers among positive mothers were plotted in a distribution curve. Unpaired t-test was used to analyse the differences in mean age among IgG positive and negative mothers. To analyse the difference in proportions Chi-square test was used. A p-value of <0.05 was considered significant.

RESULTS

Among the 251 samples tested from mothers, IgG antibodies to *T. gondii* at titres of ≥ 35 IU/ml were detected in the sera of 53 (21.1%) of them. The titres ranged between 35 IU/ml – 350 IU/ml with a mean ± standard deviation of 167±86 IU/ml. The distribution of titres conformed to normal distribution pattern [Table/Fig-1].



[Table/Fig-1]: Distribution of anti-*Toxoplasma* IgG titers among the 53 positive mothers.

The demographic, socio-economic, and behavioural characteristics of mothers along with seroprevalence of *Toxoplasma* IgG antibodies are presented in [Table/Fig-2]. Majority of the mothers (58 %) were in the first half of third decade of life. Housewives constituted 80 % and most of them (95%) were literate. The population belonged to middle and lower socio-economic classes. A sizeable proportion (43%) belonged to lower socio-economic strata. The study participants in general consumed salad, drank untreated water, and rarely owned cats.

Characteristics of mothers	Total tested N=251	No. positive for Taxoplasma IgG antibodies, n(%)	p-value*
Age group			
18-20	44	6(13.6)	0.143
21-25	145	34 (23.4)	
26-30	53	9 (16.9)	
31-35	9	4(44.4)	
Occupation			
Housewives	201	44 (21.9)	0.330
Daily wage manual labourers (Coolie)	17	5 (29.4)	
Farmers	8	2 (25)	
Others†	25	2 (8)	
Education			
Literate	238	48 (20.2)	0.116
Illiterate	13	5 (38.5)	
Socio-economic Status			
Middle class	144	23 (16)	0.021
Lower class	107	30 (28)	
Owning cat			
Yes	35	7 (20)	0.862
No	216	46 (21.3)	
Consumption of Salad			
Yes	191	37 (19.4)	0.228
No	60	16 (26.7)	
Drinking untreated water			
Yes	157	30 (19.1)	0.316
No	94	23 (24.5)	

[Table/Fig-2]: Demographic, socio-economic, and behavioural characteristics of mothers along with seropositivity for *Toxoplasma* IgG antibodies.
†Tailor, Nurse, Teacher, Engineer, Clerk, Pharmacist
*Chi-square Test; p-value of <0.05 was considered significant

There was no statistically significant difference between the mean age of the seropositive and seronegative mothers (t=1.298 and p=0.195). The prevalence of antibodies among the mothers belonging to lower socio-economic strata was 28% as compared to 16% among those belonging to middle classes (class 3 and 4). This higher prevalence among women of lower socio-economic strata was statistically significant (p=0.021). There was no significant association with seropositivity and occupation, literacy, owning of cats, and consumption of salad, or drinking untreated water [Table/Fig-2]. The obstetrical characteristics of mothers along with seroprevalence of *Toxoplasma* IgG antibodies are presented in [Table/Fig-3]. Multigravida outnumbered primigravida, majority of them had term gestation and they delivered normally. There was no significant association with seropositivity and gravid status, gestational age, previous history of abortion, and mode of delivery (p =0.231, 0.174, 0.451, 0.724 respectively).

Cord blood samples from 5 (2%) of the 251 babies gave a positive anti-*Toxoplasma* IgM antibody reaction. The Optical Density (OD) values of the positives were just above the cut-off value: they were on an average 0.043±0.015 above the cut-off value of the test in which they were included. IgG antibodies to *T. gondii* could only be detected in the cord blood sample of 1 of these 5 babies. Anti-*Toxoplasma* IgM positivity and IgG antibodies in the cord blood of these babies and in the serum samples from their respective mothers is presented in [Table/Fig-4].

The IgM positivity in the cord blood samples of the newborns were considered as false positive reaction in 4 cases, because there was no evidence of maternal infection at all: The serum samples simultaneously collected from their mothers lacked IgG and IgM anti-*Toxoplasma* antibodies. Though both, the cord blood samples

Characteristics of mothers	Total tested, N=251	No.positive for *Toxoplasma* IgG antibodies, n(%)	p-value*
Gravida			
Primigravida	113	20(17.7)	0.231
Multigravida	138	33(23.9)	
Gestational age			
Term	230	51(22.2)	0.174
Preterm	21	2(9.5)	
Previous history of abortion			
Present	39	10(25.6)	0.451
Absent	212	43(20.3)	
Mode of delivery			
Normal delivery	175	38(21.7)	0.724
Caesarean section	76	15(19.7)	

[Table/Fig-3]: Obstetrical characteristics of mothers along with seropositivity for *Toxoplasma* IgG antibodies.
*Chi-square Test; p-value of <0.05 was considered significant

Case	IgM in Baby's cord blood	IgG in Baby's cord blood	IgG in Mother's serum	IgM in Mother's serum	Inference
1	+	-	-	-	False positive
2	+	-	-	-	False positive
3	+	-	-	-	False positive
4	+	-	-	-	False positive
5-1	+	+	+	-	False positive
5-2*	-	-	N.D	N.D	

[Table/Fig-4]: Anti-*Toxoplasma* IgM and IgG antibodies in the cord blood of babies and sera from their respective mothers.
*Venous blood sample of the baby collected at 11 months on follow up, N.D = Not Done

from the baby and mother's serum sample had IgG antibodies in one case, there was no four-fold difference in titres between them (case 5: [Table/Fig-4]) and on follow up of this child at 11 months of age neither IgG nor IgM antibodies to *T. gondii* could be detected in the serum sample; there were no clinical features of congenital toxoplasmosis either.

DISCUSSION

We report here a seroprevalence of 21.1% for anti-*Toxoplasma* IgG antibodies among the rural pregnant women tested from Kolar region, southern Karnataka. Thus, 79% of pregnant women who lacked anti-*Toxoplasma* antibodies are at the risk of acquiring primary infection during pregnancy. Seropositivity rates comparable to that found in our study, have also been reported by earlier studies from India [3,9]. However, the seroprevalence rates do not seem to be uniform throughout the country. It is reported to be as high as 77% in Kumaon region of Himalayas, 48% in Northeast India, and 37% in Coastal Karnataka [4,9,11].

The prevalence of *Toxoplasma* antibodies may vary in different populations depending upon climatic and socio-economic conditions, and behavioural patterns [9]. In our study we observed that there was a significant association between seropositivity and lower socioeconomic strata. Similar observation has been made in an earlier study from Assam [12].

We observed IgM positivity in 5 (2%) of cord blood samples tested, but their validity could not be established; they were considered as false positives. Such false positive reactions in ELISA for anti-*Toxoplasma* IgM antibodies are well documented [13,14]. We emphasize that one should evince care in reporting IgM positive ELISA reactions for the diagnosis of toxoplasmosis.

One of the mothers in our series had anti-*Toxoplasma* IgG antibodies in her serum without the presence of anti-*Toxoplasma* IgM antibodies, indicative of infection in the past. However, considering the possibility of latent infection, we followed up her baby whose cord blood gave positive reaction for anti-*Toxoplasma* IgM and IgG antibodies. On follow up, we could not find any clinical or serological evidence for vertical transmission of toxoplasmosis. Though vertical transmission of toxoplasmosis has been documented in India, to the best of our knowledge, there are no studies on vertical transmission of toxoplasmosis involving a series of mother-baby pairs. In this direction, our study may be considered as a pilot study. As the vertical transmission of toxoplasmosis is known to be directly proportional to the prevalence of antibodies among women in the child bearing age, studies similar to ours need to be undertaken at least in high prevalence areas of the country [11].

Recently a higher frequency of vertical transmission of toxoplasmosis by using polymerase chain reaction for detection of *T. gondii* DNA in umbilical cord tissue samples of neonates has been reported from Libya [15]. Such studies based on molecular diagnosis along with serological studies may contribute towards more accurate estimations of vertical transmission in the population.

LIMITATION

We could not detect any vertical transmission of toxoplasmosis among the 251 cord blood samples of newborns tested; but a false positive IgM reaction was encountered in 5(2%) of the samples tested. If we had detected even 1 case of vertical transmission among 251 newborns, then the vertical transmission rate would have been 0.4 %. Thus the vertical transmission rate in Kolar region appears to be less than 0.4 %. Secondly, we have tested the serum samples from mothers and cord blood samples from newborns for anti-*Toxoplasma* antibodies of IgG and IgM classes only. Inclusion of tests to detect anti - *Toxoplasma* IgA and IgE class of antibodies, in addition, would have increased the sensitivity and specificity of detection of recent *Toxoplasma* infections in mothers and vertical infection in newborns.

CONCLUSION

Our data shows that only about one fifth of pregnant mothers from Kolar region of Karnataka are naturally immune to toxoplasmosis and a large proportion, nearly 80% women are at risk of toxoplasmosis during pregnancy. In this context it becomes necessary to screen pregnant women routinely to detect recent *toxoplasma* infections. It is also essential to check the newborns for evidence of congenital toxoplasmosis. These efforts would pave way for prevention of infections during pregnancy and treatment of vertical infections.

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