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Seroprevalence of Toxoplasma Gondii among Pregnant Women Having Bad Obstetric History in a Tertiary Care Hospital of Eastern Odisha

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Abstract
Introduction: *Toxoplasma gondii infection is a major risk factor in women having bad obstetric history. If vertically transmitted it can result in adverse pregnancy out comes ranging from abortion to congenital anomalies.*
Objective: *The current case control study is undertaken to evaluate the prevalence of T.gondii and the associated environmental and personal factors that may contribute to infection, among pregnant women in the eastern part of Odisha.*
Materials and Methods: *Serum samples of 120 pregnant women having bad obstetric history and 60 pregnant women without such history of age group 20 to ≥ 40 years attending a tertiary care Hospital in Cuttack district of Odisha were tested for anti-Toxoplasma IgG and IgM antibodies by ELISA. Epidemiological data of the women was collected through a standard questionnaire.*
Results: *The prevalence of anti-Toxoplasma IgG was 17.5% in cases and 13.3% in controls, and of IgM was 2.5% in cases and 1.7% in controls. Sero prevalence increased steadily from 20-24 yrs (13%) to 30-34 yrs (30%) and most women were in second trimester. Though statistically significant association was observed between T. Gondii seroprevalence and women of low socio economic status living in urban areas, no such association was observed with other risk factors like contact with cat and soil or consumption of meat, raw salad and contaminated water.*
Conclusion: *As Toxoplasma seroprevalence among poor urban women is considerable and support the concern that women may be vulnerable to such infection all pregnant women should be screened routinely and be provided preconception health education regarding Toxoplasmosis to prevent primary infection.*
Keywords: *Toxoplasma gondii, bad obstetric history, seropositivity, ELISA*

Introduction

Toxoplasma gondii is an obligatory intracellular protozoan parasite. Cats and wild Felines are the only definitive host while other worm blooded animals including humans are intermediate hosts[1]. Infection is acquired by ingestion of viable tissue cysts in uncooked or under cooked meat or oocysts excreted by cats that contaminate food and water or by vertical transmission[2]. It was estimated that one third of the world's population is infected by *T.gondii* [3].Studies show that about 90% of infections in immune competent humans are asymptomatic.

While up to 10% present with fever, malaise, head ache with cervical lymphadenopathy or ocular disease[4]. In immune suppressed patients, toxopl-

asmosis can cause severe encephalitis by acute infection or reactivation of latent infection [5]. Infection during pregnancy may cause spontaneous abortion, still birth, or congenital anomalies such as microcephaly, hydrocephaly, intrauterine growth retardation, chorioretinitis in the offspring depending on the gestational age, when maternal infection is acquired. It may present late in childhood as deafness, blindness and mental retardation[6]. Congenital infections acquired during the first trimester are more severe than those acquired in the second and third trimester[7]. Anti-parasitic treatment during pregnancy may reduce the risk of transmission to the foetus if it is diagnosed early [8]. For diagnosis, isolation of *T. gondii or* demonstration of DNA by PCR are confirmative methods but, these are practically difficult[9]. Hence, Toxoplasmosis is mostly diagnosed by detection of specific antibodies by Latex agglutination test, indirect fluorescent antibody test or the specific IgM and IgG-based ELISA[10]. Following an acute infection IgM antibody titres rise from 5 days to weeks, reaching a peak after 1 to 2 months then rapidly decline. But, in many cases IgM antibody may persist for years[11]. IgG antibodies appear later than IgM, detectable within 1 to 2 weeks and reach the peak after 3 to 6 months following acute infection. They will be detectable for years, even throughout life after acquiring infection [12]. If IgG is positive and IgM is negative, this indicates an old infection. If both IgG and IgM are positive, this indicates possibility of a recent infection acquired within a year or a false-positive test result. If IgG and IgM are both negative, this indicates the absence of infection or extremely recent acute infection.[12][13] If acute infection is suspected, repeat testing is recommended within 2 to 3 weeks in which,4-fold rise in IgG antibody titres confirms a recent infection[14][15]. If interpretation of IgG and IgM titers are difficult to establish acute infection, IgG avidity test should be done. High avidity implies that infection occurred at least 5 months before testing [12].

Since anti-toxoplasma antibody testing is not routinely done in many parts of country, information regarding infection, diagnosis and interpretation of test results is lacking. As there was limited data on seroprevalence of Toxoplasmosis in pregnant women in eastern Odisha, the present study was carried out to evaluate the disease prevalence, and its association with known risk factors.

Materials and Methods

Study design, area and population

This prospective case control study was conducted from September 2014 to October 2016 in a tertiary care Government Hospital at Cuttack District of Odisha.

Cuttack a part of eastern India has a tropical wet, monsoon rainforest type climate. Average rain fall is 1700mm, humidity of 78% and average annual temperature is 27.5^0C. Coordinates of the district are, Latitude 20° 30' 0" N and longitude 85° 50' 0" E.

180 pregnant women above 19 years of age having gestation within 28 weeks, who attended the Antenatal and Gynaecology Unit of our Hospital were included in the study. The BOH (bad obstetric group) group consisted of 120 women with a history of abortion, IUD or still birth, IUGR; preterm delivery or congenital anomalies and control group consisted of 60 women without history of any adverse pregnancy out come. Women with other medical conditions which could cause miscarriage such as hypertension, diabetes mellitus, syphilis, rhesus incompatibility, and consanguinity were excluded from the study. Similarly women, having AIDS or severe autoimmune disorder e.g Rhumatoid arthritis, or receiving cancer chemotherapy and other forms of immunosuppressive therapy, were excluded from study.

Informed written consent was obtained from all the participants. Ethical clearance for the study was obtained from the institutional Ethics Review Committee.

Data collection: Epidemiological data of the participants such as age, residence type, socio-economic status, period of gestation, obstetric history as well as inforation regarding association with known risk factors for infection such as contact with cats or soil, consumption of raw salads, undercooked meat or contaminated water and past history of blood transfusions or organ transplantation were collected using an interviewer-administered questionnaire.

Laboratory detection of anti Toxo IgG and IgM antibodies

Two millilitres of venous blood was collected aseptically from each participant. Serum was separated by centrifugation at 3000 rpm for 10 minutes, labelled and stored at -20^{0}C until tested. All the sera were tested for the presence of *Toxoplasma gondii* specific antibodies IgM and IgG using ELISA kit of Delta Biologicals (Subsidiary of ERBA Diagnostics–ITALY) according to the manufacturer's guidelines. The results were read by a Micro well reader and compared in parallel with positive and negative controls. Optical density was read at 450 nm on an ELISA reader. Cut-off points and antibody index calculations were done according to manufacturer's instructions .When the absorbance of the sample was higher than that of cut-off, the sample was considered positive for the presence of specific IgM/ IgG .The ratio between the OD value of the sample and that of the cut-off was calculated. The sample was considered positive, if the ratio is >1.2, negative if the ratio is <0.8 and border line positive, if the ratio is ± 20% of cut-off (0.8-1.2).

Statistical Analysis

Data was entered in excel sheet and imported into software Statistical Package for the Social Sciences (SPSS version 21) for analysis. Binomial data were evaluated by chi-square to test statistically significant differences. The odd ratio (OR) and its 95% confidence interval (CI) were used to estimate the strength of the association between the *T. gondii* infection and the associated risk factors. Incidences (IgM positivity) and prevalence (IgG positivity) rates are expressed as percentages. P-values less than 0.05 were considered statistically significant.

Results

Serum of total 180 pregnant women attending Antenatal and Gynaecology Unit of the Hospital, were screened for anti Toxoplasma IgG and IgM antibodies by ELISA.

Over all Toxoplasma seropositivity among pregnant women in this study was 16.7% (30/180). Seropositivity was higher in women with bad obstetric history (BOH), (22/120:18.3%) than the control groups (08/60:13.3%). In the BOH group 21(17.5%) women were positive for Toxo-IgG and 3(2.5%) women for Toxo-IgM antibody. Among those three IgM positive women, two were also positive for IgG, and one was negative for IgG. Similarly in the control group 8 women were positive for Toxo-IgG(13.3%) and one woman for Toxo-IgM(1.7%) who was also, positive for IgG. IgM seroprev-alance rate among IgG positives is 9.5%(2 /21) in BOH groups which, indicates incidence of recent Toxoplasma infection (Table-1).In the BOH group maximum seroprevalance (30%) was observed in women between 30 to 34 yrs but, maximum participants were in age group between 25 to 29 yrs, who had seroprevalance of 18.9%.In the control group, women between 30 to 34 yrs, also had maximum seroprevalance (14.3%) (Table-2). In the BOH group highest seroprevalance was also observed in 2nd Trimester (22%), followed by 1st Trimester (18.75%) and 3rd Trimester (9%). Pre term delivery and abortion (67/120:55.8%) were most common bad obstetric history, but Toxo-plasma seroprevalance was highest in women with history of abortion (33.3%) followed by IUD (16%) and preterm delivery (14.7%) (Table-3&4).In BOH group, Seroprevalance were more, in urban areas (13/61:21.3%) than rural areas (9/59:15.25%). Again in urban areas, women of low socioeconomic status had more seropreva-lance, (45.8%) than their rural counterparts (21.7%) (Table-5).

Table-1: Seropositivity of Toxoplasma-IgG and IgM in different ages among BOH and control groups

Type of groups	No of sera Only IgG +ve(%)	No of sera Only IgM +ve(%)	No of sera both Ig M & Ig G + ve(%)	Total No of sero-positives(%)	Total no of sero-negatives(%)	Total	P- value
BOH group	19(15.8)	1(0.8)	2(1.7)	22(18.3)	98(81.7)	120(100%)	
Control group	7(11.7)	0	1(1.7)	08(13.3)	52(8.7)	60(100%)	0.396

Table-2 : Sero positivity of Toxoplasma-IgG and IgM in different ages among BOH and control groups

Age group	no of cases (BOH group)	Sero positivity	P–value	no of cases (Control group)	Sero positivity	P –value
20-24	23	3(13%)		10	2(20%)	
25-29	37	7(18.9%)		29	4(13.8%)	
30-34	30	9(30%)		14	2(14.3%)	
35-39	27	3(11.1%)		06	0(00%)	
≥40	3	0		01	0(00%)	
Total	120	22(18.3%)	0.311	60	08(13.3%)	0.831

Table-3 : Seropositivity of Toxoplasma IgG and IgM in different trimesters among BOH groups.

Age group	No of cases	Sero-positivity(%)
1st Trimester	48	9 (18.75%)
2nd Trimester	50	11 (22%)
3rd Trimester	22	2 (9%)
Total	120	22(18.3%)

Table-4: Association of bad obstetrical events with Seropositivity among BOH groups.

Type of BOH	No of Cases	No of Seropositives (%)	No of Seronegatives (%)	P- value
Abortion	33	11 (33.3%)	22(66.7%)	
IUD/StillBirth	25	4 (16%)	21(84%)	
Pre term delivery	34	5 (14.7%)	29(85%)	0.098
IUGR	19	1 (5.3%)	18(94.7%)	
Congenital anomalis	9	1 (11.1%)	8(88.9%)	
Total	120(100%)	22 (18.3%)	98(81.7%)	

Table 5 : Association of various risk factors with Toxoplasma Seropositivity among BOH and control group

Risk factors	No.of cases (BOH)	No. of seropositives	Odds Ratio (95% CI)	P – value	No. of cases (Control)	No of seropositives	Odds Ratio (95% CI)	P – value
Contact with cat			0.809				2.556	0.628
Yes	19	03	(0.214-3.060)	1.0	08	02	(0.417-15.653)	
No	101	19			52	06		
Contact with soil			0.476				4.600	0.167
Yes	19	02	(0.102-2.233)	0.525	09	03	(0.87-24.316)	
No	101	20			51	05		
Consumption of Under cooked meat							-	-
Yes	00	00	-	-	00	00		
No	120	22			60	08		
Consumption of Raw salads							-	-
Yes	120	22	-	-	60	08		
No	0	00			00	00		
Consumption of Contaminated water			0.471	0.776			1.714	1.000
Yes	10	01	(.057-3.923)		05	01	(0.167-17.626)	
No	110	21			55	07		
Resident type			1.505				0.529	0.655
Urban	61	13	(0.589-3.843)	0.391	38	04	(0.118-2.370)	
Rural	59	9			22	04		
Socio-economic status				0.001				0.593
Low	47	16			36	04	-	
Middle	52	03	-		14	03		
High	21	03			10	01		
History of blood transfusion/organ transplantation			1.3 (0.251-6.731)	1.000			-	1.000
Yes	09	02			2	00		
No	111	20			58	08		

Discussion

Various epidemiological studies show that prevalence of *T. gondii* infection in pregnant women varies substantially among countries. In European countries, prevalence varies from 9 to 67%[16].In Asian countries, low prevalence were found in Korea(0.8%) and Vietnam(11.2%) but, high prevalence were found in Nepalese ,and Indian populations[17][18][19][20]. As reported previously seroprevalence of toxoplasmosis in Indian pregnant women varies between 5% and 80%.[20][21] Over all Toxoplasma seropositivity in this study was 16.7%(30/180) among pregnant women viz was less than the average pan India Toxoplasma-sero prevalence (22.4%), where Singh et.al(2013) observed IgG prevalence of 37.3% in South India, 21.2% in East India , 19.7% in North India and 8.8% in West India and of IgM ranged from 0.4% to 2.9% in four study zones[22].In our study the sero prevalence of *T. gondii* infection in the BOH group was 18.3% vs 13.3% in the control group which was similar to prevalence rate of 20% in spontaneous abortion verses 5% in control, as observed by Anubhuti et.al [23]. In this study all the IgM positive sera were IgG positive, except one in BOH group which was IgG negative. This woman could have acquired very recent Toxoplasma infection where Ig G titer has not yet risen. In the current study among the BOH group, 0.8% were seropositive for only IgM antibodies which indicates acute infection, 15.8% were seropositive for only IgG antibodies which indicate chronic infection, and 1.7% were seropositive for both IgM and IgG antibodies which indicates possible recent past infection of not more than a year. Our results were lower than, results of Sucilathangam.G et.al[24] where over all IgG sero prevalence was 23% and of IgM was 3.8%. Similarly among the control group we observed, IgG seropositivity of 11.7% and of IgM ,1.7 % . These results agreed with the low prevalence rate for IgG (6.7%) and IgM (1.7%)among healthy pregnant women, as reported by Amany M. Kamal etal[25] . But Borkakoty et al, from North East part of India reported high IgG seroprevalence (36.8%) and IgM seroprevalence (5.9%) in women without history of adverse pregnancy outcome.[26] The difference in prevalence rate can be explained by variations in geographical and climatic conditions between different areas as the oocysts sporulates better in hot and humid climate.

In this study seroprevalence among BOH group was highest in women between30 and 34 yrs (30%) than younger age groups and maximum seropositive women were in second trimester (IgG 20% and IgM 2%). These results were in accordance with results of Singh et.al, where prevalence rate steadily increased from 18.1% in women of 18-25yr to 40.5% in women of >40 yrs, and IgM seropositivity was maximum in second trimester (2.25%) [22].

No participant in our study was consuming raw/ under cooked meat in traditional home cooking. But, all were consuming raw salad. Most of the urban women were using drinking water supplied by municipality and the rural women from tube well or dug well. In the present study statistically no significant relationship was observed between the prevalence of *Toxoplasma* infection and risk factors like eating raw/undercooked meat and raw salad or drinking contaminated water, or contact with cats or soil, which was similar to reports of Khurana *et al* where there was no correlation between risk factors and seropositivity of toxoplasmosis in pregnancy[27]. But study by Chintapalli and Padmaja in south India showed a significant correlation with history of contact with pet animals 60% ($P < 0.05$), in pregnant women [28]. Non association of risk factors with seroposi-tivity may be explained by the fact that these risk factors play a limited role in this region due to different religious, cultural, and eating behaviour of our participants.

In our study seroprevalence in pregnant women having BOH from urban areas (21.3%) were higher compared to rural areas (15.2%).This can be explained as urban habit of eating improperly cooked poultry/mutton or contaminated junk foods and water from street vendors could be a

source for *T. gondii* transmission. Similarly in BOH group, women of low socioeconomic status (34%) had more seroprevalance than women of mid (5.7%) and high socioeconomic status (14.3%)(P <.05) which was in accordance with seropositivity of 66.3% in low, verses 33.7% in high and mid socio-economic status(P<.05) as reported by Borkakoty et al[26].Another observation in the same group was that women of low socioeconomic status living in urban areas, had more seroprevalance (45.8%) than their rural counterparts (21.7%).It seems that unhygienic poor living condition, unhealthy food habits and lack of health education regarding Toxoplasma transmission in women of low socio economic status staying in overcrowded urban slums probably favour Toxoplasma transmission.

The current study has several limitations, which must be considered before drawing any conclusion. As this is an institutional study in an urban set-up, it may not correctly represent the general population. For confirmation of acute infection in women showing both IgM and IgG seropositivity, neither ELISA could be repeated, nor IgG avidity test could be performed. Lastly pregnancy outcome of seropositive participants after anti toxoplasma therapy could not be documented. However, being the first seroprevalence study among pregnant women in this area it will help in estimating the magnitude of Toxoplasma infection and association of potential risk factors with disease, and help in patient management.

Conclusion

The current study has revealed that Toxoplasma infection during pregnancy can lead to adverse foetal out comes. Early serologic screening for primary Toxoplasma infection should be offered routinely to all pregnant women in first trimester. Pregnant woman suspected of recent infection should be confirmed at a toxoplasmosis reference laboratory, using accurate tests and correct interpretation. All women who are pregnant or planning a pregnancy should be made aware of, specific hygienic and dietary recommendations to prevent primary Toxoplasma infection.

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