

CLINICO-MICROBIOLOGICAL STUDY OF TOXOPLASMOSIS IN PREGNANT FEMALES WITH BAD OBSTETRIC HISTORY (BOH)

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ABSTRACT

BACKGROUND

Context- Toxoplasmosis is an infection caused by a microscopic parasite called *Toxoplasma gondii*. Although, the infection generally causes a mild symptomless illness in people with healthy immune systems, it is of risk during pregnancy as it can infect the growing foetus in utero.

The aim of the study is to assess the seroprevalence of IgG and IgM antibodies for toxoplasmosis in women with normal pregnancy, pregnant women with one abortion and pregnant women with two or more abortions (BOH). The seropositivity was analysed according to number of abortions, age, parity and trimester of pregnancy.

MATERIALS AND METHODS

Serum samples were collected from pregnant women attending antenatal clinic with demographic data. Patients were divided into three groups- Normal pregnant women without any history of abortion (N), pregnant patients with one abortion (A1) and those with two or more abortions (A2) that is those with a Bad Obstetric History (BOH). 83 serum samples were subjected for determination of toxoplasma IgM antibodies and 81 for toxoplasma IgG antibodies. The study was carried out over a period of two years from November 2012 to October 2014.

Settings and Design- Experimental and analytical study.

RESULTS

Toxoplasma IgM prevalence was 1.2% and that of IgG was 27.16%. The distribution showed increase of IgG and IgM with increase in the number of abortions. In normal group patients (N), IgG was mostly positive for patients with gestational age 2nd trimester (13-28 weeks). The distribution also showed that in this group IgG was positive for those in the age group of 20-25 years. In group, A1 patients IgG was equally positive for those with gestational age 2nd trimester (13-28 weeks) and 3rd trimester (29-40 weeks). In this group, IgG was mostly positive for those in the age group of 20-25 years and 26-30 years, respectively. The distribution for group A2 patients showed an increase of IgG antibodies for those with gestational age 1st trimester (1-12 weeks). The patients of this group also showed increased IgG antibodies in the age group 20-25 years and 26-30 years.

CONCLUSION

High prevalence of toxoplasma IgG antibodies indicates previous exposure or asymptomatic infection. There was only one case of IgM antibody and the patient belonged to group A2, which is highly suggestive of BOH due to toxoplasmosis. The prevalence of toxoplasma in the community could be because of non-vegetarian food habits of the patients or consumption of contaminated food and vegetables. Early detection of toxoplasmosis in pregnant women can reduce severity of the disease in the newborn.

KEYWORDS

Toxoplasma gondii, Toxoplasmosis, Bad Obstetric History (BOH).

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BACKGROUND

Toxoplasmosis is a protozoan infestation caused by *Toxoplasma gondii*. *Toxoplasma gondii* is a coccidian parasite having an intestinal phase in cat of the family Felidae particularly *Felis catus* (domestic cat) and an extraintestinal phase in mouse, man or other animals.^{1,2}

Serological survey indicates that about 80% of all primary infections are asymptomatic due to immune system. It may lead to transient illness characterised by lymphadenopathy, fever, fatigue, arthralgia, dermatosis, malaise, headache and myalgia. Toxoplasmosis is most

dangerous to two populations- immunocompromised patients and fetuses whose mother acquires acute infection during pregnancy. In immunocompromised individuals, it can cause life-threatening infections such as encephalitis, pneumonia and chorioretinitis.³

The spectrum of outcome of congenital toxoplasmosis ranges from subclinical infection to intrauterine death. There may be damages to central nervous system (cerebral calcification, hydrocephalus, microcephaly), chorioretinitis, low birth weight, enlarged liver and spleen, jaundice, anaemia, petechiae and eye damage. Children who are apparently normal at birth may develop the disease later in life.⁴

Aim

The aim of the study is to assess the seroprevalence of IgG and IgM antibodies for Toxoplasmosis in women with normal pregnancy, pregnant women with one abortion and pregnant women with two or more abortions (BOH).

The seropositivity was analysed according to number of abortions, age, parity and trimester of pregnancy.

MATERIALS AND METHODS

The study was an experimental and analytical study carried out in the Department of Microbiology, MGM Medical College, Kamothe, Navi Mumbai, over a period of two years from November 2012 to October 2014. Ethical clearance was obtained from institutional ethics committee prior to study. Blood samples were collected from pregnant women attending antenatal clinic and patients admitted in antenatal ward of the hospital after taking written informed consent to perform ELISA test for Toxoplasma IgM and IgG on the serum samples.

The IgM and IgG Elisa kit was manufactured by Delta Biologicals a subsidiary of ERBA Diagnostics and supplied by Transasia Bio Medicals Ltd. The Toxoplasma IgM Elisa Kit bearing lot no-257-A with expiry date 2015-07 had a sensitivity of 99.9% and specificity of 99.0%. The Toxoplasma IgG Elisa Kit with lot no-204-D and expiry date 2015-05 had both sensitivity and specificity of 100%. The test was performed as per instructions of the manufacturer.

IgG Elisa- The controls consisted of one negative control and 5 calibrators as positive control. Validity and test results were calculated as per literature of the manufacturer.

Validity Criteria

- OD of negative control was <0.6 times the OD of calibrator 1.
- OD of C1 was more than 0.2 at 450 nm.
- OD of C5 was more than 1.5.

Cut-off corresponds to calibrator 1. Ratio between OD value of sample and that of cut-off was >1.1 and was considered as positive result.

IgM Elisa- The controls consisted of two Negative Controls (NC), two Positive Controls (PC) and two cut-off calibrators. The mean absorbance of NC, cut-off calibrator and PC was calculated.

Validity Criteria

- Blankwell-Blank absorbance was <0.050 at 450/630.
- NC- Mean absorbance after subtracting blank absorbance was <0.020.
- Cut-off calibrator- Mean absorbance after subtracting blank absorbance was >0.020 and <0.450.
- PC- Mean absorbance after subtracting blank absorbance was >0.500.

The index value was calculated to obtain the qualitative specimen results.

The cut-off value was obtained by subtracting the blank absorbance from the mean absorbance of the cut-off calibrator.

The index value was calculated by dividing the specimen absorbance by the cut-off value.

Index value was >1.1 and was considered reactive.

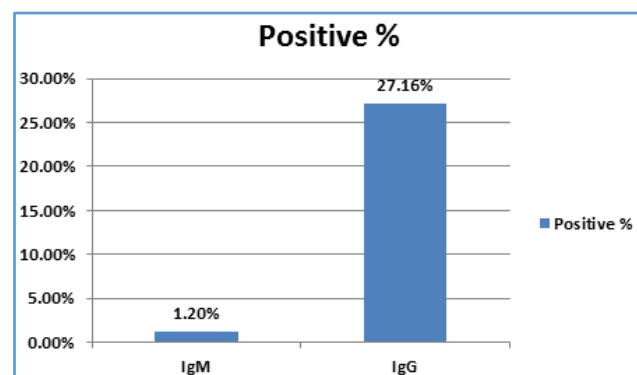
A predefined proforma was used to collect the data regarding age of the patient, gestational age (trimester) and number of abortions. The result of ELISA test was then subjected to statistical analysis.

RESULTS

A total of 83 samples were taken for detection of IgM antibodies against toxoplasma, out of which, 24 samples belonged to group N, which consisted normal ANC patients, 32 belonged to group A1, which consisted ANC patients with one abortion and 27 belonged to group A2 consisting of ANC patients with two or more abortions (BOH). Whereas, a total of 81 samples were taken for detection of IgG antibodies against Toxoplasma out of which 24 samples belonged to group N, 35 samples belonged to group A1 and 22 samples belonged to group A2. Prevalence of IgM positivity was 1.2% and that of IgG was 27.16% (Table 1). The distribution of abortion has shown that the prevalence of IgM increase from 0 to 3.7% and IgG from 20.83% to 40.9% with increase in number of abortions (Table 2). Only one patient out of the total of 83 subjected for determination of IgM antibodies against toxoplasma was found to be positive. This patient belonged to age group 26-30 years (28 years), gestational age second trimester and parity 1. The age group distribution has shown high rate of IgG positive for group N patients belonging to age group of 20-25 years (60%), for group A1 patients, high IgG positive is seen in age group of 20-25 years and 26-30 years (37.5% each group) and for group A2 patients IgG positive for those in age group of 20-25 and 26-30 (33.3% each group) (Table 3). The gestational age distribution show that IgG was positive for group N patients with gestational age 2nd trimester (13-28 weeks) (80%) in group A1 patients IgG was equally positive for those with gestational age 2nd trimester (13-28 weeks) (50%) and 3rd trimester (29-40 weeks) (50%). The distribution for group A2 patients showed a high positive rate of IgG antibodies for those with gestational age 1st trimester (1-12 weeks) (44.44%) (Table 4).

Antibody	No. of Samples	No. of Positive Samples	Positive (%)	P value
IgM	83	1	1.2%	>0.05 not significant
IgG	81	22	27.16%	0.0185 significant

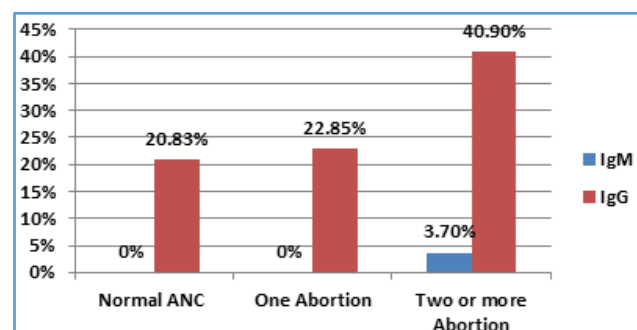
Table 1. IgM and IgG Positivity in Total Number of Subjects



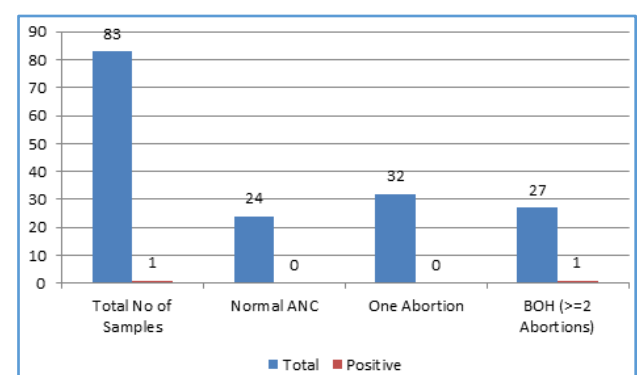
Graph 1. IgM and IgG Positivity

Abortion Distribution	IgM	IgG
Normal ANC	0/24 = 0%	5/24 = 20.83%
One abortion	0/32 = 0%	8/35 = 22.85%
Two or more abortion	1/27 = 3.7%	9/22 = 40.9%

Table 2. IgM and IgG Positivity as per Three Groups of Patients



Graph 2. IgM and IgG Positivity Increase with Increase in Number of Abortions

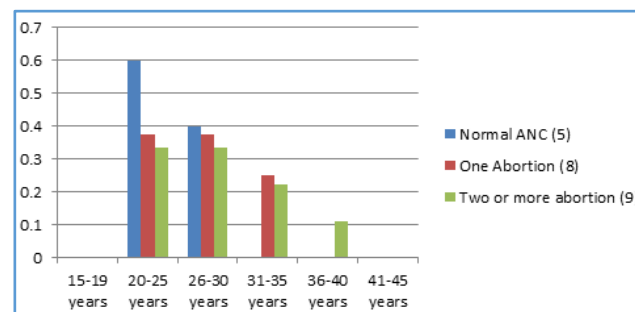


Graph 3. IgM and Abortion

Age Distribution	Normal ANC (5)	One Abortion (8)	Two or More Abortions (9)
15-19 years	0 (0%)	0 (0%)	0 (0%)
20-25 years	3 (60%)	3 (37.5%)	3 (33.3%)
26-30 years	2 (40%)	3 (37.5%)	3 (33.3%)

31-35 years	0 (0%)	2 (25%)	2 (22.2%)
36-40 years	0 (0%)	0 (0%)	1 (11.11%)
41-45 years	0 (0%)	0 (0%)	0 (0%)

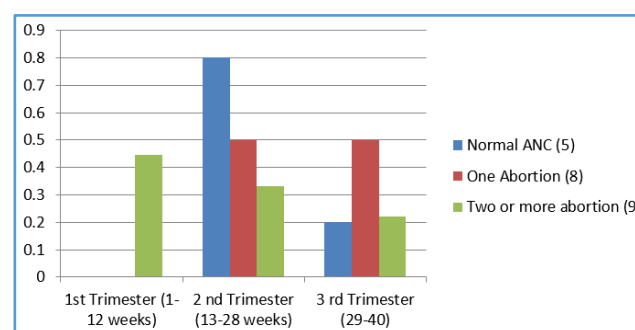
Table 3. Age Distribution of Subjects (IgG)



Graph 4. Age Group Distribution of Subjects (IgG)

Gestational Age	Normal ANC (5)	One Abortion (8)	Two or More Abortion (9)
1 st trimester (1-12 weeks)	0 (0%)	0 (0%)	4 (44.44%)
2 nd trimester (13-28 weeks)	4 (80%)	4 (50%)	3 (33.33%)
3 rd Trimester (29-40)	1 (20%)	4 (50%)	2 (22.22%)

Table 4. Gestational Distribution of IgG Positivity



Graph 5. Gestational Age Distribution Showing IgG Positivity

DISCUSSION

The toxoplasma IgG and IgM antibodies were tested by ELISA method. In this study, out of 81 cases subjected to Toxoplasma IgG ELISA, 22 cases (27.16%) are positive for toxoplasma specific IgG antibodies (p value- 0.0185 significant). Out of 81 cases, 5 cases are of Normal Pregnancy (NP) without abortion. The seroprevalence of IgG in this group is 20.82% (5/24). Similarly, out of 81 cases, 35 cases are patients with history of one abortion. The seroprevalence of IgG in the group 22.85% (8/35). Out of 81 cases, 9 patients are with a history of 2 or more abortions (A2) (BOH) with an IgG seroprevalence rate of 40% (9/22). The present study shows that seroprevalence for IgG increases with number of abortions (Table 2). Presence of IgG antibodies indicates prior exposure. However, IgG seropositivity does not increase with gestational age (Table 4).

A total of 83 cases were subjected to Toxoplasma IgM ELISA and only one case 1.2% (1/83) was found to be positive for Toxoplasma-specific IgM-antibodies. This patient

belonged to A2 group with history of three abortions. The presence of toxoplasma IgM antibodies in this patient indicates recent or current infection.

The geographical distribution of prevalence of toxoplasmosis in pregnant women has been evaluated by different authors in India and abroad. The toxoplasma IgG prevalence in normal pregnancy in our study is 20.83. For IgG prevalence studies in normal pregnancy, our findings are close to Ahmed QI et al⁵ from Kashmir who reported a toxoplasma IgG prevalence of 27% in normal pregnancy. Higher values are reported by Denoj Sabastian et al⁶ from Kerala (67.7%) and Srirupa Pal et al⁷ from Kolkata (45%). However, Senthamarai et al⁸ from Kanchipuram have reported a much lower values of 8%.

The Toxoplasma IgM prevalence in Normal Pregnancy (NP) in our study is found to be 0%, which is similar to 0% toxoplasma IgM prevalence reported by Senthamarai et al⁸ from Kanchipuram. However, Ahmad QI et al⁵ and Srirupa Pal et al⁷ have reported a prevalence of 1.12% and 17.5%, respectively.

The Toxoplasma IgG prevalence in BOH group (A2) in our study is found to be 40.9%, which is closer to a prevalence of 58.14% reported by Kh Sulochana Devi et al⁹ from Imphal (Manipur). Other workers have reported a much lower IgG prevalence in BOH group such as 26% prevalence by Srirupa Pal et al,⁷ 13.09% by Senthamarai et al,⁸ 8% by Padmavathy et al¹⁰ from Bangalore and a 4.2% IgG prevalence by D. Turbadkar et al¹¹ from Mumbai. The toxoplasma IgM prevalence in BOH group in our study is 3.7%, which is closer to the findings reported by Kh Sulochana Devi et al⁹ (3.1%), Senthamarai et al⁸ (7.14%) and Padmavathy et al¹⁰ (5.8%). Higher values were reported by D. Turbadkar et al¹¹ (10.5%), Srirupa Pal et al⁷ (18%), Chopra et al¹² (39%) and by Denoj Sabastian et al⁶ (50.7%).

Our study on Toxoplasma seroprevalence indicates that the prevalence of Toxoplasma IgG antibodies increases with the age and the number of abortions (NP=20-30 years, A1=20-35 years, A2=20-40 years). The toxoplasma IgG antibodies were found in the 2nd and 3rd trimester for NP and A1 group. However, in A2 group, the IgG prevalence was found in all the three trimesters of pregnancy (1st, 2nd and 3rd). Prevalence of IgG antibodies also increased with the parity of the patient (NP=0-1, A1=0-3, A2=0-4 parity).

Only one out of a total of 83 patients was found to be positive for toxoplasma IgM antibodies. This patient belonged to A2 group, age group 26-30 years, gestational age 2nd trimester and parity 1 and had a history of 3 abortions.

CONCLUSION

Our studies on toxoplasma seroprevalence indicate that the prevalence of toxoplasma IgG antibodies increases with the age and the number of abortions (NP=20-30 years, A1=20-35 years, A2=20-40 years). The Toxoplasma IgG antibodies were found in the 2nd and 3rd trimester for NP and A1 group. However, in A2 group, the IgG prevalence was found in all the three trimesters of pregnancy (1st, 2nd and 3rd). Prevalence of IgG also increased with the parity of the

patient (NP=0-1, A1=0-3, A2=0-4 parity). High prevalence of toxoplasma IgG antibodies indicate previous exposure or infection.

There was only one case of IgM antibody and the patient belonged to group A2, age group 26-30 years (28 years), gestational age 2nd trimester and parity 1 and had a history of 3 abortions, which is highly suggestive of BOH due to toxoplasmosis. The prevalence of toxoplasmosis in the community could be because of non-vegetarian food habits of the patients. Early detection in pregnant women can reduce the severity of the disease in the newborn.

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