Original Article

Seroprevalence and Clinical Correlates of *Toxoplasma gondii* Infection among Pregnant Womens in Tertiary Care Hospital

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Abstract

Background: Toxoplasmosis is caused by an infection with protozoan parasite *Toxoplasma gondii*. Acute infection in pregnant women may be transmitted to the fetus and cause severe illness. Most infected new born have no symptoms at birth, but if left untreated serious clinical manifestation can develop during childhood and early adulthood. Because congenital toxoplasma infection does not usually produce recognizable sign of infection in infancy and non-specificity of the symptoms, we were concerned by the fact that most case remain untreated, therefore we have used immunoglobulin M (IgM) avidity enzyme-linked immunosorbent assay (ELISA) for screening infants, and identify who should receive therapy.

Materials and Methods: A total of 90 pregnant women were included in the study. The group consists of the mother of bad obstetric history, and clinical conditions suggestive of toxoplasmosis. Blood sample collected from all these mothers and were screened by ELISA for IgM antibodies.

Results: Among 90 women, maximum age group was from 20 to 24 years i.e., 36 (40%) followed by 34 (37.77%) from the age group of 25-29 years.18 were seropositive for IgM toxoplasmosis, and abortion was the most common event seen followed by preterm delivery. Maximum patients were on a mixed diet, i.e., 13 (72.22%).

Conclusion: Congenital toxoplasmosis is a preventable disease, and it emphasizes the importance of early prenatal serological tests, and to take preventive measures when necessary, in order to avoid a dramatic fetal disease. It should be mandatory to screen every pregnant females and infants, and initiation of judicious treatment on time can, thus be provided to prevent morbidity and mortality due to toxoplasmosis.

Keywords: Immunoglobulin M enzyme-linked immunosorbent assay, Pregnancy, Toxoplasmosis

INTRODUCTION

Toxoplasmosis is one of the most common parasitic infections seen in humans. Approximately one-third of the population is exposed to this parasite while it is dangerous for mothers infected during pregnancy and infants.[1]

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Toxoplasmosis is usually diagnosed by serological tests by detection of specific immunoglobulin M (IgM) and IgG antibodies. A positive IgM titer establishes recent infection where-as negative IgM result virtually rules out recently acquired infection.[1] Acute infection with toxoplasma during pregnancy and its potentially tragic outcome for the fetus continues to occur worldwide despite the fact that it can be prevented worldwide.[2] The seroprevalence in pregnant women on world wide scale varies from 7 to 51.3%.[3] Intrauterine infection remains one of the major challenges for obstetricians during pregnancy. Toxoplasmosis, other (syphilis, varicella-zoster, parvovirus B19), rubella, cytomegalovirus and herpes infections have been studied the world over to establish its co-relationship with bad obstetric history. It has been proved beyond doubt that toxoplasmosis

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infection during pregnancy is associated with poor obstetric outcome resulting in significant consequences for the fetus such as spontaneous abortion, late intrauterine fetal death, early and late fetal growth retardation, prematurity, live born infant with evidence of disease. Early detection with serological examination and treatment in pregnancy can reduce the hazard substantially by preventing the transmission of infection from mother to baby.

Thus, the present study was conducted to determine the seroprevalence and clinical correlates of *Toxoplasma gondii* infection in pregnant women.

MATERIALS AND METHODS

After obtaining Institutional Ethical Committee approval the present clinical, prospective study of seroprevalence and clinical correlation of *T. gondii* infection, was carried out in Department of Microbiology a Tertiary Care Institute, during the period December 2011 to October 2013. Total of 90 pregnant women indoor as well as outdoor patients referred from obstetricians, who were having strong index of clinical and/or radiological suspicion for the toxoplasmosis infection were included in our study. The relevant history, clinical findings and investigations were noted. Exclusion criteria comprised of patients who were not willing to participate in the study.

Methodology

The study participants were explained about the study protocol and involved tests in the language of their understanding. After the informed consent, they were enrolled for this study. Approximately, 5-6 ml blood was collected under all aseptic precautions, and it was then labeled correctly and was centrifuged at 3000 rotation/min for 10 min.[4] Serum was transferred in the sterile labeled vials, and these were stored at −20°C. Before performing the enzyme-linked immunosorbent assay, (ELISA) the samples and ELISA kit was brought to the room temperature.

Test Details[5]

Name of the test used*:* ELISA (enzywell toxoplasma IgM - Diesse - Italy).

Principle of the Test

The test for the assay of toxoplasma IgM is based on the principle of the capture of these Igs and the subsequent identification of those which are specific, making use of their ability to bind an antigen conjugated to peroxidase. The capture is performed using monoclonal antibodies bound to the solid phase (microtiter wells). The antigen is composed of purified, inactivated and sonicated tachyzoite labeled with peroxidase bound to specific and anti-toxoplasma monoclonal antibodies.

Procedure

Bring the kit and sample at room temperature before the start of the procedure. Prepare the required number of strip.

Prepare the washing buffer by diluting the wash buffer 10 × (100 ml + 900 ml H_2O). Prepare the immunocomplex by adding the conjugate to the antigen (volume shown on the label).

Dilute samples 1:101 distributing 10 µl of serum into 1 ml of diluents. Dispense 100 µl of each diluted sample per well. Place undiluted control in a strip (100 µl in each well). The minimum requisite is 1 negative control, 2 cut off, 1 positive control. Leave one well for blank, performed using 100 µl of the substrate mixture. Wells are covered with protective film and incubated for 45 min at 37°C. After washing 4 times for 30 s (300 µl), add 100 µl of immunocomplex (antigen-anti *T. gondii* monoclonal antibodies labeled with peroxidase) to each well and incubate again for 45 min at 37°C, covering the well with the protective film. The plate is washed again 4 times as described above. Finally, substrate is distributed 100 µl/well and incubated for 15 min at room temperature. After 15 min at room temperature, the enzymatic reaction is stopped by adding 100 µl of stop solution. The adsorbance (optical density [OD]) is read at 450 nm or 450/620 nm within 30 min.

Scheme of Test Procedure

Step 1: Place 100 µl of diluted sample/controls in the wells of the strips
1. Incubate for 45 min at 37°C
2. Wash 4 times (300 µl)

Step 2: Add 100 µl of immunocomplex to each well
1. Incubate for 45 min at 37°C
2. Wash 4 times (300 µl)

Step 3: Add 100 µl of substrate to each well
1. Incubate for 15 min at room temperature.

Step 4: Add 100 µl of stop solution
1. Read absorbance at 450 nm within 30 min.

Test Validation

Substract the value of the blank (≤0.150) from all other readings. The OD values of the control cut-off serum tested in triplicate must be within 25% of the mean value. Disregard any abnormal value and recalculate the mean. The positive control must have an OD at least 1.5 times that of the cut-off serum. The ratio between negative control and cut-off must be ≤0.6. The OD cut-off must be ≥0.2 at 450 nm and 0.16 at ≥450/620 nm.

Interpretation of Results

If the absorbance of the sample is higher than that of cut-off, the sample is positive for the presence of specific IgM. Calculate the ratio between the OD value of the sample and that of the cut-off.

The sample is considered,

Positive, if the ratio is >1.2.

Negative, if the ratio is <0.8.

Doubtful, ±20% of cut-off.

ELISA test was put for detection of IgM antibodies and its level for *T. gondii* infection. Presence of IgM antibodies does indicate ongoing current infection which may range from last 7 to 10 days. Positive and negative findings of ELISA were correlated with clinical findings and/or radiological findings. Data were recorded in the proforma and analysed statistically by using Student's *t*-test, standard error of the difference between two means and Chi-square test. The IBM SPSS statistics for windows, version 19.0. (Armonk, NY: IBM corp.) model was used. $P < 0.05$ was considered as statistically significant and $P < 0.01$ was considered as highly significant.

RESULTS

In this study, 90 serum samples from pregnant women visiting antenatal care OPD who were suspected to be having toxoplasmosis were evaluated for the presence of anti-toxoplasma IgM antibodies by using ELISA method.

Maximum age group was from 20 to 24 years i.e., 36 (40%) followed by 34 (37.77%) from the age group of 25 to 29 years.

Maximum patients were on a mixed diet, i.e., 13 (72.22%) patients followed by contact with pets and soil were present in 7 (38.88%) and 8 (44.44%) patients respectively (Table 1 and Figure 1). Some of them had exposed to more than one risk factors.

Among IgM toxoplasma positive, the common obstetric events were seen in abortion, i.e., 8 (44.44%) followed by preterm in 4 (22.22%) cases (Figure 2).

In 18 (20%) patients were seropositive for IgM toxoplasma. Maximum seropositivity was seen in age group of 20-24 years i.e., 9 (50%) patients, followed by 6 (33.33%) patients in age group of 25-29 years (Figure 3).

DISCUSSION

In pregnant women, who were clinically suspected to be having toxoplasmosis, majority were from age group of 20 to 24 years i.e., 36 (40%) cases followed by 34 (37.77%) cases from age group of 25 to 29 years in our study (Table 2). Similarly, study conducted by Chintapalli and Padmaja,[6] showed that majority of suspected cases of

Table 1: Analysis of various risk factors in IgM toxoplasma positive pregnant women

Risk factor	Number of pregnant women with toxoplasmosis (*n*=18) (%)
Contact with pet animals	7 (38.88)
Mixed diet	13 (72.22)
Past history of toxoplasmosis	0 (0)
Contact with soil	8 (44.44)

IgM: Immunoglobulin M

Table 2: Age-wise distribution of pregnant females

Age (years)	Number of cases (%)
15-19	8 (8.88)
20-24	36 (40)
25-29	34 (37.77)
30-34	12 (13.33)
Total	90 (100)

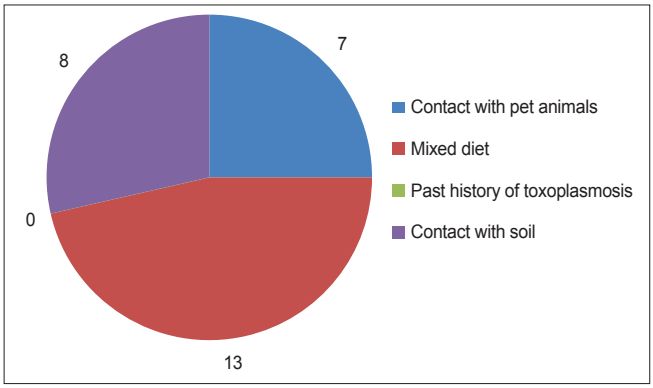

Figure 1: Analysis of various risk factors in immunoglobulin M toxoplasma positive pregnant women

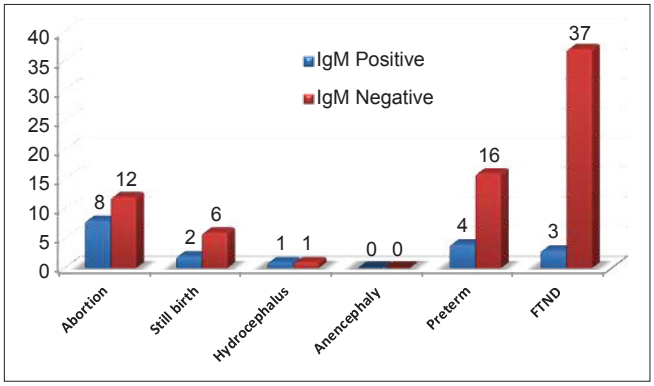

Figure 2: Various obstretic events in pregnant patients

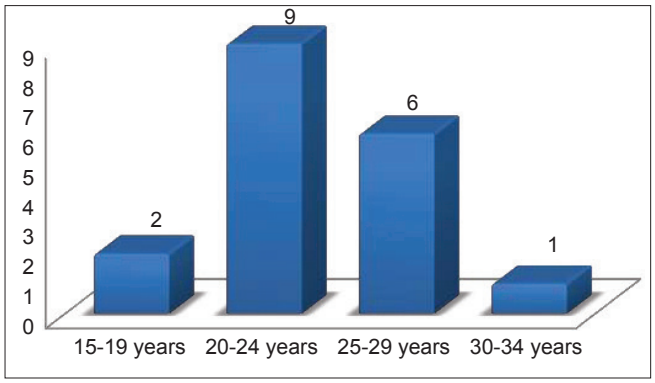

Figure 3: Age-wise distribution of pregnant females with immunoglobulin M enzyme-linked immunosorbent assay positive toxoplasmosis

toxoplasmosis was from 20 to 24 years of age 26 (32.5%), while the study by Khurana *et al.*[7] stated that most of the women suspected with toxoplasmosis infection belonged to the age group 25-29 years. However the study conducted by Fouladvand *et al.*[8] stated that there was no significant difference in the seroprevalence of toxoplasma antibody between different age groups. The mean age in their study was 21 years.

Toxoplasmosis mainly spreads through ingestion of under cooked meat and meat products, contact with pet animals like cats whose feces are infected with oocysts of toxoplasma, mother to child transmission (congenital toxoplasmosis) if mother is infected during pregnancy. Study by Chintapalli and Padmaja 2013[6] showed a significant correlation with history of contact with pet animals 60% ($P < 0.005$), in pregnant patients. A study carried out by Khurana *et al.* in 2010[7] reported that there was no correlation between risk factors and seropositivity of toxoplasmosis in pregnancy. Similarly study by Fouladvand *et al.*[8] found no statistically significant association between seropositivity of toxoplasma and area of residency, educational status, availability of drinking water and raw meat consumption habit.

Toxoplasmosis during pregnancy causes congenital fetal infection with possible fetal loss due to abortion, still birth and congenital malformations of which abortion is the major cause of fetal loss. Table 3 shows the various obstetric events occurring among the pregnant females suspected with toxoplasmosis. Among 18 toxoplasma IgM positive the common obstetric event was abortion i.e. 8 (44.4%) cases followed by preterm delivery 4 (22.22%) cases, hydrocephalus was found in 1 (5.55%) and 2 (11.11%) were with still birth. None of the pregnant women was showed anencephaly on routine ultrasonography. A similar study conducted by Khurana *et al.*[7] and Zargar *et al.*[9] shows that 33.33% and 34.5% of infected pregnant females had miscarriage in first trimester which occurred due to major

Table 3: Obstretical events in pregnant patients

Symptoms	IgM positive (%)	IgM negative (%)	No. of cases (%)	*P* value
Abortion	8 (44.44)	12 (16.66)	20 (22.22)	0.0227
Still birth	2 (11.11)	6 (8.33)	8 (8.88)	0.6579
Hydrocephalus	1 (5.55)	1 (1.38)	2 (2.22)	0.3618
Anencephaly	0 (0)	0 (0)	0 (0)	1.000
Preterm	4 (22.22)	16 (22.22)	20 (22.22)	1.000
FTND	3 (16.66)	37 (51.38)	40 (44.44)	0.0086
Total	18 (20)	72 (80)	90 (100)	-

FTND: Full term normal delivery

Table 4: Age-wise distribution of pregnant females with IgM ELISA positive toxoplasmosis

Age (years)	Number of IgM positive pregnant women (%)
15-19	2 (11.11)
20-24	9 (50)
25-29	6 (33.33)
30-34	1 (5.55)
Total	18 (100)

IgM: Immunoglobulin M, ELISA: Enzyme-linked immunosorbent assay

congenital abnormalities. Also study by Chintapalli, and Padmaja[6] found that the abortion constituted the major clinical case of pregnancy wastage when compared to stillbirth, intrauterine device, congenital malformation like hydrocephalus and anencephaly.

Out of 90 pregnant females suspected with toxoplasmosis, 18 females were positive for IgM antibody i.e., 20%, and maximum seropositivity was seen among the patients ranging between 20 and 24 years of age (Table 4). Similarly study conducted by Chintapalli and Padmaja[6] showed 20% seropositivity for toxoplasma specific for IgM antibodies with maximum cases from age range of 20-24, while Fouladvand *et al.*[8] found 23.4% seropositivity for IgM toxoplasma and commonly seen in age range of 21-25 years, which is concordant with our study. However, Yasodhara *et al.*[10] and Yelikar and Bhat[11] reported 18.3% and 16.67% of reactive IgM antibodies against toxoplasma respectively.

CONCLUSION

- Primary infection with toxoplasmosis in pregnant women can lead to the adverse outcome that are initially in apparent or asymptomatic and thus difficult to diagnose on clinical grounds. It is evident that the maternal infection with Toxoplasma plays a critical role in pregnancy wastage. If the mother of infants with congenital toxoplasmosis could reliably identify exposure to *T. gondii*, it would provide strong support for eliminating this disease by educating pregnant women about risk factors

- Congenital toxoplasmosis is a preventable disease, and it emphasize the importance of early prenatal serological tests, and preventive measures when necessary, in order to avoid a dramatic fetal disease
- It should be mandatory to screen every immunocompromised patient, pregnant females and infants for toxoplasmosis, and initiation of judicious treatment on time can, thus be provided to prevent morbidity and mortality due to toxoplasmosis.

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