

Seroprevalence of toxoplasmosis in antenatal women with bad obstetric history attending antenatal OPD in a tertiary hospital in Assam

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Abstract

Background: Toxoplasmosis has long since been incriminated with pregnancy wastage. In India, studies clearly show the rise in toxoplasma infection over last few decades. The present study is an effort to revisit the trend of maternal toxoplasmosis in this part of the country presently.

Material and Method: Serum samples collected over one year from 90 pregnant women were tested for IgG and IgM antibodies against *Toxoplasma gondii* by ELISA. 60 pregnant women with history of two or more foetal wastage in the form of abortions, premature labour, stillbirth, congenitally malformed fetus, intrauterine growth retardation, intra uterine death or neonatal death comprised the study group. Control group consisted of 30 healthy pregnant women with two or more normal pregnancies without any bad obstetric history (BOH).

Results: Overall prevalence of toxoplasmosis based on IgG seropositivity was 38.9% demonstrating very high prevalence of maternal toxoplasmosis. Positivity was higher in the study group with higher positivity among asymptomatic study cases.

Conclusions: High prevalence of toxoplasmosis found among pregnant women in the study is clearly indicative of toxoplasma endemicity in this region. Accurate and timely diagnosis is vital for prompt institution of therapy and hence there is a genuine role of screening pregnant women for Toxoplasmosis in this part of India.

Keywords: Toxoplasmosis, ELISA, Pregnancy Wastage.

Introduction

Maternal infections and infestations play a critical role in adverse pregnancy outcome. Among the many etiologies incriminated with pregnancy wastage, toxoplasmosis is well recognized and has garnered increased concern over the last few decades with recent studies indicating that prevalence of maternal toxoplasmosis in India may be a lot higher than previously considered.

Toxoplasmosis is the disease caused by infection with the obligate intracellular parasite *Toxoplasma gondii*.^(1,2) The disease manifests mainly in two forms, the congenital toxoplasmosis and the acquired toxoplasmosis. Acute toxoplasmosis is usually asymptomatic and self limited and goes unrecognized in 80 to 90% of adults and children with acquired infection.^(1,2,3) Congenital toxoplasmosis occurs when a woman becomes infected during pregnancy. The severity of the disease depends on the stage of pregnancy when she becomes infected and encompasses a wide spectrum of clinical disease in congenitally infected children. Acquired toxoplasmosis or postnatal acquired infection is mostly asymptomatic.

Toxoplasmosis has been associated with habitual abortion, prematurity, intra uterine growth retardation (IUGR), still birth, obscure lymphadenitis, congenital infections like chorioretinitis, uveitis, macular degeneration, hydrocephalus, microcephaly and hepatomegaly. Mild disease may consist of slightly diminished vision only, whereas severe disease in children may present with the most striking and

characteristic symptoms of chorioretinitis, hydrocephalus and intracranial calcification which are known as the "Classical triad" of the disease.⁽⁴⁾ Infection in early pregnancy may result in fetal wastage, intra uterine death (IUD) or stillbirth. Immunocompromised adults are another group susceptible to *Toxoplasma* infection and toxoplasmosis which is mostly asymptomatic, may present with devastating disease like encephalitis in those with depressed immunity.⁽¹⁾

Toxoplasma gondii was discovered in 1908 by Nicolle and Maceaux in a North American rodent (*Ctenodactylus gundii*), by Splendor in a rabbit and by Darling in man. The infection is transmitted to man by ingestion of undercooked meat of infected animals harboring the tissue cysts or foodstuff contaminated by cat feces and by transplacental transmission.⁽⁵⁾ The risk of transmission to fetus is highest if mother acquires infection in the last trimester and it is most severe if maternal infection occurs in the first trimester.⁽⁶⁾ The risk of infection increases with the duration of pregnancy. Studies suggest that when mothers are infected in the first trimester 14% fetuses become infected but infection is more severe (i.e. fetal wastage) as compared to 29% in the second trimester and 59% in the third trimester to nearly 100% during the last weeks.⁽⁷⁾

Diagnosis of toxoplasma infection may be established by serological tests, amplification of specific nucleic acid sequences (i.e. polymerase chain reaction), histological demonstration of the parasite or its antigens (i.e. immunoperoxidase staining), isolation of the parasite from blood or body fluids after sub-inoculation

of sample into peritoneal cavity of mice. Serological tests like IgG and IgM ELISA that detect antibodies in serum are widely used and preferred as they are highly specific, sensitive and reproducible methods that are available commercially.⁽⁸⁾

In recent years the incidence of toxoplasmosis in human beings is being increasingly reported from all over the world including India. In India, studies clearly show the rise in toxoplasma infection over the last few decades.⁽⁹⁾ Similar rise in incidence might well be expected in the north eastern part of India, where the climate is conducive to the toxoplasma oocyst to survive for long periods. The present study "Seroprevalence of toxoplasmosis in antenatal women with bad obstetric history attending antenatal OPD in a tertiary hospital" has been carried out as an effort to throw some light on the present trend of the disease in this part of the country keeping the following **aims and objectives** in mind:

1. To find the prevalence of Toxoplasmosis in pregnant women with past history of pregnancy wastage.
2. To find the prevalence of Toxoplasmosis in healthy pregnant women with previous full term deliveries.

Material and Methods

In the present study which is a prospective case control study based on simple random sampling analysed by descriptive epidemiology, sample size is 90, of which 60 were included in the study group and 30 comprised the control group. Study group consisted of pregnant women with a history of atleast two or more foetal wastage (pregnancy loss) in the form of abortions, premature labour, still birth, congenitally malformed fetus, intrauterine growth retardation, intra uterine death or neonatal death selected randomly from among patients attending antenatal OPD. Control group consisted of apparently healthy pregnant women with two or more normal pregnancies without any bad obstetric history (BOH).

Serum samples collected over one year from June 2011 to May 2012 from the 90 pregnant women were tested for IgG and IgM antibodies against *Toxoplasma gondii* by ELISA using the Labor Diagnostika Nord GmbH & Co. KG (LDN) Toxoplasma IgG ELISA and LDN Toxoplasma IgM ELISA kits and procedures described in the kit instruction manual were followed.

Cases from both the study group and control group were interviewed for detail clinical history and other relevant data and entered into the case Proforma. Informed consent was taken from every case. The study was done with the approval of the Institutional Ethics Committee. Odds ratio was calculated and P- value was calculated using Chi-square test.

Results

In the present study prevalence of toxoplasmosis based on IgG seropositivity in the study group was found to be 46.7% against 23.3% in the control group.

Seropositivity for IgM was 11.7% in the study group and 6.7% in the control group. 4 cases in the study group and none in the control group had both IgG and IgM positive (Fig. 1). The overall IgG seroprevalence (i.e. study + control group) is 38.9%.

The total seropositivity considering the test positive cases i.e. IgG+/IgM+/ both+ was found to be 31(51.7%) cases in the study group and 9(30%) cases in the control group. Overall seropositivity (study group + control group) in this study was thus 44.4%.

In the study group 32 cases were negative for Toxoplasma IgG antibody with a concentration of ≤ 10 IU/ml. Of the 28 cases that were positive, only one case had a high positive titre of >200 IU/ml and the rest had titres in the range of 11 – 200 IU/ml.

In the control group, 23 cases had titres ≤ 10 IU/ml (negative) and none of the cases had titres >200 IU/ml. The 7 positive cases had titres from 11 – 200 IU/ml.

Pregnant women in the reproductive age group of 16 – 45 years comprised the study population with distribution as seen in Table 1.

Highest IgG seropositivity (12 cases) was seen in the 21 – 25 years age group in the study group. In the control group, 7 cases were positive for IgG antibody and among them maximum cases i.e. 6 belonged to the 26 – 30 years age group.

In the study group highest number of cases were with gravida 3 and expectedly showed highest number i.e. 16 for IgG positivity. Out of the 4 cases that had both IgG and IgM positive, 3 had gravida 3 and 1 had gravida > 4 . In the control group also majority of the cases were gravida 3 and IgG was positive highest in 6 cases. No cases in the control group showed simultaneous IgG or IgM positivity.

In the study group highest number of cases (34) as well as highest number of IgG positive cases (17) were seen in women with gestation period above 24 weeks. The IgM seropositivity was however seen to be maximum in cases of women with gestation weeks between 13 – 24 weeks. All the 4 cases that had both IgG and IgM positive were in the gestation period of above 24 weeks. In the control group also highest number (16) of pregnant women studied were in the gestation period of above 24 weeks. Also out of the 7 IgG positive cases, 3 i.e. 42.9% belonged to this group. Out of the 2 cases positive for IgM, 1 case had gestation period above 24 weeks.

Amongst the 60 cases in the study group, 26 women presented with different symptoms like bleeding, per vagina, decreased fetal movement, IUD etc. Of these 10 i.e. 38.5 % were positive for IgG and 3 i.e. 11.5% were positive for IgM. In the asymptomatic group (total 34), 18 i.e. 52.9% were positive for IgG and 4 i.e. 11.8% were positive for IgM. Out of the 4 women who had both IgG and IgM positive only one was symptomatic.

There were altogether 26 symptomatic cases in the study group with bleeding per vagina and decreased fetal movement being the commonest complaints. Many cases

also presented with more than one symptom. Highest seropositivity for IgG was seen for decreased fetal movement while highest IgM positivity was seen for bleeding per vagina. The solitary symptomatic patient with both IgG and IgM positive presented complaints of decreased fetal movement. Fig. 2 shows the spectrum of presenting complaints among the symptomatic cases.

Women with BOH in the study group were found to have history of 2–4 pregnancy wastages. Most of the cases (42) gave history of 2 pregnancy wastage. In this group 21 i.e. 50% were IgG positive. All the IgM positive cases belonged to this group (16.7%). All the 4 cases having both IgG and IgM positive had 2 pregnancy wastages in their past obstetric history

Quantitative estimation of IgG antibody in relation to the number of pregnancy wastage revealed that majority of cases with history of 2 pregnancy wastage had titre values between 11 – 35 IU/ml. In this group one case had the highest titre of all and measured >200 IU/ml.

The spectrum of pregnancy wastage widely varied with spontaneous abortions being the commonest event of BOH. Expectedly the number of cases and IgG & IgM were all highest for cases with spontaneous abortions. The spectrum of pregnancy wastage along with respective seroprevalences are seen in Table 2.

In both the study and the control group majority of cases belonged to the low socio-economic group (LSG) with 16 IgG+ LSG cases forming 26.6% of the study group and 12 IgG+ middle/high socio-economic group (M/HSG) cases accounting for 20% of the study group. However within each category of both study and control group prevalence of IgG seropositivity was greater in women of M/HSG than those in LSW.

In study as well as control groups rural women were greater in number than the urban women. In the study group IgG seroprevalence was higher among rural population (50%) than the urban (40%), whereas in the control group prevalence of IgG positivity was higher in the urban group (33.3%).

The prevalence of toxoplasmosis was also investigated in relation to contact with animals particularly cat. Contact with other domestic animals or no contact with animals was also studied as seen in Table 3. In the study group 25% of the total IgG+ cases gave history of contact with cats. Higher IgG seropositivity was found in cases with contact with other domestic animals or no contact with any animals although cats are the definitive hosts for *Toxoplasma gondii*.

The distribution of vegetarian and non-vegetarian was similar in the study and control groups with more non-vegetarians than vegetarians. Seropositivity for IgG in the study group was 45.5% among the vegetarians and 46.9% among the non-vegetarians. In the control group 40% of vegetarians and 20% of non-vegetarians tested positive for IgG.

Table 4 shows that Ultrasonography (USG) gravid uterus was done in most (50) of the cases in the study

group except a few (10). Majority of the USG i.e. 56.7% of the study group were within normal limits and no abnormality detected (NAD). Among cases with NAD in the USG 50% were IgG+. Among those with positive findings in the USG, intra uterine growth retardation (IUGR) was the commonest finding, found in 4 cases of which however only one was IgG positive and another was IgM positive. 2 out of 3 cases of incomplete abortion were positive for IgG. 6 i.e. 60% of the 10 cases with no USG tested positive for IgG.

In both the study and control groups distribution of blood groups among the cases were similar with the commonest blood group being O+, followed by A+, B+ and AB+ (Rh negative blood groups were excluded from study). In the study group positivity for IgG was 54.5% among the blood group O+ cases followed by 40% among the blood group A+ cases.

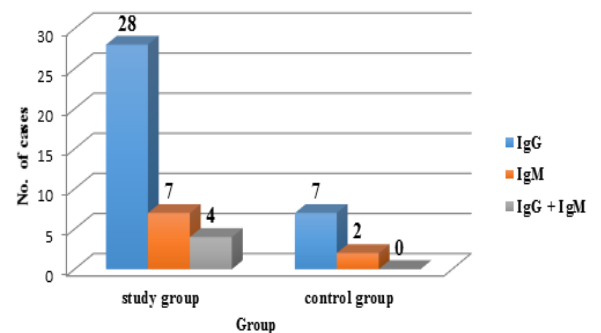


Fig.1: Seroprevalence of maternal Toxoplasmosis

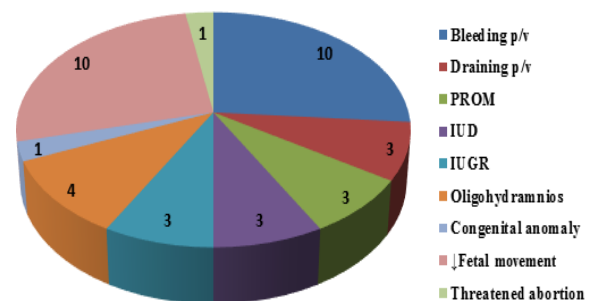


Fig. 2: Spectrum of presentation in the symptomatic cases in the study group

Table 1: Age-wise distribution in the study group and control groups

| Age group (in years) | Total cases studied (90) | | |
|----------------------|--------------------------|-----------------|-------------------|
| | No. of cases (%) | Study group (%) | Control group (%) |
| 16 – 20 | 3(3.3) | 3 (5) | 0 |
| 21 – 25 | 27(30) | 23(38.3) | 49(13.3) |
| 26 – 30 | 39(43.3) | 23(38.3) | 16(53.3) |
| 31 – 35 | 17(18.9) | 8(13.3) | 9(30) |
| 36 – 40 | 3(3.3) | 3(5) | 0 |
| 41 – 45 | 1(1.1) | 0 | 1(3.3) |

| | | | |
|-------|----|----|----|
| Total | 90 | 60 | 30 |
|-------|----|----|----|

Table 2: Distribution of Toxoplasma antibodies in relation to the nature of pregnancy wastages.

| No. of pregnancy wastage | No. of cases | IgG | | IgM | |
|--------------------------|--------------|-----------|----|----------|----|
| | | + | - | + | - |
| Spontaneous abortions | 38 | 17 (44.7) | 21 | 5 (13.1) | 33 |
| Missed abortions | 5 | 3 (60) | 2 | 0 | 5 |

| | | | | | |
|-------------------------|----|----------|----|----------|----|
| Still births | 7 | 5 (71.4) | 2 | 1 (14.3) | 6 |
| IUD | 20 | 8 (40) | 12 | 3 (15) | 17 |
| Neonatal deaths | 13 | 6 (46.1) | 7 | 0 | 13 |
| IUGR | 8 | 6 (75) | 2 | 1 (12.5) | 7 |
| PROM | 4 | 0 | 4 | 1 (25) | 3 |
| Congenital malformation | 8 | 3 (37.5) | 5 | 1 (12.5) | 7 |

Table 3: Seroprevalence in relation to contact with cats or other domestic animals or neither

| Contact with | Study group | | | | | Control group | | | | |
|--|--------------|---------|----|---------|----|---------------|---------|---|---------|----|
| | No. of cases | IgG | | IgM | | No. of cases | IgG | | IgM | |
| | | + | - | + | - | | + | - | + | - |
| Cats | 14 | 7 (50) | 7 | 0 | 14 | 9 | 1(11.1) | 8 | 2(22.2) | 9 |
| Other animals (cattle, goat, dog, poultry) | 22 | 9(40.9) | 13 | 3(13.6) | 19 | 14 | 5(35.7) | 9 | 0 | 14 |
| None | 24 | 12 (50) | 12 | 4(16.6) | 20 | 7 | 2 (8.3) | 5 | 0 | 7 |

Table 4: Findings of ultrasonography gravid uterus in the study group

| USG gravid uterus findings | No. of cases (%) | IgG | | IgM | |
|----------------------------|------------------|-----|----|-----|----|
| | | + | - | + | - |
| NAD | 34 (56.7) | 17 | 17 | 3 | 31 |
| Incomplete abortion | 3 (5) | 2 | 1 | 1 | 2 |
| IUD | 3 (5) | 1 | 2 | 1 | 2 |
| IUGR | 4 (6.7) | 1 | 3 | 1 | 3 |
| Congenital malformation | 1 (1.6) | 0 | 1 | 0 | 1 |
| PROM | 2 (3.4) | 0 | 2 | 0 | 2 |
| Fetal ascites | 1(1.6) | 0 | 1 | 0 | 1 |
| Oligohydramnios | 2 (3.4) | 1 | 1 | 0 | 1 |
| No USG done | 10 (16.7) | 6 | 4 | 1 | 9 |

Discussion

Reproductive failure due to recurrent abortions or other forms of pregnancy wastage like IUD, IUGR, still births or congenital anomaly has since long been attributed to infection by the intracellular parasite *Toxoplasma gondii*. Factors like absence of serological data before pregnancy, low number of patients and uncontrolled studies are hindrances in assessing the true picture of toxoplasmosis related adverse pregnancy outcomes.⁽¹⁰⁾ There is a considerable difficulty in conducting a well-controlled prospective study, which is essential to establish a causal relationship between toxoplasmosis and abortion and this study is an effort to find out the frequency of involvement and the role of chronic toxoplasmosis or active toxoplasmosis in repeated pregnancy wastage in this part of the country.

Isolation of *T. gondii* from the endometrium even several weeks after abortion does not prove congenital toxoplasmosis. *Toxoplasma gondii* has been found in the uteri of non-pregnant women also. Moreover even when placenta is infected, the fetus may escape infection.⁽¹⁰⁾ Isolation of the parasite from the product of conception is the most reliable method to arrive at the diagnosis of toxoplasmosis, but it is not always possible and failure of isolation does not exclude toxoplasmosis. Most workers in recent years preferred the ELISA test and its modification, to establish the diagnosis of recent active toxoplasmosis.⁽¹¹⁾ Most of the studies on sensitivity and specificity of ELISA test reveal a very high degree of sensitivity.

A highly sensitive IgM capture ELISA and IgG ELISA technique were applied in this study, for diagnosis of active toxoplasmosis and *T. gondii* infection respectively.

In the present study the seroprevalence of maternal Toxoplasma IgG was found to be 46.7% in the study group and 23.3% in the control group. The rates are much higher than the ones found in a prior study (unpublished) carried out in this institution, which reported a IgG seropositivity of 15% and 3.3% in study and control groups. The odds in favour of having IgG positive in the study group is 2.875 times the odds in favour of IgG positivity in the control group and p value=0.018. Thus, the odds in favour of IgG positivity in the study group is significantly greater than the odds in the control group.

Turbadkar D et al. (2003) who tested 380 serum samples from pregnant women having bad obstetric history for IgG antibodies by ELISA and found anti-Toxoplasma IgG antibodies positive in 42.1% cases.⁽¹²⁾

The present study has shown higher prevalence than this study.

Thapliyal N *et al.* (2005) while studying TORCH infection over a nine months period in 20 pregnant women with bad obstetric history in Kumaon region by ELISA found 55% IgG seropositivity for toxoplasmosis.⁽¹³⁾ In this study prevalence rate is much higher than found in the present study.

BJ Borkakoty *et al.* (2007) studied 112 pregnant women with bad obstetric history in Assam and found seroprevalence of 44.6% anti Toxoplasma IgG antibodies among pregnant women with history of pregnancy wastage.⁽¹⁴⁾ Mousa DA *et al.* (2011) evaluated serum samples, from 143 pregnant women with previous adverse pregnancy outcome in Libya and found 64 i.e. 44.8% were positive for Toxoplasma IgG antibody.⁽¹⁵⁾ Munmun DS *et al.* (2012) studied 105 pregnant women with BOH in Andhra Pradesh and found the Toxoplasma IgG seropositivity to be 49.52% in the study group.⁽¹⁶⁾ The findings of these studies are closely similar to the present findings.

BJ Borkakoty *et al.* (2007) who studied 68 pregnant women without bad obstetric history in Assam found seroprevalence 36.8% anti Toxoplasma IgG antibodies.⁽¹⁴⁾ Munmun DS *et al.* (2012) studied 105 women with normal previous pregnancies (control group) in Andhra Pradesh and found the Toxoplasma seropositivity to be 12.38% in the control group.⁽¹⁶⁾

In the present study the seroprevalence of maternal Toxoplasma IgM was found to be 11.67% in the study group and 6.7% in the control group.

Mousa DA *et al.* (2011) evaluated serum samples, from 143 pregnant women with previous adverse pregnancy outcome in Libya and found 8.4% were positive for Toxoplasma IgM. All of those with high IgG and IgM positivity gave a positive history of habitual abortion.⁽¹⁵⁾ This study is also quite similar to the present study.

Akoijam *et al.* (2002) carried out a study among primi gravid women in a district of North India, to determine the seroprevalence rate of Toxoplasma gondii infection using ELISA test. Out of the 503 women screened 210 women (41.75%) were seropositive for Toxoplasma gondii infection.⁽¹⁷⁾ The findings of this study was far higher than the present study.

In a study by Srirupa P *et al.* (2011) in Kolkata 50 pregnant women with bad obstetric history and 40 primi gravid women were investigated for anti - *T.gondii* antibodies and seroprevalence was found to be higher amongst primi gravid women (IgG 45%) than women with BOH (26%) contradicting the present study and almost all Indian studies on the topic so far.⁽⁹⁾

The incidence and prevalence reported by various workers from different parts of the world and India vary widely. This can be attributed to lack of uniformity in screening protocol for diagnosis of Toxoplasmosis. Also different screening methods followed by different workers, differences in food habits and differences of

prevailing conditions are factors contributing to such variance in different populations. Moreover there is paucity of well-controlled longitudinal studies and hence fluctuations may arise across studies.

Pregnant women in the reproductive age group of 16 – 45 years comprised the study population in this study and highest IgG seropositivity was seen in the 21 – 25 years age group in 12 cases accounting for 53.3% unlike BJ Borkakoty *et al.* (2007), Assam who found increase in seroprevalence of toxoplasmosis with increasing maternal age.⁽¹⁴⁾ Munmun DS *et al.* (2012) also found that the seroprevalence showed gradual increase with advancing age in their study.⁽¹⁶⁾ Also in a study by Qublan *et al.* (2002) higher seroprevalence of toxoplasmosis was seen with higher parity which is in agreement with the present study.⁽¹⁸⁾ However in this study a P-value=0.15, revealed seroprevalence of Toxoplasmosis in relation to parity is insignificant among the study and control group and a P value=0.429, revealed insignificant difference in seropositivity for Toxoplasmosis with increasing events of pregnancy wastage.

The spectrum of pregnancy wastage widely varied with spontaneous abortions being the commonest event of BOH. Spontaneous abortion was present in 38 of the 60 cases in study group and among them 17 i.e. 44.7% were seropositive for IgG. IUD was the next commonest and present in 20 cases among which 40 % (8 cases) were IgG positive. In a similar serological study for TORCH infections in women with bad obstetric history in Nagpur by Jalgaokar SV (2006) maximum number of cases of abortion (27.27%), intrauterine growth retardation (9.37%), intrauterine death (17.64%) and preterm labor (18.18%) was associated with toxoplasma infection.⁽¹⁹⁾

In the present study IgG seroprevalence was higher among rural population (50%) in the study group whereas in the control group prevalence of IgG positivity was higher in the urban group (33.3%). A seroepidemiological study of Toxoplasmosis was carried out in different sections of population of Chandigarh by Mohan *et al.* (2002). They observed a significant higher rate in the slum area (10.5%) as compared to females in the urban area (2.55%).⁽²⁰⁾

The prevalence of toxoplasmosis was also investigated in relation to contact with animals particularly cat as well as in relation to food habits (vegetarian/ non vegetarian). Akoijam *et al.* (2002) studying primi gravid women in a district of North India, to determine the seroprevalence rate of *Toxoplasma gondii* infection did not find any statistical difference in women who had contact with cats and who did not.⁽¹⁷⁾ Also no statistical difference was seen in prevalence of *T. gondii* infection among vegetarians and non-vegetarians.⁽¹⁷⁾ Both these findings are similar to this study.

In this study, the seroprevalence of Toxoplasmosis in relation to contact with cat and other animals was found to be insignificant as P value= 0.91. While

analyzing seroprevalence of Toxoplasmosis with no history of contact with animals P value=0.5, which is again statistically insignificant. Regarding food habits P value=0.535 in the study group and P value=0.173 in the control group, thus in both groups the findings are insignificant.

Conclusion

A well-controlled cohort study will bring to light a better picture of the comprehensive maternal and fetal affliction attributable to *Toxoplasma gondii* infection. Preventive interventions like mass awareness about this infection and its consequences are essential. Also proper hygiene and good antenatal care are efficient in curbing the prevalence of Toxoplasmosis in pregnancy, rendering a healthy and disease free offspring.

References

- Dubey JP. Toxoplasmosis. In: Cox FEQ, Wakelin D, Gillespie SH, Despommier DD, editors. Topley Wilson's Microbiology and Microbial Infections Parasitology, 10th edition. New York: Oxford University Press. 2010. p. 303–18.
- Kasper LH, Kim K. Toxoplasma Infections. In: Kasper DL, Fauci AS, Longo DL, Hauser SL, Jameson JL, Loscalzo J, editors. Harrison's Principles of Internal medicine, 18th edition. USA: McGraw Hill; 2012. p. 1722-9.
- Chatterjee KD. In Textbook of Parasitology, 13th edition. New Delhi: CBS Publisher; 2009. p. 129-33.
- Eichenwald HF. A Study of Congenital Toxoplasmosis. In: J Chr. Siim editor. Human Toxoplasmosis. Munksgaard, Copenhagen; 1960. p. 41- 9.
- Hutchison WM. The nematode transmission of *Toxoplasma gondii*. Trans R Soc Trop Med Hyg 1967;61:80-9.
- McCabe, R and Remington, JS. Toxoplasmosis: the time has come. N Engl J Med 1988;318:313–5.
- Desmots G., Couvreur J. Congenital Toxoplasmosis. A prospective study of 378 pregnancies. N Engl J Med 1974;290:1110-6.
- Forbes BA. Serologic Diagnosis of Infectious Diseases. In Forbes BA, Sahm DF, Weissfeld AS, editors. Bailey and Scott's Diagnostic Microbiology, 12th edition. China: Elsevier; 2007. p. 167-8.
- Pal S, Das N, Pal D. Sero-prevalence and risk factors of *toxoplasma gondii* in pregnant women in Kolkata India. Journal of Recent Advances in Applied Sciences 2011; 26:27-33.
- Ferguson DJP, Hutchison WM. An ultrastructural study of the early development and tissue cyst formation of *Toxoplasma gondii* in the brains of mice. Parasitol Res 1987;73:483–491.
- Montoya, JG. Laboratory diagnosis of *Toxoplasma gondii* infection and toxoplasmosis. J Infect Dis 2002;185:S73–S82.
- Turbadkar D, Mathur M, Rele M. The seroprevalence of the TORCH infections in women with bad obstetric histories. Indian J Med Microbiol 2003;21(2):108–10.
- Thapliyal N, Shukla PK, Kumar B, Upadhyay S, Jain G. TORCH infection in women with bad obstetric history—a pilot study in Kumaon region. Indian J Pathol Microbiol 2005;48:551–3.
- Borkakoty BJ, Borthakur AK, Gohain M. Prevalence of *Toxoplasma gondii* infection amongst pregnant women in Assam, India. Indian J Med Microbiol 2007;25:431–2.
- Mousa DA, Mohammad MA, Toboli AB. *Toxoplasma gondii* infection in pregnant women with previous adverse pregnancy outcome. Med J Islam World Acad Sci 2011;19:95–102.
- Sarkar MD, Anuradha B, Sharma N, Roy RN. Seropositivity of Toxoplasmosis in Antenatal Women with Bad Obstetric History in a Tertiary-care Hospital of Andhra Pradesh. India J Health Popul Nutr 2012;30:87–92.
- Akoijam BS, Shashikant, Singh S, Kapoor SK. Seroprevalence of *Toxoplasma* infection among primigravid women attending antenatal clinic at a secondary level hospital in North India. J Indian Med Assoc 2002;100:591–2.
- Qublan HS, Jumaian N, Abu-Salem A, Hamadelil FY, Mashagbeh M, Abdel-Ghani F. Toxoplasmosis and habitual abortion. J Obstet Gynecol 2002;22:296–8.
- Surpam RB, Kmlakar UP, Khadse RK, Qazi MS, Jalgaonkar SV. Serological study for TORCH infections in women with bad obstetric history. J Obstet Gynaecol India 2006;56:41–3.
- Mohan B, Dubey ML, Malla N, Kumar R. Seroepidemiological study of toxoplasmosis in different sections of population of Union Territory of Chandigarh. J Commun Dis 2002;34:15-22.