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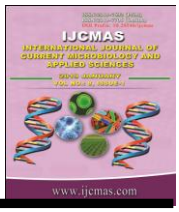

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Serological Study of Toxoplasmosis by Line Immune Assay (LIA) in Women with Bad Obstetric History

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A B S T R A C T

One of the tragedies that confront the pregnant woman and physician providing obstetric care is the occurrence of fetal death. It is reported that toxoplasmosis, one among protozoal infections have a high incidence in pregnancy, sometimes causing fetal death. Toxoplasmosis is caused by a coccidian parasite, *Toxoplasma gondii* which is highly prevalent both in humans and warm-blooded animals. The study was intended to observe the prevalence of Toxoplasmosis in pregnant women presenting with bad obstetric history (BOH). A total of 100 antenatal women with bad obstetric history were included in the study and tested for toxoplasmosis infection. All the samples were screened by Line Immuno Assay (LIA) using recomLineTORCH ScreeningI gG/IgM antibody kits against TORCH agents. Out of the 100 antenatal women in the study group, 4 were seropositive for IgM, 31 cases were positive for IgG and 5 cases were positive for both IgM and IgG of *Toxoplasma*. Out of 100 patients, Toxoplasma was seen in36.36% in abortion, 53.84% in congenital anomalies, 40% in neonatal death, 33.33% in intrauterine death and 7.6% in congenital anomalies.LIA was found to be a sensitive serological test for diagnosis of Toxoplasmosis in pregnant women with BOH.

Introduction

Toxoplasmosis a zoonotic protozoal infection is caused by a coccidian protozoan parasite *Toxoplasma gondii* (Singh, 2003). Cats including wild Felidae are the definite host and all other warm-blooded animals including humans are intermediate hosts (Giannoulis *et al.*, 2008). The infection has a worldwide distribution. Approximately one-third of all humanity has been exposed to this parasite. Although asymptomatic in immunocompetent adults, it can cause severe disease manifestations and even death in immunocompromised patients. If acquired during pregnancy, it may cause damage to the fetus as an acute infection, which is one of the important reasons for bad obstetric history (BOH) in pregnant women. Intermediate hosts may acquire infection by consuming raw or undercooked flesh from other intermediate hosts, or by ingesting oocysts from the environment. Environmental sources of *T. gondii* (oocysts) include soil, water, shellfish, fruits, and vegetables (Abu-Madi *et al.*, 2010). Maternal infection usually results from ingestion of oocysts shed into the environment or from ingestion of bradyzoites

or tachyzoites contained in meat or meat products (Giannoulis *et al.,* 2008). Acute infection in adult humans and in pregnant women goes unrecognized in as many as 90% of cases, because either it is subclinical or symptoms are nonspecific and it is falsely taken as a viral illness. The most common manifestations are non-tender lymphadenopathy, fatigue, headache, malaise and myalgia. The infection is usually self-limited and requires no treatment. First trimester fetal infection on the other hand, often results in miscarriage, stillbirth, or severe sequelae in the newborn. The risk of congenital toxoplasmosis depends on the timing of the mother's acute infection. The different rates of transmission and outcomes are most likely related to placental blood-flow, the virulence and amount of *T. gondii* acquired, and the immunologic ability of the mother to restrict parasitaemia. The overall rate of transmission of maternal infection to the foetus is about 45%. Of these, 60% are subclinical infections, 9% resulting in death of the foetus, and 30% have severe damage (Sarkar *et al.,* 2012). Serologic tests represent the most commonly used method to establish the diagnosis; documentation of recent seroconversion is the best evidence of recent infection. The diagnosis of Toxoplasma infection is done by detection of specific antibodies by enzyme linked immunosorbent assay (ELISA). The line immunoassay (LIA) for the determination of antibodies to individual antigens is a development of the enzyme-linked immunosorbent assay (ELISA) in which the antigens to be tested are adsorbed onto a nylon test strip. Presence of specific IgM antibodies indicates recent infection as the cause of BOH in pregnant women leading to congenital malformations, abortions and still births. IgM antibodies may appear within the first week of infection and generally decline within a few months; however, they sometimes persist for years after the initial infection. The detection of Toxoplasma specific immunoglobulin G (IgG) antibodies indicates chronic infection (Gilbert R *et al.,* 2003). IgG antibodies appear within one to two weeks of infection, peak in six to eight weeks and then decline over the next two years; they remain detectable for life.

Toxoplasmosis is, thus, a significant risk factor for bad obstetric outcome and a major cause of congenitally-acquired infection, leading to a high degree of intrauterine fetal death and morbidity of the newborn. Treatment of a pregnant woman who acquires infection at any time during pregnancy reduces the chance of congenital infection in her infant by approximately 60%. Therefore, early diagnosis of toxoplasmosis is essential to start appropriate treatment on time to reduce the transplacental transmission.

Most probably due to lack of facilities in isolating etiological agents causing BOH and the prohibitive cost of commercial diagnostic kits, studies analyzing the role of maternal infection in the causation of BOH are less in number. Almost all of the available information on Toxoplasmosis from India is obtained from women with pregnancy wastage. In this context, the present study was carried out on antenatal women with BOH. The study was intended to observe the serological prevalence of toxoplasmosis in antinatal women with BOH.

Materials and Methods

Recomline TORCH Screening IgG kit and recomLine TORCH Screening IgM kit was procured from the manufacturer MIKROGEN Diagnostik, Germany.

Source of data

The present study was a type of cross sectional study conducted at the Department

of Microbiology, J.J.M Medical College and SSIMS& RC, Davanagere, Karnataka, during August 2015 and July 2016 with a total of 100 antenatal women with bad obstetric history presenting to Chigateri General Hospital, Bapuji Hospital attached to J.J.M. Medical College and SSIMS&RC, Davanagere. The institutional ethical committee clearance was obtained to conduct the study.

Method of collection of data

Blood samples from 100 women with bad obstetric history presenting to Chigateri General Hospital, Bapuji Hospital attached to J.J.M. Medical College and SSIMS&RC, Davanagere were tested for toxoplasmosis infection.

A standard case proforma was maintained and study documented under the following important headings;

• Biodata: It included patient's name, age, occupation, address, contact number, OP/IP number.
• Obstetric history: It included previous unfavorable fetal outcome in terms of two or more consecutive spontaneous abortions, history of intrauterine fetal death, intrauterine growth retardation, stillbirth, early neonatal death and/or congenital anomalies.

Note:

Inclusion criteria-Antenatal women with BOH.

Exclusion criteria-Non-pregnant women, antenatal women without bad obstetric history

Method of sample collection

3ml of whole blood samples from 100 antenatal women with bad obstetric history were collected under aseptic precautions by venipuncture using sterile disposable syringes. After centrifugation, clean serum was transferred into vials and stored at 4-8°C.

Processing of serum samples

All the sera samples were tested for Toxoplasmosis infection by Line immunoassay for IgG and IgM antibodies.

Test protocol

All reagents were prepared as per manufacturer's recommendation and brought to room temperature for at least30 minutes before beginning the test. The test strips were placed in 2ml of ready to use washbuffer-A. 20µl of undiluted serum samples were pipetted on to the test strip for each incubation mixture. This was incubated for1hr with gentle shaking.

Diluted serum was siphoned from individual well and 2ml of ready for use wash buffer-A was pipetted into every well. It was washed for 5min with gentle shaking and then the wash buffer-A was siphoned off. Washing step was repeated thrice. Then 2ml ready-to-use conjugate solution was added and incubated for 45min with gentle shaking. Washing step was repeated thrice. Substrate reaction 1.5ml of ready-to-use substrate solution was added and incubated for 8min with gentle shaking. The substrate solution was removed and washed thrice briefly with deionisied water. The strips were dried between 2 layers of absorbant paper for 2hrs before analysis.

Interpretation of test results

Recomline TORCH Screening IgG/IgM test (Figure 1).

Interpretation of this test was done by two-band strategy as described below.

The presence or the absence of specific antibodies to an infectious agent is indicated by major band (lysate antigen).

Time duration of past infection of Toxoplasma and CMV, type specificity of HSV, protective immunity of Rubella is indicated by ancillary band.

Recomline TORCH Screening IgM test

Interpretation of this test was done by single band strategy. The test strips contain one band per infectious agent thus enabling the identification of specific IgM class antibodies to Toxoplasma, Rubella, CMV and HSV type 1 and 2.

RecomLineTORCH Screening IgG assay is supported by the recomLineTORCH Screening IgM assay; hence both are done together (Table 1).

Validation of test

Quality control of the test was assessed by the presence of three bands, namely; Reaction control band, Antibody class band and Cut-off control band. A positive result indicates presence of IgM or IgG antibodies to *Toxoplasma gondii*.

A negative result indicates no immunity to Toxoplasma. These individuals are presumed to be susceptible to a primary infection.

The same procedure was also repeated for IgG antibodies.

Statistical analysis

Statistical analysis was done using Microsoft excel. The data analysis involved transcription, preliminary data inspection, content analysis and interpretation. Percentages were used in this study to analyze variables.

Results and Discussion

The history of 100BOH cases encountered in the current study is as shown in Table 2.

The age group of the patients was from 19 to 38 yrs. Maximum number of BOH cases belonged to the age group of 19-24 years. Age-wise distribution of cases is shown in Table 3.

Table 4 shows the seropositivity of IgM, IgG and both IgM, IgG among various Toxoplasma positive cases.

Table 5 shows distribution of Toxoplasmosis infection in various BOH patients with different presentations.

In our study Toxoplasmosis was most common agent responsible for BOH. Abortion was the commonest mode of presentation of BOH, followed by still birth and IUD. Toxoplasma was the agent responsible for abortions in majority of the cases.

Prenatal screening for antibodies to *Toxoplasma,* Rubella, CMV, Herpes simplex virus and other agents like *Treponema pallidum*, HIV is a routine practice in many parts of the world and is commonly referred to by the acronym TORCH (Binnicker *et al.*, 2010).

In recent years increasing evidence is available which shows that infection by Toxoplasma induces fetal loss in women. The preferential liking of toxoplasma is for nucleated cells of muscle, intestinal epithelium and placenta.

Table.1 *Toxoplasma gondii* specific interpretations of the test results

IgG Lysate	IgG P30	IgM	Interpretation
Negative	Negative	Negative	Seronegative no immunity
Negative	Negative	Positive	Suspicious of seroconversion (primary infection)
Positive	Negative	Positive	Suspicious of chronic infection with reinfection.
Positive	Positive	Negative	Suspicious of past infection. Infection more than 3 months ago
Positive	Negative	Negative	Suspicious of past infection. Time of infection not determinable
Negative	Positive	Not specified	No diagnostic statement possible

Table.2 Different presentations of BOH cases

BOH	N= 100	Percentage
Abortion	44	44%
Still birth	23	23%
Intrauterine death	15	15%
Congenital	13	13%
Neonatal death	05	5%

Table.3 Age-wise distribution of BOH cases

Age group in years	Number of BOH cases	Percentage
19-24	44	44%
25-30	42	42%
31-35	12	12%
>35	02	2%

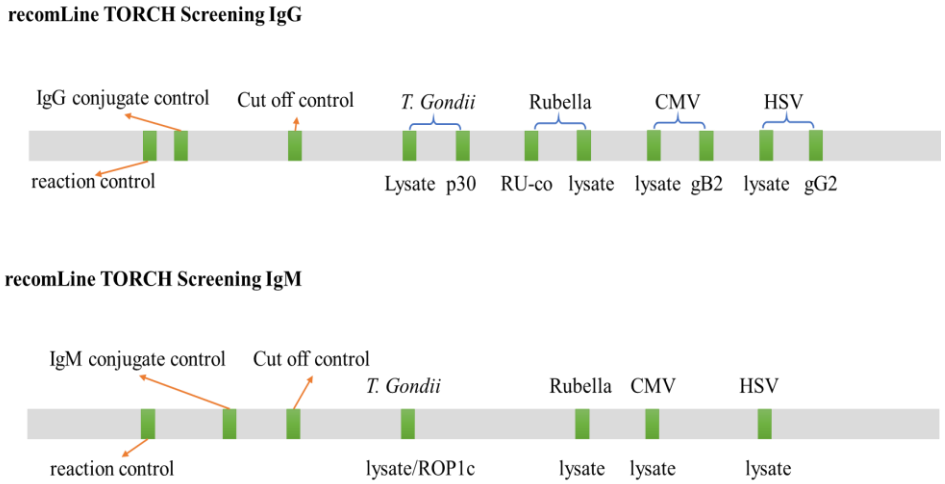
Table.4 Seropositivity of Toxoplasmosis infection

Disease	IgM	IgG	IgM&IgG	Total
Toxoplasma	04 (36.36%)	31 (39.24%)	05(83.33%)	40(41.66%)

Table.5 Distribution of Toxoplasmosis infection in various BOH cases

BOH	Toxoplasmosis
Abortion	16 (40%)
Still birth	10 (25%)
IUD	05 (12.5%)
Congenital anomaly	07 (17.5%)
Neonatal death	02 (5%)

Fig.1 Recomline TORCH test



If the mother contracts the disease during pregnancy, it will be congenitally acquired by transfer through the placenta, causing abortions, stillbirths, congenital malformations (Parija, 2008). Through serology the disease can be diagnosed. Recent infection is indicated by the demonstration of Toxoplasma specific IgM antibodies and chronic infection is by detection of Toxoplasma specific IgG antibodies.

Abortion was the commonest mode of presentation of BOH in our study and the similar results were obtained from a study by Surpam *et al.*, in which 30% presented as abortion (Surpam *et al.*, 2010).

In our study the age group of 19-24 years showed the presence of maximum number of BOH cases with 44% of cases belonging to this age group, followed by 42% in the age group of 25-30 years. This was similar to the results of a study by Sood *et al.*, in which 46% of cases were in the age group of 21- 25 years (Sood *et al.*, 2009).

In this study out of 100 cases of BOH cases 96% were positive for TORCH infections. This was similar to a study by Padmavathy *et al.*, in which 98% of the BOH cases were positive for TORCH cases *Toxoplasma* accounting for 41.66% of cases of all the TORCH positive cases (Padmavathy *et al.*, 2013), this goes in accordance with the study by AL-Taie (AL-Taie, 2010). Out of 100 patients, Toxoplasma was seen in36.36% in abortion, 53.84% in congenital anomalies, 40% in neonatal death, 33.33% in intrauterine death and 7.6% in congenital anomalies. The values are comparatively higher in this study when compared with other studies done by indirect fluorescent antibody test and indirect haemagglutination. The high sensitivity of the test system used in the present study to detect Toxoplasma antibodies is responsible for obtaining the high seropositivity rate (Singh, 2003).

Out of the 100 antenatal women in the study group, 4 were seropositive for IgM, 31cases were positive for IgG and 5 cases were positive for both IgM and IgG of Toxoplasma. 60 cases were seronegative for both IgG and IgM antibodies which shows that the BOH was not due to Toxoplasma infection, but may be due to other infections like CMV (cytomegalovirus), Herpes simplex and Rubella (Zargar *et al.*, 1999). Occasionally active Toxoplasma infection can persist from one pregnancy to the next and

such infection may be the cause repeated miscarriages or still births (Chintapalli *et al.*, 2013). In such cases *T. gondii* may encyst in the uterine endometrium and is stirred into activity by the process of placentation. *T. gondii* has been isolated from abortus in one case of miscarriage. Hence it is essential to test for both IgG and IgM antibodies, as chronic infection can also lead to fetal wastage.

From this study it is observed that illiteracy, poverty, overcrowding, lack of hygiene and associated environmental factors play a crucial role for the high incidence of Toxoplasmosis. This shows that unique environmental factors in various communities, eating habits have on the transmission of this infection. The socio-epidemiological aspects of toxoplasmosis are the important contributing factors for the spread of the disease. Early diagnosis and treatment have an effective role in reducing transmission of infection from mother to baby (Allain *et al.*, 1998).

IN conclusion toxoplasmosis is amenable to treatment. Early detection with repeated serological examination and treatment in all pregnancies can reduce the hazards. Prevention of primary infection is the best choice which is based upon educating women on the modes of *Toxoplasma gondii* transmission and avoidance of risky behaviors. We strongly feel that pre-conception counseling aimed at primary prevention of toxoplasmosis can reduce the seroconversion rate during pregnancy by 60 percent.

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References

Abu-Madi MA, JM Behnke and Dabritz HA. 2010. *Toxoplasma gondii* seropositivity and co-infection with TORCH pathogens in high-risk patients from Qatar. Am J Trop Med Hyg. 1; 82(4):626-33.

Allain JP, CR Palmer and Pearson G. 1998. Epidemiological study of latent and recent infection by *Toxoplasma gondii* in pregnant women from a regional population in the UK. J Infect. 36(2): 189-96.

AL–Taie AA. 2010. Serological study for TORCH infections in women with high delivery risk factors in Mosul. Tikrit Journal of Pure Science. 15(1): 193-8.

Binnicker MJ, DJ Jespersen and Harring JA. 2010. Multiplex detection of IgM and IgG class antibodies to *Toxoplasma gondii*, rubella virus, and cytomegalovirus using a novel multiplex flow immunoassay. Clin Vaccine Immunol. 17(11):1734-8.

Chintapalli S and Padmaja IJ. 2013.Seroprevalence of toxoplasmosis in antenatal women with bad obstetric history. Trop Parasitol. 3(1):62.

Giannoulis C, B Zournatzi, AGiomisi, E Diza and Tzafettas I. 2008. Toxoplasmosis during pregnancy: a case report and review of the literature. Hippokratia. 12(3): 139-143.

Gilbert R and Gras L. 2003. European Multicentre Study on Congenital Toxoplasmosis. Effect of timing and type of treatment on the risk of mother to child transmission of *Toxoplasma gondii*. BJOG. 110(2):112-20.

Padmavathy M, M Gowri, J Malini, BL Umapathy, BV Navaneeth, M Bhatia and Harle S. 2013. Seroprevalence of TORCH infections and adverse reproductive outcome in current

pregnancy with bad obstetric history. J Clin Biomed Sci. 3(2): 62-71.

Parija SC. 2008. Text Book of Medical Parasitology. 3rd ed. Ch. 7. New Delhi, All India Publishers and Distributors, p. 17280.

Sarkar MD, B Anuradha, N Sharma and Roy RN. 2012. Seropositivity of toxoplasmosis in antenatal women with bad obstetric history in a tertiary-care hospital of Andhra Pradesh, India. J Health Popul. Nutr. 30(1):87-92.

Singh S. 2003. Mother-to-child transmission and diagnosis of *Toxoplasma gondii* infection during pregnancy. Indian J Med Microbiol. 21(2):69-76.

Sood N, S Soni, M Vegad and Gupta P. 2009. Seroprevalence of *Toxoplasma gondii* in women with bad obstetric history in Ahmedabad. Gujarat Med J. 64(64):35-7.

Surpam RB, UP Kamlakar, RK Khadse, MS Qazi and Jalgaonkar SV. 2006. Serological study for TORCH infections in women with bad obstetric history. J Obstet Gynecol India. 56(1): 41-3.

Zargar AH, AI Wani, SR Masoodi,BA Laway, DK Kakroo, MA Thokar,BA Sofi and Bashir MI. 1999. Seroprevalence of toxoplasmosis in women with recurrent abortions/ neonatal deaths and its treatment outcome. Indian J Pathol Microbiol., 42(4): 483-6.

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