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Seroprevalence of acute toxoplasmosis in women with Bad Obstetric History in a tertiary care hospital in Uttarakhand

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ABSTRACT
Background: *Toxoplasma infection is an important cause for bad obstetric history and congenital infections. The risk of congenital infection depends upon the timing of maternal infection. The clinical implications are manifold but can be prevented by early diagnosis and treatment.*
Aim: *To assess the results and significance of IgM and IgG antibodies with IgG avidity test for diagnosis of toxoplasmosis in antenatal women with and without bad obstetric history (BOH) and to find out the prevalence of toxoplasmosis among pregnant women attending our hospital.*
Material and methods*: This was a prospective study where subjects were divided into study and control group (100 each). Serum samples were collected and Anti-toxoplasma IgM, IgG and IgG avidity of antibodies were analyzed using automated method (VIDAS, bioMerieux). Results thus obtained were analyzed statistically.*
Results*: 200 antenatal women were divided into control and study group based on presence or absence of bad obstetric history. Maximum cases belonged to 21-25 years i.e., 37% and 34% in study and control group respectively. Maximum seropositivity was observed in low socioeconomic class with 23% (18/78) in study group. With 24.13% abortion was the dominating BOH in the study group with seropositivity for Toxoplasma (both IgM and IgG avidity). Taking IgM and IgG avidity results collectively, the prevalence of recent toxoplasmosis in the subjects studied was found to be 9.5% (19/200).*
Conclusion*: It is recommended to screen all women of reproductive age group for toxoplasmosis during preconception period irrespective of BOH using IgM, IgG as well as IgG avidity tests. The strategy of screening followed by treatment of toxoplasmosis would reduce the burden of fetal loss and congenital anomalies.*
Keywords: *Congenital toxoplasmosis, abortions, antenatal diagnosis, TORCH, stillbirth, IgG avidity*

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I. INTRODUCTION

T. gondii is an obligate intracellular protozoan parasite infecting approximately one-third of the exposed population.[1] *T. gondii* has a broad range of intermediate hosts namely, mammals, birds and reptiles while members of family Felidae are the definitive hosts. Being worldwide in distribution with wide host range, high rate of infection and ability to coexist with the host makes *T. gondii* one of the most successful parasites on earth.[2, 3] Ability to coexist establishes life-long chronic toxoplasmosis in healthy individuals. But high infection rate makes it a serious threat to human health.[4]

There are three infective stages of *T. gondii:* the sporozoite stage in oocysts found in cat faeces, the rapidly dividing tachyzoites seen during acute infection and the slowly dividing bradyzoite stage in cysts during latent infection.[5, 6] Humans acquire infection by ingestion of oocysts that have contaminated food and water or by ingestion of improperly cooked meat containing tissue cysts. Vertical transmission from mother to fetus occurs transplacentally when uninfected mother acquires toxoplasma infection during pregnancy.*T. gondii* can also infect the fetus during delivery. Rarely, organ transplantation, blood transfusion, and laboratory acquired toxoplasmosis are also described.[7] In cases of congenital toxoplasmosis, the risk of severity of illness decreases as the gestational age advances in relation to the timing of maternal infection i.e., foetus is at highest risk when mother is infected during first trimester.[8]

Congenital infection or reactivation of a latent infection during immune dysfunction leads to fatal outcome. The prevalence rates vary significantly across India, with the highest (37.3%) in South India and the lowest in West India (8.8%), East India 21.2% and 19.7% in North India.[9] Majority of cases of toxoplasmosis during pregnancy are subclinical, however in some the serious outcomes in the form of abortions, blindness at birth, or severe

cognitive impairmentat birth may be observed.[4] Other complications of congenital toxoplasmosis reported in infants range from relatively mild signs, such as prematurity, peripheral retinal scars, sensory deficits, developmental delay, impaired psychomotor performance, and mental retardation to the classic triad of signs consisting of hydrocephalus, intracerebral calcification, and chorioretinitis.[10, 11] On the other hand, there is a reduced possibility of congenital infection by 60% if the pregnant woman is treated early for toxoplasmosis.[10]
Toxoplasma-specific IgM and IgG antibodies are the serological tests commonly employed for diagnosing toxoplasma infection. A positive IgG titre is sufficient to establish that a patient has been infected with *T. gondii* but a negative IgM result virtually rules out a recently acquired infection, unless sera are tested so early when an antibody response has not yet developed or is undetectable.[3]

With this background, the present study was carried out to determine the seroprevalence of acute toxoplasmosis in antenatal women with bad obstetric history (BOH) and to compare it with that in antenatal women having previous uneventful obstetric history, attending the antenatal clinic of a tertiary care hospital in Uttarakhand. An attempt to assess the results and significance of IgM and IgG antibodies with IgG avidity test for diagnosis of toxoplasmosis in specimens obtained from antenatal women was also carried out. Since it is still not a common practice in developing country like India to include *Toxoplasma* infections in the differential diagnosis in such cases.

Study design

This prospective, comparative study hospital based study was conducted in the Department of Microbiology in collaboration with Obstetrics & Gynecology department of our hospital for a period of one year from Jan 2017 to Dec 2017.

Ethical clearance was granted by committee of our institute. Patient confidentiality was maintained by excluding their name and registration number while compiling the data and giving a specific identifier number.

An informed consent was taken from the patient or from the guardian (in case of minor) in the prescribed format.

Study group: A total of 100 samples from antenatal cases with clinically diagnosed bad obstetric history attending outpatient clinics of Obstetrics Department as well as those admitted in Obstetrics ward of our hospital were included as study group.

Control group: A total of 100 samples from healthy antenatal cases without any bad obstetric history attending outpatient clinics of Obstetrics Department as well as those admitted in Obstetrics ward of our hospital were included as control group of the study.

Selection criteria of subjects

***Inclusion criteria for study group*:** Pregnant women of all age groups with previous history of abortions, intrauterine fetal death, stillbirths, preterm deliveries, unexplained early neonatal death, congenital anomalies: chorioretinitis, hydrocephalus were included in study group.

***Exclusion criteria for study group*:** Non-pregnant ladies with BOH, woman unwilling to participate in the study, pregnant women with previous uneventful pregnancies were excluded from the study.

***Inclusion criteria for Control group*:** Pregnant women of all age groupswith no previous bad obstetrics history were included in this study.

***Exclusion criteria for Control group*:** Women in antenatal period with bad obstetrics history, primigravida and women unwilling to participate in the study were excluded from the study.

The socio economic status in the study was defined as per Kuppuswamy's socio economic status scale.[12] At the time of sample collection all demographic details and relevant clinical data was collected in the specially designed Case Recording Form for each participant.

II. MATERIAL AND METHODS

Blood specimens were drawn under aseptic conditions by venipuncture which was collected in a vacutainer vial without anticoagulant. Serum samples were separated through centrifugation which was later aliquoted and stored at -20^0 C until further investigation. The serum was tested for Toxoplasma IgM and IgG antibodies using automated method (VIDAS, bioMerieux).Test Kits used were VIDAS Toxo IgM (TXM) (bioMerieux), VIDAS Toxo IgG (TXG) (bioMerieux) and VIDAS Toxo IgG avidity (TXGA) (bioMerieux).

Standards for calibration and positive and negative controls were run for validation of the test procedure and kits. The manufacturer's instructions were followed for performance of the tests. Serum samples from all subjects of study as well as control group were tested for IgM and IgG antibodies against *Toxoplasma*.

The samples found to be higher than 300 IU/ml for *Toxoplasma* IgG antibodies were further tested for *Toxoplasma* IgG avidity as per manufacturer's protocol.

Results thus obtained were analyzed using Chi square test for their statistical significance.

III. RESULTS

Out of total 100 patients each in the study and control group the maximum number of cases included were found in 21-25 years of age group, with 37% in study group and 34% in control group. The maximum number of cases in study group i.e., 82/100 and 64/100 cases in the control group belonged to the lower socioeconomic class.

When clinical spectrum of bad obstetric history among cases studied was analyzed, among the subjects in the study group dominant past history of abortions was 58/100. (Table no1)

Table no 1: Clinical spectrum of Bad Obstetric History of cases in study group (n=100)

Clinical conditions in study group	BOH
Abortions	58
Still births	13
Intra Uterine Death	16
Hydrocephalus	11
Congenital malformation	9
Preterm labour	2
Unexplained death	4

The results of IgM antibodies against Toxoplasma revealed that in the study group, 12/100 subjects were positive while in the control group only 1/100 subject was found positive for IgM antibodies against *Toxoplasma*.

In the study group, 35/100 subjects were found positive for IgG antibodies against *Toxoplasma* while in the control group only 4/100 subjects were found positive for IgG antibodies against *Toxoplasma*.

Table no 2 shows that in the study group, 10/100 (10%) subjects were positive for both IgG and IgM antibodies and 2/100 (2%) subjects were positive only for IgM antibody. Statistical analysis revealed that with a p value of <0.05 the results were significant.

Table no 2: Comparison of IgM and IgG antibodies against *Toxoplasma* in study group

	IgG positive	IgG negative
IgM positive	10	2
IgM negative	25	63

Chi square: p value <0.05 Significant

In the control group, none of the subjects were positive for both IgG and IgM antibodies whereas 17/100 subjects were positive only for IgG antibodies. While 1/100 subject was positive for IgM antibody only and 82/100 subjects were both negative for both IgG and IgM antibodies for against *Toxoplasma*.It was observed that 52.63% (10/19) Seropositive cases (IgM & IgG avidity) for *Toxoplasma* also belonged to the age group of 21-25 years.

The comparison of titres of IgM and IgG antibodies in the study group showed that 10/100 patients were positive for IgM antibodies as well as IgG antibodies with a titre of >300IU/ml. Statistical analysis revealed that with a p value of <0.05 the results were significant. (Table no 3)

Table no 3: Comparison of IgM and IgG antibodies titre against *Toxoplasma* in study group

	IgG positive >300 IU/ml	IgG positive<300 IU/ml
IgM positive	10	2
IgM negative	7	26

Chi square: p value <0.05 Significant

Only samples with IgG antibody titres>300IU/ml were analysed for IgG avidity. In the study group, 7/17 (41.17%) subjects were found positive for IgG avidity antibodies while in the control group only 1 sample tested was found positive for IgG avidity antibodies against *Toxoplasma*. (Table no 4)

Table no 4: Results of avidity of IgG antibodies against *Toxoplasma (n=18)*

	IgG avidity positive	IgG avidity negative
Study group	7	10
Control group	1	0

Since only those serum samples with IgG titre of >300IU/ml were considered for IgG avidity test as per the protocol. Therefore, this analysis was performed on 28 samples only. The comparison of results of IgM antibodies and IgG avidity in the study group showed that only 1/28 samples was positive both for IgM and IgG

avidity antibodies. The observations were found to be not significant statistically with a p value of >0.05. (Table no 5)

Table 5: Comparison of IgM and IgG avidity antibody results against *Toxoplasma* in study group (n=28)

	IgG avidity positive	IgG avidity negative
IgM positive	1	11
IgM Negative	6	10

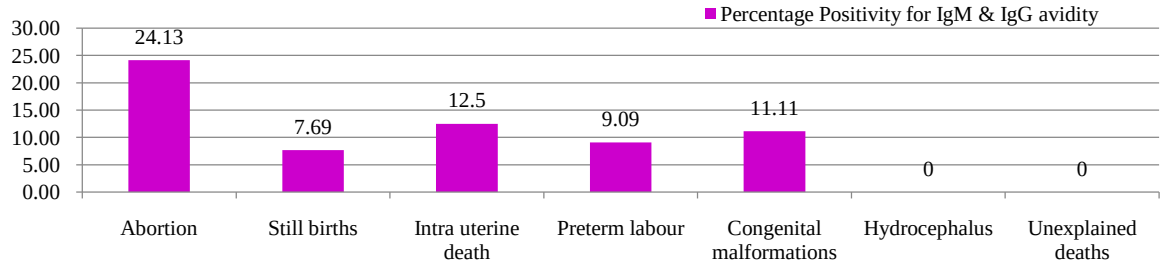
Chi square: p value >0.05 Not significant

In the control group, similar comparison revealed that none of the subjects was positive for both IgM as well as avidity of IgG antibodies, and only one sample out of two had IgG titre>300IU/ml which was also found positive for IgG avidity.

In subjects belonging to lower socioeconomic strata 18/78 (23.07%) subjects were found seropositive for IgM & IgG avidity. None of the cases belonged to upper socioeconomic class. On statistical analysis these observations were found to be significant (p value <0.05)

BOH and their comparison with seropositivity for IgM and IgG avidity in the study group showed thatmaximum seropositivity with 24.13% (14/58) was observed in cases with history of abortions while all cases with a history of hydrocephalous and unexplained deaths were seronegative. (Fig no 1)

Fig no 1: Comparison of percentage positivity of IgM & IgG avidity results with various Bad Obstetric Histories in the study group



IV. DISCUSSION

TORCH group of infectious agents represent *Rubella*, *Cytomegalovirus*, *Herpes* viruses and *Toxoplasma gondii*. In India, awareness about these infections and their association with congenital anamolies is poor. Mostly women who seek medical attention, or are referred by obstetricians for toxoplasma related investigations, are those who have had an undesirable pregnancy outcome.[4] A study conducted in general population of Uttarakhand reported 34.84% IgG serpositivity and 6.33% IgM seropositivity of toxoplasmosis.[13]

In most of the studies including the present, maximum number of antenatal cases belonged to 21-25 years of age group and 26-30 years.[14, 15, 16] This is the average age for marriage in India and also the average child bearing age group.

A total of 19% serum samples from study group were found positive for *Toxoplasma* (IgM & IgG avidity, suggesting acute or ongoing infection), of which 52.63% (10/19) cases belonged to the age group of 21-25 years. Among the studies of other workers [14, 15, 18] it was observed that for acute toxoplasmosis only IgM titres were considered, however in the present study both IgM and IgG avidity were taken into account. Deka S reported the overall seropositivity of *Toxoplasma gondii* infection as 41.2%. while IgG in 38.2% and IgM was raised only in 13.3% pregnant women in a study in Uttarakhand.[17] The difference could be due to the duration of study as well as the sample size.

Being a charitable hospital where this study was conducted the majority of study participants belonged to lower socioeconomic class. Hence, it is not possible to comment on the prevalence of toxoplasmosis in higher socioeconomic class in this study. Some women had more than one type of clinical condition pertaining to BOH, of which the dominant past history of abortions was reported by 62% subjects. Similar observation by other workers suggest predominance of abortion, stillbirths, congenital anomalies, preterm delivery as the major features of BOH linked to toxoplasmosis. (Table no 6)

Table no 6: Comparison of commonest clinical conditions of BOH observed by various workers

Study	Year of study	Details of BOH	% of Positive Cases
Surpam R B et al [19]	2006	Abortion	27.7%
		Still Births	17.64%
		Congenital anomalies	4.76%
		Preterm deliveries	18.18%
N Sridevi et al [18]	2013-14	Abortion	53.85%
		Still Births	19.23%
		Congenital anomalies	3.85%
		Preterm deliveries	7.69%
Present Study	**2017**	**Abortion**	**62 %**
		Still Births	**13%**
		Congenital anomalies	**9%**
		Preterm deliveries	**15%**

A relationship between abortions and evidence of acute toxoplasmosis has emerged internationally also as the commonest presenting feature of BOH with 33.7% [19] and 30.4% [20]. The significant relationship between antenatal women with bad obstetric history (study group) and presence of IgM antibodies in 12% cases suggests evidence of recent exposure or ongoing active infection as observed in the present study. This result is in concurrence with the studies of N Sridevi et al as16.25% [18], Surpam RB et al 14.66% [19] and Esboei BR et al 10.4%. [21]

In the present study, 25 subjects who were IgG positive, had history related to BOH to be placed in study group, probably due to late phase of infection or due to wide gap in between the past and present pregnancies the lady had seroconverted and can be interpreted to be safe from the impending problems of toxoplasmosis. Sixty three cases belonging to study group might have had past BOH due to some cause other than toxoplasmosis.

In the control group, on comparison of results of IgM and IgG antibodies against *Toxoplasma*, none of the subjects were found to be positive for both IgG and IgM antibodies whereas 17% subjects were positive only for IgG antibodies and assuming that the individuals had developed IgG antibodies prior to their previous pregnancies. However, 1/100 subject was positive for IgM antibody only signifying the importance of screening for toxoplasmosis even in the absence of any significant history. Statistical analysis could not be performed on this data due to certain limitations. It is recommended to perform a screening of a large population of healthy women of reproductive age group in future.

Timing of the onset of the infection is crucial in pregnant women, especially risk for the fetus increases on post-conceptional acquisition ofinfection. In such cases IgG avidity detection is helpful.[16] A positive IgG avidity is an important tool to guide towards acute toxoplasmosis which can lead to poor outcome of pregnancy even with high titres of IgG. Positive IgG avidity in antenatal cases with bad obstetric history has greater significance suggesting comparatively recent infection hence pre-conceptual screening for acute toxoplasmosis is suggested to counsel these women for future pregnancies.

Detection of anti-*Toxoplasma*-specific IgM antibodies is a sensitive indicator of an ongoing or recent infection, but diagnosis of primary infection with *T. gondii* in early pregnancy can be improved by determination of anti-*Toxoplasma* IgG avidity. In this study two cases were detected to be having acute toxoplasmosis even in the control group.

The diagnostic accuracy of low avidity in relation to IgG positivity produces a significant negative likelihood ratio which indicates strong evidence to rule out old *Toxoplasma* infection suspected by the presence of positive IgG antibodies.[22] In the present study the maximum seropositivity (IgM and IgG avidity) was observed in cases with history of abortions 27.45% (14/51).Similar observations were recorded by other workers from India and abroad.[14, 15, 18]

In France [23] and Austria [24], screening for toxoplasmosis is mandatory in prenatal care. The serological tests for Toxoplasma however, have certain limitations. The detection of antibodies in immunocompromised individuals may be difficult.[25] IgM may persist for longer than expected periods and discrimination between recent and older infections may be a problem. This is an important factor when diagnosing toxoplasmosis in immune compromised individuals as the presence of IgG indicates a risk for the reactivation of a latent infection, and IgM indicates the possibility of an acute infection. [26]

In pregnant women, positive IgM results indicate the likely acquisition of infection during gestation and a positive IgG and negative IgM result indicates a previous infection.[27] Avidity tests have helped to overcome this problem as they help differentiate between recently and distantly acquired infections. Avidity tests are based on the fact that during acute infections, IgG antibodies bind antigen relatively weakly and therefore have a low avidity. Chronic infections, have more strongly-binding antibodies and therefore have a high avidity.[27, 28]

V. CONCLUSION

The transplacental transmission of toxoplasmosis may lead a large spectrum of complications. The infection is preventable and treatable, therefore early serological screening for *Toxoplasma* antibodies in mothers can reduce the perinatal morbidity and mortality.[1, 6]

IgG avidity has an important role to play in the form of defining recent or old toxoplasmosis in a single sample itself since IgM antibodies against *Toxoplasma* can persist sometimes, for years together. Taking IgM and IgG avidity results collectively, the prevalence of recent toxoplasmosis has been found to be 9.5% (19/200).

The risk factors for toxoplasmosis are more prevalent in low socioeconomic class due to poor living and hygienic conditions. However, in the present study the details of such risk factors were not included. Integrated public health strategies and awareness programs concerning many other infections will go a long way in reducing their prevalence.

In the present study, abortions and low socioeconomic status were found to be the key factors observed in relation to recent toxoplasmosis. One case each was found positive in the healthy control group for IgM as well as IgG avidity. However, an elaborate study in future for detection of IgM, IgG and IgG avidity in the general population with a higher number is suggested to find the true relevance of screening all women of reproductive age group for toxoplasmosis during preconception period irrespective of BOH. Being a hospital-based study many other presumptive risk factors like closeness to domestic or wild animals or familial clustering due to household effect etc. could not be analyzed due to unavailability of information. Therefore, larger community-based prospective studies can fill these gaps. Due to lack of awareness regarding hand and food hygiene, presence of comorbidities and non-vegetarian diet, the toxoplasmosis is an endemic infection. However, screening and treatment would reduce the burden of fetal loss and congenital anomalies.

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