

Association of Toxoplasma Gondii Infection with Spontaneous Abortion

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Abstract:

Objective: To evaluate whether there is any association of Toxoplasma gondii infection with spontaneous abortion.

Materials and Methods: This observational case control study was conducted in the Department of Obstetrics and Gynaecology, Dhaka Medical College Hospital, Dhaka (DMCH), over a period of one year from 31st December 2008 to 30th November 2009. A total 91 pregnant women were selected for this study. Among them 46 patients of spontaneous abortion were selected as case and 45 healthy pregnant women were selected as control purposively. Spontaneous abortion cases were diagnosed on the basis of history, clinical examination and USG findings. Patients with medical disorder, Rh-incompatibility, multiple pregnancy, cervical incompetence, septic abortion, threatened abortion, fibroid with pregnancy and uterine anomaly were excluded from the study. The clinical examination was done by standard method. Blood was collected from each subject, serum was separated. Main outcome measure was presence of toxoplasma antibodies in spontaneous abortion cases. Antibody was detected by ELISA method. A p value of <0.5 was considered as significant.

Results: In both groups there was no difference in age, gestational age, socioeconomic status, living standard and educational status.

Toxoplasma specific IgM was detected in higher percentage of women with spontaneous abortion (15.2%) than with controls (0%), which is statistically significant (<0.05). Seroprevalence for past Toxoplasma infection (IgG) was similar in both the groups, 17.4% and 8.9% for case and control respectively ($p>0.05$). Though there is no association between seropositivity and level of education, place of living, contact with cat, occupational status, parity and past history of spontaneous abortion, seropositivity is higher in the poor socioeconomic group.

Conclusion: Seroprevalence for recent Toxoplasma gondii infection is higher in women with spontaneous abortion.

Key Words: Seroprevalence, Toxoplasma gondii , spontaneous abortion.

Introduction:

Toxoplasmosis is caused by the obligate intracellular parasite *Toxoplasma Gondii*¹. The sexual form of the parasite lives in the gut of the definitive host, the cat,

where it produces oocysts. After a period of maturing in the environment, these oocysts become the source of infection for secondary hosts, which may ingest them. In the secondary hosts (which include man,

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cattle, sheep, pigs, rodents and birds) there is disseminated infection. Following a successful immune response the infection is controlled, but dormant parasites remain encysted in host tissue for many years. The life cycle is completed when carnivorous felines eat infected animal tissue².

It has been estimated that up-to one third of the world's population is infected by *Toxoplasma gondii*³. In developed countries toxoplasmosis is the most common protozoal infection; around 22% of adults in UK are seropositive⁴. In countries like India or Brazil 42-60% of pregnant female are seropositive for toxoplasma⁴.

Toxoplasmosis can be transmitted to humans by three principal routes. It is estimated that approximately 50% of the toxoplasmosis cases in the United States are caused by eating inadequately cooked meat containing tissue cyst form of the organism⁵.

A second means of acquiring toxoplasmosis is by inadvertently ingesting the oocysts that cats have passed in their feces, either from a cat litter box, from infected soil where a cat has defecated or from unwashed fruits or vegetables that have contracted infected soil. A third means of transmission is transplacentally, when a primary infection occurs during pregnancy.

Maternal risks of toxoplasmosis in the immunocompetent gravida are minimal and 90% of infections are asymptomatic and self limited. Symptoms most commonly resemble infectious mononucleosis with posterior cervical or axillary lymphadenopathy that is discrete, nontender and non-suppurative. Other maternal symptoms may include malaise and muscle pain. Fetal risks, on the other hand, vary widely, depending on the trimester of acquisition and presence or absence of maternal treatment. In general the earlier the infection is acquired, the lower the risk of fetal transmission, but the greater the risk of serious sequelae to the fetus when congenital infection does develop⁶.

Maternal seroconversion occurring at or before five weeks gestation has been reported to have minimal to no risk of fetal infection, where as seroconversion in the third trimester may result in congenital infection in as many as 60% of neonates⁷. As many

as 85% of congenitally infected neonates have subclinical infections that will later surface as chorioretinitis, hearing loss or developmental delays. Serious sequelae noted at or after birth, including intrauterine death, neurologic abnormalities, hydrocephalus, cerebral calcification, and chorioretinal scars with or without severe visual impairment, which may be reduced by the use of prenatal antibiotic therapy even though the rate of congenital infection may not change⁸.

Spontaneous abortion is the most common complication of pregnancy and is defined as the passing of a pregnancy prior to completion of the 20th gestational weeks. It implies delivery of all or any part of the products of conception, with or without a fetus weighting less than 500 gm. Although the true incidence of spontaneous abortion is unknown, approximately 15% of clinically evident pregnancies and 60% of chemically evident pregnancies end in spontaneous abortion⁹.

An abnormal karyotype is the leading cause of spontaneous abortion. Other suspected causes of spontaneous abortion include infection, anatomic defects, endocrine factors, immunologic factors and maternal systemic illness. In a significant percentage of spontaneous abortion, the etiology is unknown⁹.

Primary toxoplasma infection acquiring during pregnancy may result in severe damage to the fetus including spontaneous abortion, IUGR, still birth, early neonatal death or congenital toxoplasmosis. Data indicate, however, that although chronic toxoplasmosis is associated with abortion it is not a common event¹⁰. Silveria et al recently reported a case of maternofetal transmission in a preconceptionally immunized woman¹¹. This finding could be accounted for by a down regulation of the T-cell mediated immune response that is observed during pregnancy¹².

Acute and latent toxoplasma gondii infections during pregnancy are mostly diagnosed by serological test including detection of antitoxoplasma gondii specific IgM and IgG antibodies. Antibody screening programs aimed at the diagnosis of T.gondii infection among pregnant women have therefore been introduced in several countries¹³. In European countries, prevalence of toxoplasma gondii infection in pregnant woman varies from 9-67%¹⁴. While prevalence as high as

41.8 to 53.4% in pregnant women have been reported in Indian, Malaysian and Nepalese population ¹⁴. In France and Austria, where the incidence of toxoplasmosis is much higher than in the United States, universal screening is mandatory ¹⁵. In the United States, although universal screening has been frequently discussed, it is not generally practiced. Current evidence does not support routine screening for toxoplasmosis in UK ¹⁶.

Toxoplasma infection is assumed to be one of the causes of spontaneous abortion. In Bangladesh the research on toxoplasmosis is a few though it is high risk area due to prevalence of domestic cat. Therefore, this case control study has been designed to investigate toxoplasma antibodies in a group of women with spontaneous abortion versus normal pregnancies and to assess whether there is an association between toxoplasmosis and spontaneous abortion.

Materials and Methods:

This observational case control study was conducted in the Department of Obstetrics and Gynaecology, Dhaka Medical College Hospital, Dhaka (DMCH), over a period of one year from 31st December 2008 to 30th November 2009. A total 91 pregnant women were selected for this study. Among them 46 patient of spontaneous abortion were selected as case and 45 healthy pregnant women were selected as control purposively.

A detailed history was taken from the patients including age of patients, gestational age, occupation, educational level, husband's and patient's monthly income, previous history of abortion, previous history of delivery of congenitally abnormal baby, complication during present pregnancy, history of medical and gynaecological disorders, previously done investigations etc. Socioeconomic status was classified by occupation, monthly income and educational level. A complete general and obstetric examination was done by per abdominal and per vaginal examination. Spontaneous abortion cases were diagnosed by history, clinical examination and USG findings. Normal healthy pregnant women were taken as control. All the controls were observed up to 20 weeks for any evidence of abortion. Informed written consent was taken from both the groups.

With usual aseptic precaution 5 ml of venous blood was collected from each subject from the antecubital vein using disposable plastic syringe. Blood sample from spontaneous abortion cases was drawn immediately after or within 7 days of abortion. Serum was separated by centrifugation for 5 minutes at 3000 rpm at room temperature immediately after the blood was allowed to clot for 30 minutes. Separated serum was allocated in different eppendrop and preserved immediately at -20⁰c temperature for subsequent analysis.

Anti-Toxoplasma IgG and IgM antibodies were investigated in the department of Microbiology,

The qualitative immunoenzymatic determination of IgM class antibodies against *Toxoplasma gondii* was based on the ELISA (Enzyme Linked Immunosorbent Assay) technique. The *Toxoplasma* IgM ELISA is an IgMm - capture ELISA.

The quantitative immunoenzymatic determination of IgG – class antibodies against *T. gondii* was also done by ELISA technique.

If only IgM or both IgM and IgG are positive, then the person was considered as infected within past 6 months. If only IgG is positive it is considered that the person is infected at least 6 months before.

Patients with medical disorder, Rh-incompatibility, multiple pregnancy, cervical incompetence, septic abortion, threatened abortion, fibroid with pregnancy and uterine anomaly were excluded from the study. Main outcome measure was presence of toxoplasma antibodies in spontaneous abortion cases. Ethical clearance and permission for the study was taken from appropriate authority.

Data were processed and analyzed using SPSS version 16.0. Chi-square (χ^2) test, unpaired students t-test, and Fisher's exact test were done for test of significance where appropriate. A p value of <0.05 was considered as significant.

Results:

The findings of the present study shows the fact that the case and control populations were similar in age, socioeconomic status, educational status, place of living, occupational status, gestational age and without any significant difference between the groups.

Table-I
Characteristics of the patients

Characteristics	Case (46)		Control (45)		Significance
	Mean \pm SD		Mean \pm SD		
Age (Yrs)	24.43 \pm 4.17		24.56 \pm 4.36		0.830
Gestational age - GA (Weeks)	14.11 \pm 4.33		14.29 \pm 4.49		0.997
	N	%	N	%	
<i>Socioeconomic status</i>					
Upper class	2	4.3	1	2.2	0.830
Middle class	28	60.9	29	64.4	
Poor	16	34.8	15	33.3	
<i>Place of living</i>					
Urban	19	41.3	20	44.4	0.919
Semi-urban	13	28.3	13	28.9	
Rural	14	30.4	12	26.7	
<i>Gravida</i>					
Primi	14	30.4	13	28.9	0.871
Multi	32	69.6	32	71.1	
<i>Contact with cat</i>					
Present	41	89.1	42	93.3	0.369
Absent	5	10.9	3	6.7	

Table-II
Presence of immunoglobulin in serum

Immunoglobulin	Case (46)		Control (45)		Significance
	N	%	N	%	
<i>Ig M</i>					
Positive	7	15.2	0	00	0.007
Negative	39	84.8	45	100	
<i>Ig G</i>					
Positive	8	17.4	4	8.9	0.231
Negative	38	82.6	41	91.1	

Table III
Distribution of the study subjects by Seropositivity, either IgG or IgM

Characteristics	No of women (91)	Seropositive		P value
		N	%	
<i>Contact with cat</i>				
Present	83	14	16.9	0.751
Absent	8	1	12.5	
<i>Gravidity</i>				
Primi	27	6	22.2	0.338
Multi	64	9	14.1	
<i>Previous congenital anomaly</i>				
Present	3	0	00	0.578
Absent	88	15	17	
<i>Social Class</i>				
Upper	3	1	33.3	0.036
Middle	57	5	8.8	
Poor	31	9	29.0	
<i>Place of living</i>				
Urban	39	5	12.8	0.058
Semi urban	26	2	7.7	
Rural	26	8	30.8	

Result shown by χ^2 test

Table-IX
IgG status among the past history of spontaneous abortion of the study subjects (n=21)

IgG	Case (10)		Control (11)		P value
	N	%	N	%	
Positive	1	10.00	2	18.2	.537
Negative	9	90.0	9	81.8	

Table I shows the demographic characteristics of the patient and shows no difference between the groups (> 0.05).

Table II shows that IgM was positive in 7(15.2%) patients in case group and none in control group and the difference was statistically significant (p<0.05). Whereas 8 (17.4%) and 4 (8.9%) patients had IgG positive in case and control group respectively, which shows no significant difference (p>0.05).

There is no relation of seropositivity with contact with cat, number of pregnancy and history of previous pregnancy. But significant association is found with social class and place of living (p< 0.05). Rural and lower socioeconomic people are more infected with toxoplasma gondii.

There is no relation with presence of IgG that is sign of previous infection with spontaneous abortion.

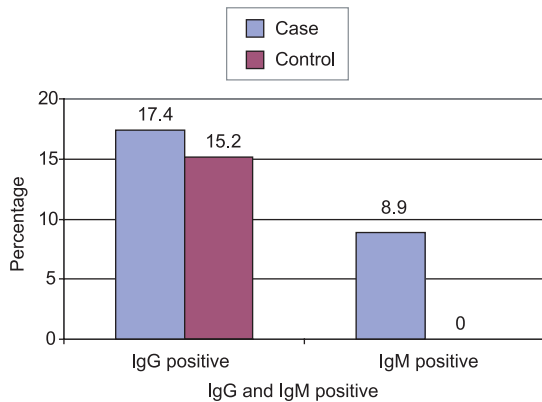


Fig 1: Bar diagram showing the IgG and IgM positive in case and control group (n=91)

This diagram (Fig 1) shows the presence of recent (Ig M) and old (Ig G) infection in both groups. Sign of old infection present in both groups without any significant difference. On the other hand recent infection is found in 15.2% cases in spontaneous abortion group only. No IgM found in control group, which indicates that

there is a strong association of spontaneous abortion and toxoplasma gondii infection.

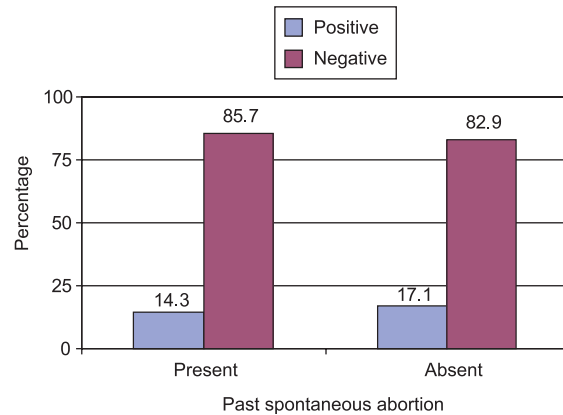


Fig 2: Bar diagram showing the seropositive status between past history of spontaneous abortion and no history of spontaneous abortion of the study subjects (n=91)

Figure 2 shows seropositive (either IgG or IgM) was found in 3 (14.3%) patients who had past spontaneous abortion and in 2 (17.1%) who had no past spontaneous abortion and the difference was not statistically significant (p>0.05) between two groups. (P= 0.756, p value reached from Chi-Square test).

Discussion:

Most acute toxoplasma infection are relatively asymptomatic, therefore, a universal challenge for obstetrician is how to identify acute infections during pregnancy. The method of choice in the diagnosis is by detection of specific antibodies in the patient's serum. Different studies have been done to see the association between toxoplasma gondii infection with spontaneous abortion. Many studies have demonstrated the relationship between toxoplasma gondii infection with spontaneous abortion^{17,18,19,20,21}, while others have refuted an association²².

The purpose of present study was to see the seroprevalence of toxoplasma gondii infection in

spontaneous abortion. A case control study with 46 cases of spontaneous abortion and 45 healthy pregnant women (control) was designed. The finding of the present study is strengthened by the fact that the case and control populations were similar in age, mostly were third decade. Socioeconomic status of both group were similar. Regarding obstetric variable like gestational age and gravidities were homogenously distributed without any significant difference between the groups.

The serological data of this study indicates that there was a clear association between acute toxoplasma infection and spontaneous abortion. IgM antibody was positive in 15.2% of cases and 0% of controls ($p < 0.05$) of our series. Strong association of presence of IgM antibody and spontaneous abortion also was found in 10.52%¹⁹, 34.5%¹⁸ and 23.3%²⁰ cases in different studies. Although other one study shows only 0.97% IgM positive in spontaneous abortion²³ majority shows strong association of IgM antibodies and spontaneous abortions¹⁸⁻²⁰.

Regarding the IgG status, 17.4% of cases and 8.9% controls were IgG positive. But the difference is not statistically significant ($p = > 0.05$). But a study shows significantly higher seroprevalence of toxoplasmosis in habitual abortion²⁴.

In this study there is no significant difference in seropositivity (IgG) between patient with past history of spontaneous abortion and patient with no such history ($p = > 0.05$). But study shows that seroprevalence rate is increased with a greater number of previous abortions²⁴.

Most of the patients had IgG positive both in case and control groups but we did not find any association between presence of IgG and abortion, as rate was similar in both groups. It indicates that old infection is not responsible for abortion. There was no relation with seropositivity with age, gestational age, gravidity and previous history of congenital anomaly. Except age same findings showed by other two studies^{14, 25} and one showed increasing seropositivity with age²⁵. Pet animals particularly cat is a major source of transmission of Toxoplasma infection in human and Muna et al showed higher seropositivity among the patients who had close contact with cat²⁵. But we did not find any association between seropositivity and contact of cat.

Is there any relation with social class and living standard of women with Toxoplasma infection? As eating of improperly washed or unwashed fruits and vegetables those have contact with contaminated soil from cat's feces might responsible for transmission of infection. Living standard and social class might have some relation. Alvarado-Esquivel et al showed significantly higher seroprevalence (14% vs 6%, $p < 0.05$) in women from lower social class than from higher social class²⁵. Nash et al showed significantly higher seroprevalence in women from rural areas²⁶. We also found higher seroprevalence in women from rural areas and from lower social class.

Conclusion:

This study has shown that seroprevalence for recent toxoplasma gondii infection is higher in women with spontaneous abortion. But this study failed to identify any association between latent toxoplasma gondii infection and spontaneous abortion.

References:

1. Ernest JM, 'Parasitic infections' in James, Weiner Steer and Gonik (eds.) High risk pregnancy: management options. Elsevier, India, Third edition, 2006; pp.710-713.
2. Finch RG, Moss P, Jeffries DJ and Anderson J 'Infectious disease, tropical medicine and sexually transmitted diseases', Kumar and Clark Clinical Medicine, 6th edn, Elsevier New York. 2005.
3. Montoya JG, Liesenfeld O. 'Toxoplasmosis'. Lancet. 2004; 363: 1965-76.
4. Todd WT, Lock Wood DNJ, Sundar S. 'Infectious diseases'. In: Boon, NA, Colledge, NR, Walker, BR and hunter, JA (eds). Davidson's Principal and Practice of Medicine. 20th edn. Elsevier, New York, 2006.
5. Roghnam MC, Fulkner CT, Lefkowitz A. Decreased seroprevalence for toxoplasma gondii in seventh day Adventists in Maryland. Am J Trop Med. 1999; 60: 790-92.
6. Hohlfeld P, Daffos F, Tlaulliez P et al. Fetal Toxoplasmosis: outcome of pregnancy and infant follow up after in utero treatment. J Pediatr. 1989; 115: 765-69.
7. Forestier F, Daffos F, Hohlfeld P, Lynch L. Infectious fetal diseases prevention, prenatal

- diagnosis, practical matters. *Presse Med.* 1991; 20:1448-54.
8. Foulon, W, Pinon JM, Stray-Pedersen B. Prenatal diagnosis of congenital toxoplasmosis: a multicenter evaluation of different diagnostic parameters. . *Am J Obstet Gynecol*, 1999; 118: 843-48.
 9. Peter S, Uzelac S, Garnel . 'Early pregnancy risk', In Decherney AH, Nathan, L, Goodwin, T, Laufer, N (eds.). *Current Obstetrics & Gynaecologic Diagnosis and Treatment*, 10th edn, McGraw-Hill, New York 2007; 259-66.
 10. Arias F, Daffary SN & Bhide AG . 'Fetal infections' *Practical guide to high risk pregnancy and delivery*. . 3rd edn. Elsevier, New Delhi, India, 2008; pp. 160-64.
 11. Silveria C, Ferreira R, Muccioli C, Nussenblatt R, Belfort R. Toxoplasmosis transmitted to a newborn from the mother infected 20 years earlier. *Am J Ophthalmol*. 2003; 136: 370-71.
 12. Garweg JG, Scherrer J, Wallon M, Kodjikian L, Peyron F. Reactivation of ocular toxoplasmosis during pregnancy. *BJOG* 2005; 112: 241-42.
 13. Kumar A, Arora V, Mathur M. Toxoplasma antibody levels in females with habitual or sporadic abortions and normal pregnancies. *Ind J Med Microbiol* 2004; 22: 276-77.
 14. Esquivel CA, Alvarez AS, Durate GS, Martinez SE . Seroepidemiology of toxoplasma gondii infection in pregnant women in a public hospital in northern Mexico. *BMC Infectious Diseases* 2006; 6: Viewed 13 July 2006, <http://www.biomedcentral.com/1471-2334/6/113>
 15. Jeannel D, Costagliola D, Niel G . What is known about the prevention of congenital toxoplasmosis? *Lancet*. 1990; 336: 359-61
 16. Overton TG . 'Antenatal care', in Edmonds DK (eds),. *Dewhurst's Textbook of Obstetrics and Gynaecology*, 7th edn, Blackwell Publishing Oxford. 2007; 39-45.
 17. Lolis D, Tzigounis V, Michalac S, Koumentakou, Kaskarelis D E. ' *Int J Gynaecol Obstet*. 1978; 15(4): 299-301.
 18. Zargar AH, Waani GR, Laway BA, Kakroo DP, Sof BA . Toxoplasmosis in women with recurrent abortions, neonatal death and its treatment outcome. *Ind J Pathol Microbiol*. 1999; 42(4): 481-83.
 19. Turbadkar D, Mathur M, Rele M. Seroprevalence of torch infection in bad obstetric history. *Ind J Med Microbiol* 2003; 21(2):108-10.
 20. Yasodhara P, Rama Lakshi BA, Lakshmi V, Krishna TP . Socioeconomic status and prevalence of toxoplasmosis during pregnancy. *Ind J Med Microbiol*. 2004; 22: 241-43.
 21. Chen KT, Esklid A, Bresnahan M, Pedersen B, Sher A, Jenum PA . Previous maternal infection with toxoplasma gondii and the risk of fetal death. *Am J Obstet Gynaecol* 2005; 193:443-49.
 22. Djurkovic-Dijakovic O. Toxoplasma infection and pathologic outcome of pregnancy. *Gynecol Obstet Inv*. 1995; 40(1): 36-41.
 23. Razzak AH, Wais SA, Saeid AY . Toxoplasmosis: the innocent suspect of pregnancy wastage in Duhok, Iraq. *Eastern Medi Health J*. 2005; 11:625-32.
 24. Muna M, Hamdani AI, and Mahdi NK. Toxoplasmosis among women with habitual abortion. *Eastern Medi Health J*. 1997; 3: 310-15.
 25. Alvarado-Esquivel C, Torru C, Liesenfeld O, Lopez CR, Martinez ES, Alvarezm S, 'Seroepidemiology in toxoplasma gondii infection in pregnant women in rural Durango, Mexico'. *J Parasitol*. 2009; 95(2):271-74.
 26. Nash JQ, Chissel S, Jones J, Warburton F, Verlander NQ . Risk factors for toxoplasmosis in pregnant women in kent, United Kingdom. *Cambridge J*. 2005; 133: 475-33.