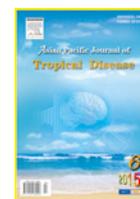


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Role of *Toxoplasma gondii* serology in patients with habitual abortionsNaushaba Siddiqui^{1*}, Haris Mansoor Khan¹, Tamkin Rabbani², Parvez Anwar Khan¹, Fatima Shujatullah¹¹Department of Microbiology, Jawahar Lal Nehru Medical College and Hospital, A.M.U. Aligarh 202002, India²Department of Obstetrics and Gynaecology, Jawahar Lal Nehru Medical College and Hospital, A.M.U. Aligarh 202002, India

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ABSTRACT

Objective: To study the prevalence of toxoplasmosis in pregnant females with history of habitual abortions.**Methods:** The study was conducted in the Department of Microbiology, Antenatal Clinic and Gynaecology, OPD of Jawahar Lal Nehru Medical College and Hospital from February 2011 to September 2012. A total of 400 patients presented with history of multiple spontaneous abortions. On basis of history and clinical examination, 48 patients are classified as high risk group for toxoplasmosis. Serum specimens were subjected to IgG ELISA, IgM ELISA and Enzywell *Toxoplasma* IgG avidity ELISA test.**Results:** Most of the patients were in the age group of 20-30 years. About 78.2% of females were from rural background. History of contact with cats was present in 56.5% of patients. About 91.3% of patients were non-vegetarian. Maximum number of patients with positive results in all the three serological tests had 2 spontaneous abortions. The prevalence of toxoplasmosis in pregnant females with history of habitual abortions were found to be 47.9%. IgM was positive in 35.4% while 16.6% of the patients had low IgG avidity antibodies indicating acute infection.**Conclusions:** Regular serological screening early in pregnancy will allow recognition of maternal infection and prompt treatment.

1. Introduction

Toxoplasmosis is a cosmopolitan disease arising from infection with the cat borne Apicomplexan, coccidian protozoan *Toxoplasma gondii* (*T. gondii*), an obligate intracellular parasite that forms cyst in mammalian tissues throughout the body[1]. It has been estimated that *T. gondii* infects upto one-third of global population[2]. The protozoan parasite is transmitted to humans by ingestion of the tissue cysts in raw or undercooked meat, particularly lamb and pork, or contact with cat faeces[3].

Congenital transmission may occur when an uninfected mother acquires primary infection during pregnancy. As most of the *Toxoplasma* infections are asymptomatic, the mainstay of diagnosis is serology. The clinical implications of *Toxoplasma* infection in pregnant women are manifold. Such patients may have spontaneous abortions, stillbirths or premature delivery in addition to other

congenital fetal anomalies[4].

Habitual abortion is defined as three or more consecutive spontaneous abortions. Habitual abortion is one of the most distressing problems in obstetrics, particularly in those women who have no successful pregnancies. The seroprevalence of toxoplasmosis infection in women with bad obstetric history (including sporadic or habitual abortions) is known to be significantly higher than those without it. A recent study from India reports a statistical difference between IgG antibody levels against *T. gondii* in habitual abortions as compared to sporadic abortions or normal pregnancies[5]. In this study we screened women with repeated abortions for prevalence of *Toxoplasma* antibodies (IgM and IgG avidity) to determine any possible aetiological relationship between abortions and *Toxoplasma* infection.

2. Materials and methods

2.1. Study group

The study was conducted in the Department of Microbiology, Antenatal Clinic and Gynaecology OPD of Jawahar Lal Nehru

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Medical College and Hospital from February 2011 to September 2012. A total of 400 patients presented with history of multiple spontaneous abortions. Clinical examination and laboratory investigations were carried out to exclude other causes of fetal wastage. All the cases were subjected to a detailed history and clinical examination. History significant for risk factors of toxoplasmosis were taken. Subjects with known causes of foetal wastage were excluded from the study. On basis of history and clinical examination, 48 patients are classified as high risk group for toxoplasmosis. This study was approved by Institutional Ethics Committee of the Faculty of Medicine, A.M.U., Aligarh. An informed consent was obtained from the patients. For serological analysis, 2 mL of venous blood was collected in a sterile container with strict aseptic precautions from each study subject. The serum was separated and stored in numbered aliquots at 20 °C till assayed.

2.2. *Toxoplasma* IgG ELISA and IgM ELISA

Quantitative determination of IgG and IgM antibodies to *T. gondii* infection was done using *Toxoplasma* IgG ELISA and *Toxoplasma* IgM ELISA kit (Calbiotech Inc.). The test was performed according to the manufacturer's instructions. The interpretation was based on cut off calculated as per the instruction provided in the manual. If the index was < 0.9, the test was considered negative, 0.9 to 1.1 equivocal and an index of > 1.1 was considered positive.

2.3. *Toxoplasma* IgG avidity ELISA

Measurement of *Toxoplasma* IgG avidity antibodies was performed and interpreted using Enzywell *Toxoplasma* IgG avidity ELISA kit (Diesse Diagnostica, Italy). The test was performed and interpreted according to the manufacturer's instructions. The percentage of avidity of the samples is expressed and calculated using the ratio between the OD found in the wells containing avidity buffer and those with wash buffer, subtracting the value of the test blank. A ratio over 35% indicates the presence of high avidity IgG antibodies; when it is lower than 30%, this indicates the presence of low avidity IgG antibodies. If the percentage is between 30% and 35%, there is a medium degree of avidity (borderline).

3. Results

The prevalence of toxoplasmosis in pregnant females with history of habitual abortions were found to be 47.9%. IgM was positive in 35.4% while 16.6% of the patients had low IgG avidity antibodies indicating acute infection. Maximum number of patients positive for all the three serological tests IgG ELISA (82.6%), IgM ELISA (82.4%) and IgG avidity ELISA (87.5%) were present in the age group 21-30 years followed by 11-20 years and 31-40 years.

No patient in the age group of 41-50 years were positive for any of the serological tests. Out of 23 patients positive for IgG ELISA, 13 (56.5%) patients had history of contact with cats, 10 (43.4%) were eating raw or unwashed vegetables, 21 (91.3%) had non vegetarian food habits, 10 (43.4%) were eating improperly cooked or minced meat products, 20 (86.9%) belonged to low socioeconomic status, 18 (78.2%) resided in rural areas, 9 (39.1%) had poor hand hygiene and none had history of blood transfusion (Table 1). Maximum number of patients had 2 spontaneous abortions (26) followed by 3 spontaneous abortions (12), 1 spontaneous abortion (7), 4 spontaneous abortions (2) and least number of patients had 6 spontaneous abortions (1). Maximum number of patients with positive results in all the three serological tests had 2 spontaneous abortions (Table 2).

Table 1

Distribution of risk factors in patients positive for serological tests [n (%)].

Risk factors	IgG ELISA (n=23)	IgM ELISA (n=17)	IgG avidity ELISA (Low avidity) (n=8)
Contact with cats	13 (56.5)	11 (64.7)	8 (100.0)
Eating raw or unwashed vegetables	10 (43.4)	8 (47.1)	4 (50.0)
Non vegetarian food habits	21 (91.3)	16 (94.1)	8 (100.0)
Eating improperly cooked or minced meat products	10 (43.4)	9 (52.0)	6 (75.0)
Low socio-economic status	20 (86.9)	15 (88.2)	7 (87.5)
Rural residence	18 (78.2)	14 (76.4)	7 (87.5)
Poor hand hygiene	9 (39.1)	8 (47.1)	7 (87.5)
Blood transfusion	0 (0)	0 (0)	0 (0)

Table 2

Distribution of patients in relation with number of abortions and positive results observed in various serological tests.

No. of spontaneous abortions / No. of patients	IgG ELISA	IgM ELISA	IgG avidity ELISA (Low avidity)
1 / 7	1	1	0
2 / 26	12	8	5
3 / 12	8	6	1
4 / 2	1	1	1
5 / 0	0	0	0
6 / 1	1	1	1
Total	23	17	8

4. Discussion

Maternal infections play a critical role in pregnancy wastage and is one of the main causes for bad obstetric history in pregnant women. The maternal *Toxoplasma* infection has been considered as a significant factor in the causation of poor pregnancy outcome. *T. gondii* is known to cause infection in utero and is often responsible for abortions, stillbirths, premature delivery and congenital malformations[6]. Intrauterine transmission occurs in approximately 25%, 54%, and 65% of untreated women who develop acute toxoplasmosis during the first, second, and third trimesters respectively. Specific maternal therapy can reduce the overall incidence of fetal infection by 50%. Fetal involvement is most severe if maternal infection is contracted early in pregnancy[7]. The extent to which toxoplasmosis causes habitual abortion is still

controversial.

In our study, the incidence of *Toxoplasma* infection was found to be relatively high (47.9%) in women with habitual abortions. The percentage of women with acute toxoplasmosis having positive IgM and IgG avidity results were found to be 35.4% and 16.6% respectively. A study done in Iraq has similar results[8]. Another study done in Libya showed a prevalence of 47.1% in females with previous abortions[7]. A study done in Kashmir, India reported a high prevalence of IgM antibody against *T. gondii* in women with repeated abortions (49.47%) compared with controls (8.88%) who did not have history of abortions[9].

However, in our study no significant relation was found between number of spontaneous abortions and seropositivity of toxoplasmosis. The highest percentages of seropositivity (100%) was found among women with history of six spontaneous abortions, followed by positivity (66.6%) among women with three spontaneous abortions, two spontaneous abortions (46.1%), 4 spontaneous abortions (25%) and the lowest (14.2%) among women with one spontaneous abortion. This corroborates the observation of Borkakoty *et al.* and Sarkar *et al.* in which the increase in the number of pregnancy wastages had no significant association with infection due to *T. gondii*[6,10].

Toxoplasmosis was most prevalent in the age group of 21-30 years. It was similar to the study done by Malarvizhi *et al.* in which the seropositivity was more in the age group of 20-40 years whereas it was lower for those below 20 years and above 40 years[11].

The high prevalence of this disease could be due to the numerous associated risk factors and many sources of infection. About 56.5% of the patients having contact with cats were positive for toxoplasmosis. It was similar to a study done in Tamil Nadu, India in which the rate of seropositivity of *T. gondii* among women who had cats as a pet animal was significantly higher (22.9%) than those without any cat in their house (7.6%)[11]. Other risk factors found to be associated with IgG seropositivity were eating raw or unwashed vegetables (43.4%), non-vegetarian food habits (91.4%), eating improperly cooked or minced meat products (43.4%), low socioeconomic status (86.9%), rural residence (78.2%) and poor hand hygiene (39.1%). The results were similar to other studies in which increased consumption of unwashed vegetables and fruits and consumption of undercooked meat were identified as possible risk factors associated with *T. gondii* infection[12,13].

In view of the high prevalence of *T. gondii* in our subjects with repeated abortions, it is recommended that every woman of reproductive age group who has history of abortions should be tested for her *Toxoplasma* serological status before next conception and treated accordingly. Women with negative serological tests are at a risk of acquiring a primary infection during pregnancy and education regarding preventive measures should be provided to them.

Conflict of interest statement

We declare that we have no conflict of interest.

References

- [1] Sakikawa M, Noda S, Hanaoka M, Nakayama H, Hojo S, Kakinoki S, et al. Anti-*Toxoplasma* antibody prevalence, primary infection rate, and risk factors in a study of toxoplasmosis in 4,466 pregnant women in Japan. *Clin Vaccine Immunol* 2012; **19**(3): 365-7.
- [2] Zemene E, Yewhalaw D, Abera S, Belay T, Samuel A, Zeynudin A. Seroprevalence of *Toxoplasma gondii* and associated risk factors among pregnant women in Jimma town, Southwestern Ethiopia. *BMC Infect Dis* 2012; **12**: 337.
- [3] Jones JL, Dargelas V, Roberts J, Press C, Remington JS, Montoya JG. Risk factors for *Toxoplasma gondii* infection in the United States. *Clin Infect Dis* 2009; **49**(6): 878-84.
- [4] Flatt A, Shetty N. Seroprevalence and risk factors for toxoplasmosis among antenatal women in London: a re-examination of risk in an ethnically diverse population. *Eur J Public Health* 2013; **23**(4): 648-52.
- [5] Kumar A, Arora V, Mathur M. *Toxoplasma* antibody levels in females with habitual or sporadic abortions and normal pregnancies. *Indian J Med Microbiol* 2004; **22**(4): 276-7.
- [6] 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**(1): 87-92.
- [7] 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.
- [8] Al-khafaji AH, Muhsen KI. Seroprevalence of toxoplasmosis among women with habitual abortion in Thi-Qar Governorate using ELISA test. *J Thi-Qar Sci* 2009; **1**(4). [Online] Available from: <http://www.iasj.net/iasj?func=fulltext&aId=47626> [Accessed on 29th March, 2015]
- [9] Zargar AH, Masoodi SR, Laway BA, Sofi BA, Wani AI. Seroprevalence of toxoplasmosis in women with repeated abortions in Kashmir. *J Epidemiol Community Health* 1998; **52**(2): 135-6.
- [10] Borkakoty BJ, Borthakur AK, Gohain M. Prevalence of *Toxoplasma gondii* infection amongst pregnant women in Assam, India. *Indian J Med Microbiol* 2007; **25**(4): 431-2.
- [11] Malarvizhi A, Viswanathan T, Lavanya V, Arul Sheeda Malar S, Moorthy K. Seroprevalence of *Toxoplasma gondii* in pregnant women. *J Public Health Epidemiol* 2012; **4**(6): 170-7.
- [12] Khan SN, Khan S, Ayaz S, Jan AH, Jehangir S, Attaullah S, et al. Seroprevalence and risk factors of toxoplasmosis among pregnant women in District Kohat, Khyber Pakhtunkhwa, Pakistan. *World Appl Sci J* 2011; **14**(7): 1032-6.
- [13] Liu Q, Wei F, Gao S, Jiang L, Lian H, Yuan B, et al. *Toxoplasma gondii* infection in pregnant women in China. *Trans R Soc Trop Med Hyg* 2009; **103**(2): 162-6.