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▣ ORIGINAL ARTICLE

IgG Avidity Antibodies against *Toxoplasma gondii* in High Risk Females of Reproductive Age Group in India

Naushaba Siddiqui[1,*], Fatima Shujatullah[1], Haris M. Khan[1], Tamkin Rabbani[2], Parvez A. Khan[1]

[1]Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, AMU, Aligarh, UP, India; [2]Department of Obstetrics and Gynecology, Jawaharlal Nehru Medical College and Hospital, AMU, Aligarh, UP, India

Abstract: *Toxoplasma gondii* is an obligate intracellular protozoan that is distributed worldwide. Recently, several tests for avidity of *Toxoplasma* IgG antibodies have been introduced to help discriminate between recently acquired and distant infections. The study was conducted in Jawaharlal Nehru Medical College and Hospital, India from February 2011 to September 2012. Serum specimens were subjected to *Toxoplasma* IgM ELISA and IgG avidity ELISA test. Out of 48 patients with abortions, 17 (35.4%) were positive for IgM ELISA, and 8 (16.6%) had low IgG avidity antibodies. Out of 48 patients with other obstetric problems, 23 (47.9%) were positive for IgM ELISA, and 17 (35.4%) had low IgG avidity antibodies. Combining both groups on avidity test, only 25 of 40 (62.5%) IgM-positive women had low-avidity IgG antibodies suggesting a recent *T. gondii* infection in these women. More importantly, 15 (37.5%) of the IgM-positive women had high-avidity antibodies suggesting that the infection was acquired before gestation The relation of IgM seropositivity with the following risk factors was not found to be statistically significant; contact with cats (0.13), non-vegetarian food habits (0.05), and low socio-economic status (0.49). While, for IgG avidity ELISA, only contact with cats (0.01) was significantly associated with seropositivity. All other risk factors have *P*-values of >0.05 (not significant). IgG avidity test when used in combination with IgM test was a valuable assay for diagnosis of ongoing or recently acquired *T. gondii* infection in India.

Key words: *Toxoplasma gondii*, toxoplasmosis, IgG avidity test, high risk pregnant women, India

INTRODUCTION

Toxoplasmosis is a cosmopolitan disease arising from infection with the cat-borne apicomplexan coccidian protozoan *Toxoplasma gondii*, an obligate intracellular parasite that forms cyst in mammalian tissues throughout the body [1]. *T. gondii* infects up to one-third of global population and a wide range of other mammalian and avian species [2,3]. Toxoplasmosis is a well-documented cause of bad obstetric history (BOH) and a major cause of congenitally-acquired infection, leading to a high degree of intrauterine fetal death and morbidity of the newborns. The clinical implications of infection due to *Toxoplasma* in pregnant patients are manifold. Such patients may have spontaneous abortions, stillbirths, intrauterine growth retardation, preterm deliveries, or fetal anomalies [4]. It has been suggested that toxoplasmosis has some unfavorable effects on reproductive capacity in both men and women. The data obtained from limited studies performed in animal models as well as in infertile couples, have supported the relationship between Toxoplasma and infertility [5].

The detection of *Toxoplasma*-specific antibodies is the primary diagnostic method to determine the infection. The immunofluorescence assay and enzyme immunoassays for IgG and IgM antibodies are the most commonly used tests today. However, these tests cannot estimate the time of infection precisely enough to properly manage the risk to the fetus of a maternal infection. Recently, a number of tests for the avidity of *Toxoplasma* IgG antibodies have been introduced to help differentiate between recently acquired and distant infections. The term "avidity" or "functional affinity" define the net antigen binding force of the populations of antibodies, and are preferable over the term "affinity" [6]. Functional affinity of specific IgG antibodies, i.e., IgG avidity, initially is low after primary antigenic challenge, but increases during subsequent weeks and months by antigen driven B cell selection [7]. Presence of low avidity antibodies usually indicates recently acquired infection. The present study was done to study the prevalence of acute *T. gondii* infection in high risk females of reproductive age group, to

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* Corresponding author (naushsid@gmail.com)

compare IgM antibody assay with IgG avidity for diagnosis of acute toxoplasmosis and to determine the usefulness of IgG avidity test in the diagnosis of toxoplasmosis in high risk females of reproductive age group in India.

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology, Antenatal Clinic, and Gynecology Out-Patient Department (OPD) of Jawaharlal Nehru Medical College and Hospital from February 2011 to September 2012. A total of 96 patients were divided into 2 study groups:

1) Abortion: Forty-eight pregnant females attending Antenatal Clinic at Jawaharlal Nehru Medical College and Hospital with history of spontaneous abortions.
2) Other obstetric problems: Forty-eight non-pregnant females attending Gynecology OPD at Jawaharlal Nehru Medical College and Hospital with complaints of infertility, intrauterine death of the baby, preterm labor, still birth of the baby, polyhydroamnios, congenital anomalies of the baby and history of visual deficits of women.

The control group comprised 15 multiparous, age-matched antenatal women attending outpatient department and antenatal clinic without bad obstetric history and who were otherwise healthy in all respect. This study was approved by Institutional Ethics Committee of the Faculty of Medicine, A.M.U., Aligarh, India. An informed consent was obtained from the patients.

Most of the patients were in the age group of 20-30 years (71 patients) followed by 11-20 years (15 patients) and >30 years (10 patients). Out of the 96 patients, 48 patients had history of multiple spontaneous abortions in previous pregnancies, 29 patients had history of primary and secondary infertility, 6 had previous history of intrauterine deaths, 6 had visual deficits, 3 had preterm labor, 1 each had history of intrauterine growth retardation, still birth, meningomyeloceole, and polyhydroamnios in previous pregnancies. Infertility was evaluated by doing thorough clinical examinations, ultrasonography, hormonal profile, and other relevant tests. All of them were interviewed to ascertain age, medical, and obstetric information. History of risk factors like contact with cats, socioeconomic background, eating habits (vegetarian/non-vegetarian), eating uncooked or minced meat products, eating raw or unwashed vegetables, hand hygiene, and blood transfusion were taken.

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℃ till assayed.

Quantitative determination of IgM antibodies to *T. gondii* infection was done using *Toxoplasma* IgM ELISA kit (Calbiotech Inc., Cambridge, Massachusetts, USA). The test was performed according to the manufacturer's instructions. The OD of the calibrator for a positive reaction should be greater than 0.25. The absorbance index for negative control should be less than 0.9, and the index for positive control should be greater than 1.2. 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. Measurements of Toxoplasma IgG avidity antibodies was performed and interpreted using Enzywell *Toxoplasma* IgG Avidity ELISA kit (DIESSE Diagnostica, Monteriggioni, Italy) was used for the measurement of *Toxoplasma* IgG avidity antibodies. The test was performed and interpreted according to the manufacturer's instructions. The percentage of avidity of the samples was 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. If the percentage is between 30% and 35%, there is a medium degree of avidity (borderline).

RESULTS

Out of 48 patients with abortions, 17 (35.4%) were positive for IgM ELISA. Out of these 17 patients, only 8 (16.6%) had low IgG avidity antibodies. Out of 48 patients with other obstetric problems, 23 (47.9%) were positive for IgM ELISA and 17 (35.4%) had low IgG avidity antibodies. So, in a total of 96 patients, 40 (41.6%) were positive for IgM ELISA and 25 (26%) had low IgG avidity antibodies (Table 1). Out of 29 patients presenting with infertility, 13 had low avidity IgG antibodies, 16 had high avidity antibodies, and 16 were IgM positive. One out of 6 patients presenting with intrauterine deaths were IgM positive, 5 had high IgG avidity, and 1 had low IgG avidity antibodies. Three (50%) out of 6 patients with visual deficits had low avidity IgG antibodies, and 3 (50%) had high avidity antibodies. One patient each with congenital anomaly in the baby and polyhydroamnios were IgM positive. All patients with IU GR, preterm labor and still births were IgM negative. All pati-

ents with complaints of intrauterine growth retardation, still births, preterm labor, congenital anomaly (meningomyelocele), and polyhydroamnios had high avidity IgG antibodies (Fig. 1). No patients in control group were positive for IgM ELISA and IgG avidity ELISA.

Table 1. Distribution of patients found positive in various serological tests

Serological tests	No. of patients (%)		
	Abortion (n=48)	Other obstetric problems[a] (n=48)	Total (n=96)
IgM ELISA	17 (35.4)	23 (47.9)	40 (41.6)
IgG avidity ELISA (low avidity)	8 (16.6)	17 (35.4)	25 (26.0)
IgM ELISA and IgG avidity ELISA (low avidity)	8 (16.6)	17 (35.4)	25 (26.6)

[a]Other obstetric problems include infertility, intrauterine death of the baby, preterm labour, still birth of the baby, polyhydroamnios, congenital anomalies of the baby, and history of visual deficits of women.

The risk factors found to be significantly associated with IgM seropositivity were eating raw or unwashed vegetables (0.03), eating improperly cooked or minced meat products (0.01), rural residence (0.01), and poor hand hygiene (0.01). The relation of IgM seropositivity with the following risk factors was not found to be statistically significant; contact with cats (0.13), non-vegetarian food habits (0.05), and low socio-economic status (0.49). While, for IgG avidity ELISA, only contact with cats (0.01) was significantly associated with seropositivity. All other risk factors had *P*-values of >0.05 (not significant) (Table 2).

DISCUSSION

In our study, 41.6% of the patients were seropositive for IgM antibodies, and 26.0% had low avidity IgG antibodies indicating acute infection. Out of 48 pregnant women with multiple

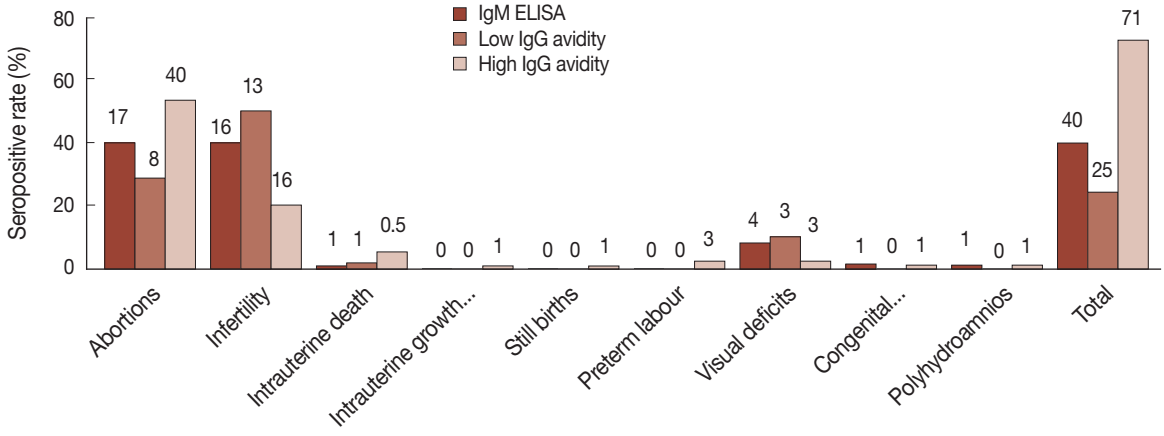

Fig. 1. Distribution of patients in relation to their presenting complaints and positive results in various serological results. Out of 48 patients presenting with complaints of abortions, 8 had low avidity IgG antibodies, 40 had high avidity antibodies, and 17 were IgM-positive. Out of 29 patients presenting with infertility, 13 had low avidity IgG antibodies, 16 had high avidity antibodies, and 16 were IgM-positive. All patients with complaints of intrauterine growth retardation, still births, preterm labor, congenital anomaly (meningomyelocele), and polyhydroamnios had high avidity IgG antibodies.

Table 2. Distribution of risk factors in patients for *Toxoplasma* IgM ELISA and low avidity IgG antibodies

Risk factors	IgM ELISA (%)	*P*-value	Odd's ratio (confidence interval)	IgG avidity (low) ELISA (%)	*P*-value	Odd's ratio (confidence interval)
Contact with cats	25/40 (62.5)	0.13	1.41 (0.36-5.40)	22/25 (88)	0.01	-
Eating raw or unwashed vegetables	18/40 (45.0)	0.03	0.26 (0.05-1.30)	11/25 (44)	0.08	1.0 (0.17-5.6)
Non vegetarian food habits	36/40 (90)	0.05	2.40 (0.22-25.0)	25/25 (100)	0.10	-
Eating improperly cooked or minced meat products	19/40 (38)	0.01	1.46 (0.41-5.15)	14/25 (56)	0.25	3.3 (0.52-21)
Low socio-economic status	35 (87.5)	0.49	1.12 (0.16-7.60)	24 (96)	0.07	-
Rural residence	28 (70)	0.01	3.00 (0.60-13.47)	21 (84)	0.48	1.5 (0.13-17)
Poor hand hygiene	17 (42.5)	0.01	1.30 (0.38-4.91)	15 (60)	0.47	6.2 (0.62-62.0)
Blood transfusion	0 (0)	-	-	0 (0)	-	-

spontaneous abortions presenting in their first trimester, 17 (35.4%) were positive for IgM antibodies. On avidity testing, only 8 of 17 (47.0%) IgM-positive women had low-avidity IgG antibodies suggesting a recent *T. gondii* infection in these women thus requiring treatment. More importantly, 9 (53.0%) of the IgM-positive women had high-avidity antibodies suggesting that the infection was acquired before gestation and no treatment is required. While screening females with other obstetric problems, out of 48 patients, 23 (47.9%) were positive for IgM ELISA and 17 (35.4%) had low IgG avidity antibodies.

Generally, detection of anti-*Toxoplasma*-specific IgM antibodies is a sensitive indicator of an ongoing or recent infection. However, false-positive IgM antibody test results have been reported in previous studies [8-11]. In such cases, the diagnosis of primary infection with *T. gondii* in early pregnancy can be improved by determination of anti-*Toxoplasma* IgG avidity, which has the ability to discriminate between recent and prior infections. Combining both groups, on avidity testing, only 25 of 40 (62.5%) IgM-positive women had low-avidity IgG antibodies suggesting a recent *T. gondii* infection in these women. More importantly, 15 (37.5%) of the IgM-positive women had high-avidity antibodies suggesting that the infection was acquired before gestation. The apparent discrepancy in detecting infection status by IgM serology and avidity tests may be due to the fact that IgM antibodies may persist for months or even years following the acute phase of an infection in some individuals; thus the presence of IgM antibodies is not always an indication of a recent infection [9]. The presence of specific *T. gondii* IgM antibodies in the chronic stage of an infection as observed in 37.5% of the IgM-positive cases in this study may have resulted in unwarranted concern and a misdiagnosis particularly in women in early pregnancy.

Among the seropositive cases, abortion (82.1%) was the most common cause of pregnancy wastage followed by intrauterine death (10.7%), congenital anomaly (3.6%), and polyhydroamnios (3.6%). The results were similar to the study done in Andhra Pradesh, India in 105 antenatal women with bad obstetric history. It was revealed that abortion (51.9%) was the commonest form of pregnancy wastage, followed by stillbirths (36.5%), premature deliveries (7.7%), congenital anomalies (1.9%), and early unexplained neonatal deaths (1.9%) [4].

Out of 29 patients with complaints of infertility, 55.1% had IgM and 44.8% had low IgG avidity antibodies (acute infection), respectively. Few studies have addressed the issue of infertility related to *T. gondii*. In another retrospective study for the comparative evaluation of *Toxoplasma* seropositivity of fertile and infertile female spouses, seroprevalence for toxoplasmosis was found to be 28.8% in infertile women [5]. In a study conducted in Ahemdabad, India, in 28 women with primary infertility, 32.1% had antibodies to *T. gondii* [12].

T. gondii is the most frequent etiology of infectious intraocular inflammation (uveitis). In some countries, up to 50% of all cases of posterior uveitis in a given population can be attributed to toxoplasmosis [13]. In our study, a total of 6 patients who presented with gynecological complaints to the OPD were accidentally diagnosed to have vision problems. On examination, they were found to have findings suggestive of chorioretinitis, viteritis, and uveitis. In a study done in Korea to assess *T. gondii* infection in 20 ocular patients with chronic irregular recurrent uveitis, 5% of the patients were found to be seropositive for toxoplasmosis [14].

Contact with cats was found to be a significant risk factor for acute infection ($P < 0.05$). It was similar to a study done in Tamil Naidu, 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%) [15].

The risk factors found to be significantly associated with IgM seropositivity were eating raw or unwashed vegetables (0.03), eating improperly cooked or minced meat products (0.01), rural residence (0.01), and poor hand hygiene (0.01). It was similar to a study done in 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 [16,17].

Thus, early diagnosis and adequate treatment of pregnant women can reduce the rate of transmission to the fetus and severity of sequelae in cases where intrauterine infection had already occurred. Generally most clinicians determine an active *Toxoplasma* infection by detecting *Toxoplasma*-specific IgM antibodies and/or by detecting a 3-fold increase in IgG antibodies (IgM-negative) in pregnant women during the first trimester. Avidity test is highly sensitive and specific for detecting a recent *T. gondii* infection in IgM-positive cases [18]. Testing with the avidity method in pregnant women during the first 16 weeks of gestation has the potential to decrease the need for follow-up sera, and also the need of performing PCR on amniotic fluid. It decreases the chance of unnecessary treatment of the mother with spiramycin or other drugs and thereby reduces costs.

CONFLICT OF INTEREST

The authors have no conflict of interest related to this study.

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