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SHORT PAPER

IgG Avidity Test for the Diagnosis of Acute *Toxoplasma gondii* Infection in Early Pregnancy

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ABSTRACT

Background: Toxoplasmosis is well known as an important infection in pregnant women. Although many serologic methods are available, diagnosis of early Toxoplasmosis may be extremely difficult. **Objective:** To detect the Toxoplasma IgG antibodies developed at the early stage of infection in pregnant women. **Methods:** 225 pregnant women, who were in the 2nd to 4th month of their pregnancy, enrolled in this study. Anti-toxoplasma IgG, IgM and IgG avidity were evaluated by ELISA method. **Results:** The patients were categorized into three groups as follows: Group A, 124 cases; IgG+, IgM+, 55.1%; group B, 99 cases; IgG+, IgM-, 44%; and group C, 2 cases; IgG -, IgM +, 0.9%. Fifty five percent of the pregnant women had positive IgG and IgM among which 7.1% had low avidity which revealed an active infection in the pregnant women. In the current study, 44% of pregnant women had positive IgG and negative IgM, all of which had high avidity, which is an indication that in our population the level of toxoplasmosis infection is high and most women have had contacts with this parasite before pregnancy. **Conclusion:** In this study, the low avidity test was 7.1% showing that the occurrence of toxoplasmosis infection is still a serious issue. Observation of 45.8% high avidity among group A suggests that either IgM has a high half-life or there is a false positive IgM as a result of rheumatologic disorders. Therefore, avidity test is important in predicting maternal toxoplasmosis which is of value in disease treatment.

Keywords: Acute Toxoplasmosis, Avidity, IgG, IgM, Pregnancy

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INTRODUCTION

Most cases of infection by *Toxoplasma gondii*, an intracellular parasite, is asymptomatic or may cause a self-limited clinical illness. Nevertheless when the pregnant woman is infected by acute *Toxoplasma gondii*, it is likely that the parasite would be transmitted to the embryo causing abortion or a severe neurological damage and congenital corioretinitis. Evaluation of IgG, IgA, and IgM to diagnose the unconfirmed toxoplasmosis is confronted with some problems. Determination of IgG is valuable, however in early stages, it turns out to be negative after a few weeks, it would become positive. On the other hand, serum IgM remains positive for several months (2).

Rheumatoid Factor (RF) also produces false positive IgM. In those cases where the pregnant woman has high toxoplasma IgG and a positive IgM for the first time, there is a need for the differential diagnosis of the unconfirmed *Toxoplasma* infection or the old infection. In cases where high positive levels of IgG and IgM are detected before pregnancy, the embryo would be protected against *Toxoplasma gondii* infection, but whenever the test becomes positive after pregnancy, the embryo is subject to a serious risk.

For the first time, the avidity test was conducted using denaturalization technique to diagnose congenital Rubellosis (3). In 1989, Hedman, introduced a new method which was based on the affinity of immunoglobulins bound to *Toxoplasma gondii* polyvalent antigens, and benefited from the high density of urea to differentiate the high affinity of immunoglobulin which was later called avidity test. This test is recently used for the detection of *Toxoplasma* IgG avidity (4). In the initial steps of *Toxoplasmosis* infection IgG avidity is low but the avidity of IgG due to the previous infections is very high. According to recent reports, when the avidity is lower than 40%, it is indicative of an initial infection or active steps and when the avidity is higher than 60%, it indicates an old infection (5,6).

In a study by Yasodhara et al., titers lower than 30% has been considered as low avidity (3). The increased titer of *Toxoplasma* IgG can also be used in the diagnosis of recent infections but its monitoring requires a long period of time which might be harmful for the embryo (2).

Several researches have supported the usefulness of Toxo-IgG avidity which is related to recent active toxoplasmosis (5-7). The timely diagnosis and treatment of toxoplasmosis during pregnancy may protect the embryo from infection and consequent damage (8).

In the current study, the importance of differential diagnosis of toxoplasmosis in the first trimester of pregnancy is evaluated.

MATERIALS AND METHODS

Patients. Two hundred and twelve pregnant women who were in the 2nd to 4th initial months of their pregnancy period and were at risk of *Toxoplasmosis* were enrolled in this study. These women referred to the Department of Infection Diseases of Sina Hospital and the Special Clinic of Tabriz University of Medical Science during a two year period (October 2008 to October 2010). They were later referred to the Medical Diagnostic Laboratory to diagnose the activeness of the infection.

ELISA Test. The levels of anti-toxoplasma IgG and IgM were measured at the beginning of pregnancy according to manufacturer's instruction (Vircell Microbiology Company). The measurement of Toxo-IgG was quantitative but that of Toxo-IgM measurement was conducted by an Index report by the computation of the cutoff point.

Avidity Test. Avidity test was performed by the method of Headman et al. using Euro-immun kit as follows: 100 µL of the patient's diluted sera were added to micro plates coated with Toxoplasma antigen. In the second step, concentrated (8M) urea solution was added to the antigen-antibody complex. After washing the excess antibody, labeled anti-IgG antibody was added to the test microplates. After 30 minute of incubation and re-washing, substrate solution was added and in the final step the reaction was stopped by adding sulfuric acid. The optical density (OD) was measured at 450 nm against the differential wavelength of 600 nm. The avidity was calculated by the following formula:

$$\text{Avidity Index (\%)} = (\text{OD of the sample treated with Urea} - \text{OD of the blank}) / (\text{OD of the sample treated without Urea}) \times 100$$

Statistical Analysis: The data were analyzed by descriptive statistical methods. Computation of diagnostic values was performed using SPSS version 14 (IBM, USA)

RESULTS AND DISCUSSION

The results of the analysis of 225 serum samples from pregnant women during the first 4 months of pregnancy are categorized into three groups. Group A, 124 cases; IgG+, IgM+, 55.1%; group B, 99 cases; IgG+, IgM-, 44%; and group C, 2 cases; IgG-, IgM+, 0.9% .

The results of the avidity tests were analyzed according to these three groups (Table 1). In the third group there were two pregnant women that, due to their negative Toxo-IgG, avidity test was not performed on their sera. In the second group, where all had negative Toxo-IgM, the avidity was high, pointing to the inactivity of the infection.

Table 1. Anti-Toxo IgG, IgM and IgG avidity in the sera of early pregnant women.

Avidity*	Group A	Group B	Group C	Total
	IgG (+), IgM (+) N (%)	IgG (+), IgM (-) N (%)	IgG (-), IgM (+) N (%)	
Low	16 (7.1)	0 (0)	0 (0)	16 (7.1)
High	103 (45.8)	99 (44)	0 (0)	202 (89.8)
Borderline	5 (2.2)	0 (0)	2 (0.9)	7 (3.1)
Total	124 (55)	(44)	(1)	225 (100)

*In this study, avidity was categorized into three groups: low avidity, lower than 40; high avidity, above 60; and border line, equal or lower than 60 and equal or higher than 40.

To diagnose *Toxoplasma* infection, negative IgG prior to pregnancy and positive IgG after pregnancy are of high importance. Since pregnant women are not usually referred to TORCH screening tests before pregnancy, the avidity test used to diagnose the active *Toxoplasma* infection during pregnancy is of a high value.

In the current study, 44% of the pregnant women had positive IgG and negative IgM and all had high avidity test results indicating that in our society the level of *Toxoplasma* infection is high and the women have contact with this parasite before pregnancy. In the first 2 to 4 months of pregnancy, 55% of the pregnant women had positive IgG and IgM and 7.1% had a low avidity test which reveals the presence of the active infection in such pregnant women.

Active *Toxoplasma* infection in 7.1% of pregnant women in our study would be considered a warning for the health care management system of the society, because of the birth of disabled newborns with congenital blindness, microcephalia and mentally handicapped, who place a big economic burden on the country and the society. On the other hand, the infection can be diagnosed and treated very promptly after a positive avidity test. In this study, all cases except those who decided to have an abortion because of *Toxoplasma* infection risk were cured and their avidity was above 62% after six months of pregnancy, showing the success of toxoplasmosis treatment.

Different studies reported different avidity titers; for instance, in one study, the avidity lower than 20% was reported as low avidity (1). Johnson and Yosodhara reported a low avidity below 30% (2,3). Emanno reported one below 40% (6) and Francoise gave the value of less than 40% (7). In our study, it was also considered lower than 40%.

In another study performed in 2004 on 37 pregnant women, the value of low avidity for IgG at the beginning of the pregnancy was observed to be 10.8%, and its high avidity was 57.2% (5). In nearly all of the studies, the samples were less than hundred cases but in our study, we examined a population of 225 pregnant women resulting in an increased validity of the results. In condition the observation of a high avidity of 45.8% in group A (IgG+, IgM+) indicates that either IgM has a high half-life or the existence of IgM in rheumatologic disorders makes it false positive. Therefore, avidity test is of prime importance and it is possible to treat the infection if a differential diagnosis of the active infection of *Toxoplasma* is performed on time.

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