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Recognition of acute toxoplasmosis with IgG avidity ELISA test in the pregnant women (the first trimester) in Qom Province, Iran, during two years (2012-2013)

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Abstract: *Toxoplasma gondii* is an obligate intracellular protozoan parasite occurring with a global distribution. Congenital toxoplasmosis results when a non-immune woman gets a primary infection during pregnancy. Chronically infected women is not transmitted the infection to their offspring. The aim of this study was to perform IgG avidity test for detection of acute toxoplasmosis in the pregnant women (The first trimester). A total of 200 human serum samples were collected from different laboratories in Qom province. The anti-toxoplasma IgG, IgM and IgG avidity were evaluated by ELISA method. The samples were divided into 2 segregated groups: Group I consisted of 25 serum samples from patients in acute phase of *Toxoplasma* infection. All the patients had the symbol of lymphadenopathy. Group II consisted of 185 serum samples prepared from patients in chronic phase of *Toxoplasma* infection. The data were analyzed by descriptive statistical methods. 20 out of 25 (80%) sera with acute toxoplasmosis showed low avidity levels and 178 out of 185 (96%) sera in chronic phase of infection showed high avidity index. 7sera had borderline ranges of AI. In this study, IgM ELISA and IgG avidity ELISA tests together could confirm the acute and chronic phases of disease completely. The IgG avidity test was created to help distinguish between past and recently acquired infection.

Keywords: Toxoplasmosis, IgG Avidity Test, ELISA, IgM

1. Introduction

Toxoplasmosis is possibly severe in 2 situations; pregnancy because of a risk of congenital toxoplasmosis and profound cellular immune deficiency status such as AIDS infection, transplantation, and malignant diseases that receive immune suppressive therapy. This obligate intracellular protozoa was identified in 1908 by Nicolle and Manceaux, in a North African rodent. Its intermediate hosts could be humans or other warm-blooded animals. The infection has a distribution worldwide. Almost one-third of the people has been confronted to this protozoan. The infection occurs usually while ingesting tissue cysts in undercooked meat, or contaminated water and food with oocysts. Because of the risk of congenital infection and its sequelae in the newborn, the diagnosis of toxoplasmosis should be done timely and correctly. When primary infection occurs during the first trimester of pregnancy, the sickness can lead to significant morbidity and mortality in the growing fetus. If the transmission occurs in the last trimester, it can cause clinical sings of congenital infection during the first decades of life. In immunodeficiency patients, the infection can lead to cerebral or extra-cerebral toxoplasmosis. Immunocompetent adults are usually asymptomatic or have symptoms such as fever, malaise, and lymphadenopathy that resolve spontaneously.

In pregnant women: Generally, acute acquired infection does not lead to expression of obvious symptoms or signs. In a minority of them may appear malaise, low-grade fever, and lymphadenopathy.

Rarely, as a result of recently acquired infection or reactivation of a chronic infection while pregnancy, women will present visual changes due to toxoplasmic chorioretinitis. In immunocompromised, chronically infected pregnant women, congenital transmission of *T. gondii* to the fetus result of reactivation of latent parasite. Therefore, it is

significant to distinguish between acute and chronic stages of infection for treatment and limitation of the effects, especially during the pregnancy. It is difficult to separate the parasite, so the diagnosis is usually based on serological methods , with detection of specific *Toxoplasma* IgG, IgM, and IgA antibodies (1-3). There are some limitations in this method; significant rise of IgG titer is not always observed especially in children and adolescents with ocular manifestation of congenital toxoplasmosis. *Toxoplasma* IgM antibody is present in some cases for years after primary infection, and it can be discerned in some diseases that have rheumatoid factors and antinuclear antibodies. Also, specific IgA antibodies can be detected after 45 months of a recorded as evidence seroconversion. On the other hand, it is well known that the strength of the bond between the antibody and epitope increases with the length of infection. The IgG avidity ELISA test could assess the avidity of specific IgG in phases of toxoplasmosis. The avidity of IgG is low in acute phase and high in chronic phase of disease. Therefore, finding out of a low IgG avidity is a reliable indicator for recent toxoplasmosis, whereas a high avidity indicates that the infection is occurred in the previous 3-5 months (4-6). The aim of this study was to perform IgG avidity test for detection of acute toxoplasmosis in the pregnant women (The first trimester).

2. Materials and Methods

2.1. Serum Sample Collection

In this descriptive crosssectional study, 200 serum samples from βHCG-positive women were randomly selected, transferred to different laboratories in Qom and examined by ELISA during two years (October 2012 to October 2013). The sera were stored at -20˚C until use. All women participating in this study gave their informed consent. The Ethical Committee of the Faculty of Medicine, Iran University of medical sciences approved this study. At first, all the sera were examined for anti-Toxoplasma IgG and IgM antibodies with ELISA test 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 cut off point. Then, they were divided into 2 segregated groups: Group I consisted of 25 serum samples from patients in acute phase of Toxoplasma infection, in which the presence of specific IgM antibodies was approved. All the patients had the symbol of lymphadenopathy. Group II consisted of 185 serum samples prepared from patients in chronic phase of Toxoplasma infection and the presence of specific IgG antibodies was confirmed.

2.2. Antigen Preparation

Tachyzoites of *T. gondii* (RH strain) were collected from the peritoneal cavity of mice that were injected 3 days earlier. Tachyzoites were washed with PBS (pH.7.2) 3 times, sonicated, and centrifuged at 12,000 *g* for 1 hr, and the supernatant was collected as the soluble antigen. The protein content was measured by the method of Bradford. The 96-well microtiter plates (Nunc Inc., Rochester, New York, USA) were coated with 5 µg/ ml of diluted protein in carbonate buffer (pH 9.6). Coated plates were placed at 4˚C over night, then washed and stored at -20˚C until use (7). Several serological tests have been used as screening tests, foe example the indirect immunofluorescent antibody test (IFAT), latex agglutination (LA), indirect hemagglutination (IHA) and ELISA with high sensitivity and specificity.In this study we did ELISA test. The detection of anti-toxoplasma antibodies by ELISA is frequently performed in numerous medical centers. The results of these tests are usually well approved by clinicians thanks to their excellent sensitivities and specificities, the rapid accessibility to results, and the considerably low costs of thetests.

2.3. Avidity ELISA

Avidity Test. Avidity test was performed by the method of Headman et al. using Euroimmun 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:

Avidity Index (%) = (OD of the sample treated with Urea – OD of the blank)/ (OD of the sample treated without Urea) × 100.

2.4. IgM ELISA

Anti-*Toxoplasma* IgM antibodies were tested by IgM-ELISA. Briefly, sera were diluted serially and added to the *T. gondii* antigen-coated microtiter plate and then anti-human IgM antibodies conjugated with HRP was added. After incubation and washing, the chromogenic substrate OPD was added, and the optical density was read by means of an automated ELISA-reader (7).

2.5. Statical Analysis

The data were analyzed by descriptive statistical methods. Computation of diagnostic values was performed using SPSS version14 (7,8) .

3. Results

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.

3.1. Acute Toxoplasmosis Group

Among 25 patients with acute toxoplasmosis, 20 (80%) had the AI as 40 and lower, and 5(20%) showed the greater AI than 40. All of the sera were positive for *Toxoplasma* specific IgG and IgM antibodies. The statistical analysis showed that there was a significant correlation between high IgM ELISA titers and low avidity of IgG ($P<0.05$).

3.2. Chronic Toxoplasmosis Group

178 (96%) of 185 sera with chronic toxoplasmosis had the AI greater than 60 and 7 (3.8%) of them had the AI among 40-60%. All the sera from this group had high titers of IgG antibodies by IgG-ELISA method and 5 (2.7%) of them had acceptable titers of IgM-ELISA. No significant correlation was found between IgG concentration and avidity index in 2 group samples($P>0.05$).

4. Discussion

Toxoplasmosis is a parasitic infection with variable prevalence in different countries. Some studies have suggested its role in abortion. Fetal infection by *Toxoplasma gondii* develops when non-immune mother becomes infected during pregnancy. Some assays have been used to evaluate *Toxoplasma* specific IgM antibodies as indicators of the acute phase infection. *Toxoplasma* IgM antibodies can be detected for 1 year or longer in some cases, so in asymptomatic individuals with stable titers of *Toxoplasma* IgG antibodies, positive IgM results are not easy to explain.

It is essential, especially during the pregnancy, to differentiate between acute and chronic stages of infection for treatment and limitation of the consequences.

The parasite isolation is usually difficult, so the diagnosis is essentially focused on serological techniques, with revelation of specific Toxoplasma IgG, IgM, and IgA antibodies.

The IgG avidity test was created to help distinguish between past and recently acquired infection. Results are founded on

the quantification of the avidity [functional affinity] of Toxoplasma-specific IgG antibodies(42). It believed that the antibodies produced have a low average affinity following an antigenic challenge. More the immune response progress, antibody affinity increases progressively over weeks or months. Previous findings with the IgG avidity method proposed that low-avidity antibodies signified recently acquired infection. After that, studies have demonstrated

evidently that low-avidity antibodies can persist for several months beyond the acute infection and because of this are not reliable for the diagnosis of recently acquired infection. According to the method used, the availability of high-avidity IgG antibodies might be used to exclude the occurrence of acute infection during the past 3-5 months. Consequently, its value is greatest when done in the first 3-4

months of gestation. A high-avidity result late in the second trimester or in the third trimester should not be interpreted to signify that the infection was notacquired in the first 3-5 months of gestation.

Diagnosis of toxoplasmosis in humans is elaborated using various techniques such as detection of anti-Toxoplasma antibodies, mouse inoculation, histological revelation of tachyzoites in tissue sections or smears of body fluid.

In 1989, Hedman, presented 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 (8). High avidity ($AI\geq60\%$) means that *Toxoplasma* infection was acquired before 3 months ago, whereas borderline avidity ($40\%<AI<60\%$) means disease at an indeterminate period, and low avidity ($AI\leq40\%$) means that the infection was acquired within the last 3 months.

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

According to this study, 80% of patients with acute toxoplasmosis had low avidity ($AI\leq40\%$) and 96% of individuals with chronic infection had high avidity ranges ($AI\geq 60\%$). It was suggested that *Toxoplasma* specific IgG avidity test that is performed on specific IgM positive samples could diagnose acute and chronic phases of toxoplasmosis (8-11). Candolfi et al. also demonstrated that measuring of IgG avidity could differentiate between the phases of toxoplasmosis. Our results showed that there was no significant relationship between the concentration of IgG antibody and the level of AI. Therefore, the strength of bonds between antibody and antigen is not dependent on antibody concentration, which was confirmed by other researchers (11,12). Meanwhile, there are a opposite relationship between IgM titer and levels of IgG AI in this study ($P<0.05$). On the other hand, Remington et al proposed that IgG avidity ELISA test is not enough to confirm acute toxoplasmosis, and it should be done in addition to IgM ELISA test. Many investigators had suggested the role of IgM detection as a key for diagnosis of acute toxoplasmosis (13). One of the limitations of IgG avidity method is recognition of reactivated toxoplasmosis in immunocompromised patients (14). 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. Several researchers have supported the usefulness of Toxo-IgG avidity which is related to recent active toxoplasmosis. The timely diagnosis and treatment of toxoplasmosis during pregnancy may protect the embryo from infection and consequent damage(9,10,11).

In the other study, 44% of the pregnant women had positive IgG and negative IgM and all had high avidity test results indicating that in their society the level of *Toxoplasma* infection was high and the women had 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 revealed the presence of the active infection in such pregnant women also the observation of a high avidity of 45.8% in group A (IgG+, IgM+) indicated that either IgM had a high half-life or the existence of IgM in rheumatologic disorders made it false positive (15-17).

In our study, IgM ELISA and IgG avidity ELISA tests together could confirm the acute and chronic phases of disease completely. The avidity test is of principal importance and it is possible to treat the infection if a differential diagnosis of the active infection of this parasite is performed on time. It may be appropriate to screen women before and during pregnancy, any raise in seropositivity should be dealt with in order to minimise the consequences of this infection.

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