The Relations Between Anti-*Toxoplasma* IgG and IgM Antibodies With Avidity Index

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Abstract
Objective: *Toxoplasma gondii* is one of the obligatory intracellular protozoan parasite with worldwide distribution. Diagnosis of acute infection is of importance in pregnancy due to congenital infection and its sequels. One of the tools for distinguishing acute from chronic infection is avidity ELISA method. This study was performed to evaluate the correlation between IgG and IgM antibodies and the of avidity index (AI).
Materials and Methods: A total of 100 serum samples were collected from different laboratories, with detectable anti-*T. gondii* IgG and IgM antibodies. Avidity IgG ELISA method was performed and AI was calculated.
Results: In the present study no relation between IgG concentration and the mean of AI was observed. The relation between the means of AI and concentrations of IgM antibody in 98% of acute sera was reverse.
Conclusion: Irrespective to titers of IgG and IgM antibodies, IgG avidity ELISA test could distinguish acute from chronic phase of *T. gondii* infection.
Keywords: *Toxoplasma gondii*, IgG, IgM, Avidity

Introduction

Toxoplasma gondii, the unicellular parasite is estimated to infect one third of population worldwide (1). *Toxoplasma* infection is asymptomatic and self-limiting illness in immunocompetent individuals (2). The infection is potentially severe in pregnancy due to the risk of congenital toxoplasmosis and in immune system deficiencies such as AIDS patients (3). Treatment strategy for *T. gondii* infection, depends on whether the infection is in acute or chronic stages (4). The avidity between antigens and specific antibodies could change during the time of infection. So, in the acute stages of *T. gondii* infection, the avidity is low and could increase during the infection (5). In this regard, a reliable indicator to detect acute toxoplasmosis is low IgG avidity, whereas high avidity means that the infection has been occurred in 3-5 months ago (6).

This study was performed to evaluate the relation between the titers of IgG and IgM antibodies and the amount of avidity index (AI).

Materials and Methods

Out of 100 human sera that were collected from different laboratories, 82 samples were positive for anti-*T. gondii* IgG antibody and 18 were positive for IgM.

Antigen Preparation

Soluble antigen was prepared as previously described (7). Briefly tachyzoites of RH strain that have been injected in Balb/c mice peritoneum were harvested 3 days later and washed with washing buffer (phosphate buffer saline, pH 7.2). For disruption of tachyzoites bodies, parasites were sonicated and then centrifuged for 1 hour at 12 000 rpm. After supernatant collecting and measurement of protein density by the method of Bradford, microtiter plates (Nunc, USA) were coated with the carbonate buffer, pH 9.6 containing 5 μg/mL of soluble protein, placed over night at 4˚C, washed and stored at -20˚C until use.

Avidity ELISA

Serial dilution of sera was prepared and each sample added to microtiter plate in duplicate row. Samples were incubated for 1 hour at 37˚C, and then washed. In washing step, one of the rows were washed with PBST (PBS, 0.05% tween 20) and the others washed with modified PBST buffer containing 6M urea. Afterward anti-human IgG conjugated with horseradish peroxidase (HRP) (Dako, Denmark) was added (1:1000 in PBST). After 1 hour of incubation and 3 times of washing the chromogenic substrate, o-phenylenediamine (OPD) (Merck, Germany) was added.

After adding sulfuric acid 20% the reaction was stopped and the absorbance read by an automated ELISA reader (BIOTEC, USA) at 492 nm. AI was calculated according to this formula:

AI = absorbance of urea washed wells/absorbance of PBS washed wells ×100.

IgM ELISA

Anti- *Toxoplasma gondii* IgM antibody was detected by

IgM-ELISA. Briefly, the serial dilution of sera from 1:10 to 1:160 in PBS, pH 7.2 was prepared and added to microtiter antigen- coated wells (Nunc, USA) afterward incubation and washing, anti-human IgM antibody conjugated with HRP (Dako, Glostrup, Denmark) was added. After 1 hour of incubation and 3 times of washing, the chromogenic substrate OPD (Merck, Germany) was added, the reaction stopped and the absorbance was recorded by means of ELISA- reader (BIOTEC, USA) at 492 nm.

For each IgG and IgM ELISA methods, 30 negative sera were tested and the amount of cut-off was calculated by this formula: X±2SD (X: the mean of the absorbance, SD: standard deviation of the absorbance for 30 samples). The absorbance more and less than the cut off were considered as positive and negative respectively.

In each IgG and IgM ELISA procedures negative and positive IgG and IgM sera were tested as control groups too.

Results
The AI percent of 72 sera out of 82 with chronic toxoplasmosis was more than 60, and 6 of them had the AI among 50- 60 (92.7% and 7.3% respectively). All the sera from this group had high titers of IgG antibodies by IgG-ELISA, and 4 of them had acceptable titers of IgM antibody too.

Acute toxoplasmosis group were positive for anti-*T. gondii* specific IgG and IgM antibodies. The AI percent of 50 and lower was seen in 16 samples in this group, whereas 2 had AI percent more than 50 (89% and 11% respectively).

In the chronic group with detectable titers of IgG antibody, the mean of AI percent in serum titers of 1:200, 1:400, 1:800, 1:1600, 1:3200 and 1:6400 was 74.3, 82.5, 76.5, 79.2, 83 and 75 respectively. There was no relation between the mean of AI and the concentration of IgG antibody in this group. In chronic group with IgG antibody, the mean of AI was more than 50 in any serum titer (Figure 1).

In the acute group with detectable IgM antibody, in 98% of samples the relation between the mean of AI and the concentration of IgM antibody was reversed. In these samples with positive IgM antibody, the mean of AI percent in serum dilutions of 1:20, 1:40, 1:80 and 1:160 was 67.3, 61, 51.9 and 30.6 respectively. In this group, by increasing the serum dilution, the mean of AI was decreased (Figure 2).

Discussion
Congenital toxoplasmosis, the infection of fetus with *T. gondii*, can lead to severe problems such as loss of fetus, encephalitis or brain and eyes lesions. This condition occurs when the mother acquires the infection during pregnancy (1). Diagnostic methods such as capture ELISA, and PCR could be useful for detecting parasite antigens and DNA in very short times after infection (8). Determination of anti-*T. gondii* IgG avidity is valuable
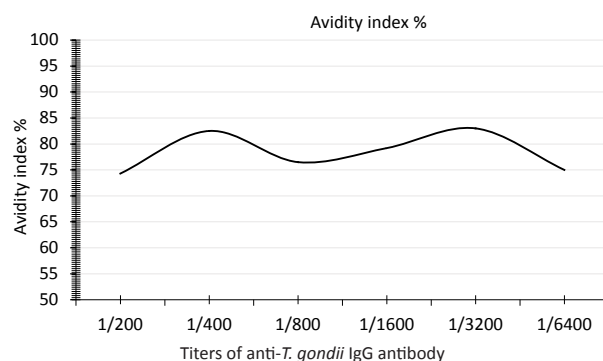
Figure 1. The Correlation Between IgG Antibody in Serum Titers and Avidity Index.
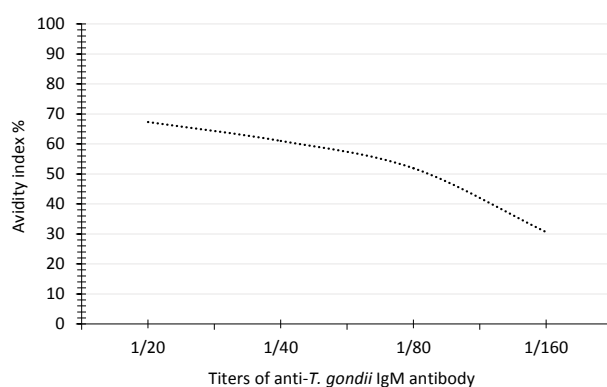
Figure 2. The Correlation Between IgM Antibody in Serum Titers and Avidity Index.

and useful tool for identifying acute from chronic positive infection in pregnant women. Avidity ELISA method is based on dissociation of hydrogen bond between antigen and antibody with urea (9). In this regard immunological markers could be distinguished by avidity immunoblotting method to differentiate acute from chronic toxoplasmosis (10).

If the *T. gondii* infection has occurred more than 3 month ago, the avidity would be high (AI ≥60%). In low avidity (AI ≤50%) the infection occurrence is within 3 month ago. In this regard, borderline avidity (50% < AI < 60%) means that the acquirement of infection is not determined (11,12). In some cases the low avidity may persist up to one year so interpretation of results of IgG avidity test in presence of IgM antibody is critical, because the low avidity does not mean the recently acquired infection here (12). In the present study there was no relation between concentrations of IgG antibody and the amount of AI, so in any serum titers for anti-*T. gondii* IgG antibody the AI was higher than 50%. In contrast, there was a negative relation between the anti-*T. gondii* IgM antibody serum titers and the AI, so by increasing the concentrations of IgM antibody the amount of AI decreased.

According to the results obtained here, IgG avidity results are irrelevant to antibody concentrations.

Ethical Issues
The Ethical Committee of Tehran University of Medical

Sciences approved this study.

Conflicts of Interests
There is no competing interest in this study.

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None to be declared.

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