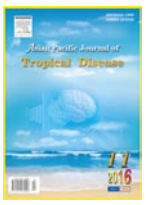
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Parasitological research doi: 10.1016/S2222-1808(16)61147-7

Diagnosis of acute toxoplasmosis in pregnant women referred to therapeutic centers of Alborz Province (Iran) using immunoglobulin G avidity ELISA technique

Lame Akhlaghi[1], Fatemeh Tabatabaie[1*], Ramtin Hadighi[1], Fatemeh Maleki[2], Fateme Hajialiani[1], Mohammad Saaid Dayer[3], Abbas Ghasemi[1], Masoud Roudbari[4]

[1]*Department of Parasitology and Mycology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran*

[2]*Department of Parasitology, Faculty of Para Medical Sciences, Iran University of Medical Sciences, Tehran, Iran*

[3]*Department of Parasitology and Entomology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

[4]*School of Public Health, Iran University of Medical Sciences, Tehran, Iran*

ARTICLE INFO

Article history:
Received 19 Jul 2016
Received in revised form 15 Aug, 2nd revised form 6 Sep 2016
Accepted 15 Sep 2016
Available online 26 Sep 2016

Keywords:
Toxoplasma gondii
Prevalence
Pregnant women
ELISA
Immunoglobulin G

ABSTRACT

Objective: To evaluate immunoglobulin G (IgG) avidity as a useful and reliable technique in diagnosing toxoplasmosis in pregnant women referring to therapeutic centers of Alborz Province (Iran) in 2014, against two other tests, IgG and immunoglobulin G (IgM) anti-*Toxoplasma*.

Methods: Serum samples (468 in total) were obtained from different therapeutic centers in Karaj City. ELISA method was used to test the anti-*Toxoplasma* avidity of IgG, IgM and IgG. The data were analyzed by descriptive statistical methods and *Chi*-square test ($P < 0.05$) using SPSS 17.0.

Results: Anti-*Toxoplasma* tests of IgM and IgG were positive in 9 and 86 samples respectively. Also, a borderline IgM was detected in 2 suspected samples. In addition, among all positive and suspected samples, 79 cases indicated high titers of IgG avidity, 7 cases were of low titers and 1 case was of a borderline titer. The prevalence of anti-*Toxoplasma* antibodies was 20%. The sera which showed high avidity index was obtained from patients at chronic phase of infection (92%) while those which showed low avidity levels were from patients at acute toxoplasmosis (77.7%).

Conclusions: This study clearly showed that acute and chronic phases of toxoplasmosis could be differentiated with the aid of IgG avidity test. This test may also assist in recognizing old and newly acquired infections.

1. Introduction

Toxoplasmosis is a parasitic disease of a worldwide distribution, endangering about one-third of human population. Due to its possible development into congenital infection and sequelae in the newborn, the disease must be timely and properly diagnosed. The occurrence of infection during the first trimester of pregnancy can expose the growing fetus to serious morbidity and mortality. Whereas, acquiring toxoplasmosis at the last trimester may lead to the development of clinical symptoms of congenital infection in exposed children during the first decade of their lives. It is, therefore, vital to recognize toxoplasmosis and its acute and chronic stages in order to treat the infection or restrain its effects, specifically during the pregnancy[1]. Although serological methods for detection of anti-*Toxoplasma* immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A antibodies provide useful diagnostic tools, there are some limitations. For example, ocular manifestation of congenital toxoplasmosis in children and adolescents is not always accompanied with increased IgG titers. In some cases,

*Corresponding author: Dr. Fatemeh Tabatabaie, Department of Medical Parasitology and Mycology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran.
Tel: +98-21-86703220
Fax: +98-21-8862 2653
E-mail: Tabatabaei.f@iums.ac.ir

The study protocol was approved by Ethical Committee of the Faculty of Medicine (Iran University of Medical Sciences). Informed written consent was obtained from all women involved in this study.

Foundation Project: Supported by Iran University of Medical Sciences (Code: 1710).

The journal implements double-blind peer review practiced by specially invited international editorial board members.

Toxoplasma IgM antibody can be detected even after a few years following primary infections. The antibody can, also, be recognized in patients with rheumatoid factors and antinuclear antibodies. However, it has been shown that as the infection becomes older, the antibody epitope bond becomes stronger. Also, ELISA test can be applied to detect specific IgG avidity at various toxoplasmosis phases, so that low and high levels of IgG avidity can indicate acute and chronic phases of toxoplasmosis respectively. Therefore, a high IgG avidity can serve as an indication of a chronic toxoplasmosis, whereas a low avidity can serve as points at a recent infection of 3–5 months old[2-4]. This study aimed to evaluate IgG avidity test for detection of acute toxoplasmosis in the pregnant women referring to therapeutic centers of Karaj City in 2014.

2. Materials and methods

2.1. Collection of serum samples

To undertake this descriptive cross sectional research, 468 serum samples were randomly obtained from beta-human chorionic gonadotropin-positive women referring to therapeutic centers in Alborz Province. The samples were then transferred to the parasitology laboratory of the faculty of Para Medical Sciences of Iran University of Medical Sciences where they were kept under standard conventional conditions for < 2 h before being centrifuged at 3 000 r/min for 15 min. The isolated sera were then frozen as per Alicot method and stored at 20 ℃ until used. Informed consents of all women involved in this study were ensured beforehand. The patients were asked to fill in a questionnaire on their demographic characteristics. The procedures of this study were also approved by the Ethical Committee of the Faculty of Medicine (Iran University of Medical Sciences). At first, ELISA test was applied to examine all sera for anti-*Toxoplasma* IgG and IgM antibodies according to manufacturer's instruction (Dia-Pro, Milan, Italy). The *Toxoplasma*-IgG was measured quantitatively but *Toxoplasma*-IgM was determined against an index reported by computing the cutoff point. The sera samples were then divided into three groups: Group I samples were obtained from patients suffering from acute toxoplasmosis; Group II samples were obtained from patients with chronic toxoplasmosis and Group III samples were obtained from patients showing borderline antibodies.

2.2. ELISA test

ELISA technique was used to measure the levels of anti-*Toxoplasma* IgG and IgM at the start of pregnancy according to manufacturer's instruction (Vircell Microbiology Company).

2.3. Avidity ELISA

Avidity tests were performed using Euroimmun kit as follows: sera were diluted before being added at 100 μL to microplates coated with *Toxoplasma* antigen. The antigen-antibody complex of microplate was then received concentrated urea solution (8 mol/L). The excess antibody was washed from the microplates and labeled anti-IgG antibody was supplemented. The microplates were incubated for 30 min and re-washed before addition of substrate solution. Sulfuric acid was final added to stop the reactions in the microplates. The measurement of optical density (OD) was performed at 450 nm against differential absorption at 600 nm. The avidity was obtained from the following formula[4-6]:

$$\text{Avidity index (\%)} = \frac{\text{OD of the sample treated with urea} - \text{OD of the blank}}{\text{OD of the sample treated without urea}} \times 100$$

2.4. Statistical analysis

The obtained data underwent statistical analysis using descriptive methods. The data were also subjected to *Chi*-square tests. Differences between variants were considered significant at $P \leqslant 0.05$. Computation of diagnostic values was performed using SPSS version 17.

3. Results

For the purpose of this study, the avidity was classified into three levels: low avidity ($\leqslant 40$), high avidity ($\geqslant 60$) and borderline ($40 \leqslant$ avidity index (AI) $\leqslant 60$). A total of 468 samples were evaluated by IgG ELISA method of which 86 samples were positive, 382 samples were negative. No borderline sample was detected. The same samples were also evaluated by IgM ELISA method of which 9 were positive, 457 were negative and 2 were borderline. Then, IgG avidity tests were carried out for all IgG and IgM positive and borderline samples. The results showed 79 samples to be high avidity, 7 to be low avidity and 1 to be borderline value.

The samples were categorized into 3 sera groups: Group I included 9 sera from patients with acute toxoplasmosis, in which specific IgM antibodies were detected and the patients of this group showed lymphadenopathy signs; Group II was formed from 86 sera from patients with chronic toxoplasmosis and these sera also contained specific IgG antibodies and Group III included 2 sera samples from patients with borderline IgM antibodies. In addition, the prevalence of anti-*Toxoplasma* antibodies was 20%. Out of 9 sera, 7 samples (77.7%) from patients with acute infections indicated low avidity levels and 79 out of 86 (sera 92%) from patients with chronic infections showed high avidity index. One sera had a borderline range of AI. By analyzing the data, we observed a significant

correlation between high IgM titers and low avidity of IgG ($P < 0.05$). But, no significant correlation was observed between IgG level and avidity index in Group II ($P > 0.05$). As per our data, measurement of IgG avidity can clearly differentiate between the acute and chronic phases of toxoplasmosis (Table 1).

Table 1

Comparative analysis of measurement of IgG, IgM and IgG avidity.

Parameters	Total	Borderline	Negative	Positive
IgG test	468	0 (0.0%)	382 (81.6%)	86 (18.4%)
IgM test	468	2 (0.4%)	457 (97.6%)	9 (1.9%)
IgG avidity	87	1 (1.1%)	79 (90.8%)	7 (8.1%)

4. Discussion

Toxoplasmosis is a worldwide parasitic infection with variable prevalence in different countries. Various techniques have been developed for *Toxoplasma* diagnosis such as anti-*Toxoplasma* antibodies detection, mouse inoculation and histological examination of tachyzoites[7].

On the other hand, several screening tests of mainly serology are used including indirect immunofluorescent antibody test, latex agglutination, indirect hemagglutination and ELISA[7,8]. In our study, we used ELISA test which is of high sensitivity and specificity. ELISA is frequently performed in many medical centers in our country for detection of anti-*Toxoplasma* antibodies. The clinicians prefer ELISA because of its excellent sensitivities and specificities, rapid accessibility and low cost. Hedman *et al*. firstly suggested a new diagnostic method in 1989 which was later called avidity test[9]. The method measures the affinity of antibodies of immunoglobulins bonding with *Toxoplasma gondii* polyvalent antigens under high urea concentration to differentiate immunoglobulin of high affinity. This method is now applied to evaluate the avidity of *Toxoplasma* IgG. A high avidity (AI $\geqslant$ 60%) serves as an indication of an old *Toxoplasma* infection acquired 3 months before the sampling, while a borderline avidity (40% < AI < 60%) indicates that the disease is at an indeterminate period. On the other hand, a low avidity (AI $\leqslant$ 40%) shows that onset of infection was within the last 3 months. Different avidity titers were reported by various authors as cutoff levels. For instance, an avidity level lower than 20% was suggested to be considered as a low avidity. Whereas, Yasodhara *et al.* suggested a low avidity to be below 30%[10]. Findal as well as Montoya and Liesenfeld agreed on a low avidity value of less than 40%[5,7]. By studying 37 pregnant women in 2004, researchers found IgG avidity varing between a low level of 10.8% and a high level of 57.2% at the beginning of pregnancy[8,9]. A pre-pregnancy negative IgG test and a post-pregnancy positive one are vital in diagnosing *Toxoplasma* infection. Since some pregnant women have not undertaken toxoplasmosis, other agents, rubella (also known as German measles), cytomegalovirus and herpes simplex screening tests prior to pregnancy, the avidity test becomes a high value to discover active toxoplasmosis during pregnancy. A number of researches that examined pregnant women have confirmed the validity of our results. However, in some cases, a high avidity of 45.8% (IgG^+, IgM^+) can be attributed to either a high half-life of IgM or the existence of rheumatoid factor. Therefore, avidity test can assist in making decision for treating toxoplasmosis if a differential diagnosis is performed on time to detect active infections. We found that 44% of pregnant women were tested positive for IgG and negative for IgM while showing high avidity values. Our results indicated that in Alborz Province, the level of *Toxoplasma* infection in pregnant women is high due to their contact with *Toxoplasma* parasite before pregnancy. Other studies showed that 55% of the pregnant women were positive for IgG and IgM whereas 7.1% of them were of low avidity levels in the first 2 to 4 months of pregnancy. This can be taken as indication for prevalence of active infections among these pregnant women[6,10,11].

However, Morris and Croxson stated that by measuring IgG avidity, they were able to distinguish between the stages of toxoplasmosis[12]. To the contrary, Remington *et al.* believed that measuring IgG avidity cannot prove acute toxoplasmosis unless it is confirmed by IgM ELISA test[13]. This has been emphasized by many investigators who proposed IgM detection for acute infection diagnosis. However, the detection of reactivated toxoplasmosis in immunocompromised patients can be considered as one of the limitations of IgG avidity method[13,14]. The elevated level of *Toxoplasma* IgG can discover recent infections but its monitoring might be harmful for the fetus as it requires a long period of time. However, toxoplasmosis must be timely diagnosed and treated during pregnancy to ensure protection of the fetus from disease and its consequences[15].

In our study, 382 pregnant women (81.6%) were negative for anti-*Toxoplasma* IgG which indicated that they were not previously exposed to the disease or IgG of sera samples at undetectable level. Also, 86 women (18.4%) were positive for anti-*Toxoplasma* IgG which showed that they were previously in contact with the agent of infection and had immunity due to their chronic infections. No intermediate titer of IgG was observed throughout this study.

IgM anti-*Toxoplasma* serum samples of all mothers were tested which showed that 457 patients (97.6%) were found negative, 9 mothers were positive and 2 mothers had uncertain results. Positive cases or intermediate (border line) might serve as indication of the current contact with the infection and the risks. This may harbor as irreparable complications for the mother and fetus. All mothers whose tests were positive or borderline for anti-*Toxoplasma* IgM, showed positive results for *Toxoplasma* IgG except for a 28-year-old first-time mother whose IgM titer was about 1.06 (positive) and IgG titer was 3.00 (negative). After carrying out IgG avidity tests on samples of mothers' sera which were either positive or borderline for *Toxoplasma* IgG and IgM

titers, 79 mothers were found of high affinity and considered to have chronic infection (previous encounter) which presented no serious risk to the mother and fetus. Also, 7 mothers had low affinity and therefore they suffered from acute infection of high risk to the mother and fetus. The only borderline case was referred to the responsible physician for proper decision on repeating her tests or prescribing her further supplemental tests.

A total of 11 mothers were tested positive or suspected for anti-*Toxoplasma* IgM test, only seven were of low IgG avidity test and their IgM tests were confirmed. Given the high affinity in one case, the possibility of active infection of the mother was ruled out. Also, one case with acute infection and borderline affinity (28-year-old mother in her first trimester whose IgM test was positive) was subjected to further complementary tests. In two cases with suspected IgM tests, acute risk of infection was rejected on the basis of IgG avidity test and high affinity.

In conclusion, our study indicated that application of both IgM-ELISA test and IgG avidity-ELISA test can assist in differentiation between acute and chronic phases of toxoplasmosis. The avidity test provides an essential diagnostic tool for treating toxoplasmosis if it is concurrently applied with a differential test to distinguish active and chronic infection of this parasite. To reduce the consequences of toxoplasmosis, it may be appropriate to monitor seropositivity in mothers before and during pregnancy.

Sometimes, further study is also required, including the sampling of the fetus (amniocentesis), which is a decisive way in parasitological study, but it is not usually welcomed by patients and their physicians due to potential risks of amniotic fluid sampling. It is, therefore, recommended that in cases of suspected toxoplasmosis, a reliable test called IgG avidity test can be used instead of doing two anti-*Toxoplasma* IgM and IgG tests. It will save time and expenditure when screening toxoplasmosis in pregnancy. Additional tests such as molecular techniques should be used only in cases that IgG avidity test involves risks to mother and fetus[12,13].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

We would like to acknowledge the finacial support of Iran University of Medical Sciences (Code: 1710).

References

[1] Leite M, Siciliano S, Rocha LS, Justa MT, César KR, Granato CF. Correlation between specific IgM levels and percentage IgG-class antibody avidity to *Toxoplasma gondii*. *Rev Inst Med Trop Sao Paulo* 2008; **50**: 237-42.

[2] Remington JS MR, Wilson CB, Desmonts G. Toxoplasmosis. In: Remington JS KJ, editor. *Infectious diseases of the fetus and newborn infant*. 7th ed. Philadelphia: Saunders/Elsevier; 2011, p. 918-1041.

[3] Murat JB, L'Ollivier C, Fricker Hidalgo H, Franck J, Pelloux H, Piarroux R. Evaluation of the new Elecsys Toxo IgG avidity assay for toxoplasmosis and new insights into the interpretation of avidity results. *Clin Vaccine Immunol* 2012; **19**(11): 1838-43.

[4] Rahbari AH, Keshavarz H, Shojaee S, Mohebali M, Rezaeian M. IgG avidity ELISA test for diagnosis of acute toxoplasmosis in humans. *Korean J Parasitol* 2012; **50**(2): 99-102.

[5] Findal G, Stray-Pedersen B, Holter EK, Berge T, Jenum PA. Persistent low *Toxoplasma* IgG avidity is common in pregnancy: experience from antenatal testing in Norway. *PLoS One* 2015; **10**(12): e0145519.

[6] Pour Abolghasem S, Bonyadi MR, Babaloo Z, Porhasan A, Nagili B, Gardashkhani OA, et al. IgG avidity test for the diagnosis of acute *Toxoplasma gondii* infection in early pregnancy. *Iran J Immunol* 2011; **8**(4): 251-5.

[7] Montoya JG, Liesenfeld O. Toxoplasmosis. *Lancet* 2004; **363**(9425): 1965-76.

[8] Tanyuksel M, Guney C, Araz E, Saracli MA, Doganci L. Performance of the immunoglobulin G avidity and enzyme immunoassay IgG/IgM screening tests for differentiation of the clinical spectrum of toxoplasmosis. *J Microbiol* 2004; **42**: 211-5.

[9] Hedman K, Lappalainen M, Seppäiä I, Mäkelä O. Recent primary toxoplasma infection indicated by a low avidity of specific IgG. *J Infect Dis* 1989; **159**: 736-40.

[10] Yasodhara P, Ramalakshmi BA, Sarma MK. A new approach to differentiate recent *vs.* chronic *Toxoplasma* infection: avidity ELISA in *Toxoplasma* serology. *Indian J Med Microbiol* 2001; **19**: 145-8.

[11] Tlamçani Z, Lemkhenete Z, Lmimouni BE. Toxoplasmosis: the value of molecular methods in diagnosis compared to conventional methods. *J Microbiol Infect Dis* 2013; **3**(2): 93-9.

[12] Morris A, Croxson M. Serological evidence of *Toxoplasma gondii* infection among pregnant women in Auckland. *N Z Med J* 2004; **117**(1189): 1-7.

[13] Remington JS, Thulliez P, Montoya JG. *Recent developments for diagnosis of toxoplasmosis*. *J Clin Microbiol* 2004; **42**: 941-5.

[14] Alvarado-Esquivel C, Niewiadomski A, Schweickert B, Liesenfeld O. Antiparasitic treatment suppresses production and avidity of *Toxoplasma gondii*-specific antibodies in a murine model of acute infection*. *Eur J Microbiol Immunol* (*Bp*) 2011; **1**(3): 249-55.

[15] Haeri MR, Jalalizadegan B, Tabatabaie F. 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). *Am J Life Sci* 2014; **2**(6-3): 18-21.