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Detection of acute *Toxoplasma gondii* infection in pregnant women by IgG avidity and PCR analysis

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Abstract. During pregnancy, *Toxoplasma gondii* can be transmitted from mother to foetus and trigger a primary infection that may be symptomatic. It is important to distinguish between recently acquired and past infections to ensure proper treatment to minimize irreversible foetal injury. We used PCR of the B1 gene to evaluate the accuracy of *T. gondii* IgG antibody avidity testing in discriminating recent from past infection. In a cross-sectional study, *T. gondii* IgG and IgM antibodies were detected by enzyme linked fluorescence assay (ELFA) in 2120 serum samples from pregnant women referred to Karaj medical laboratories, February 2013 through March 2015 with 40 samples found positive. IgM-positive samples were evaluated by IgG avidity testing and PCR to amplify the B1 gene. Avidity studies indicated 20 samples with high IgG avidity, 15 with low IgG avidity, and five showing borderline values. The B1 gene was amplified in the borderline samples, with nine of the 15 showing low avidity. The B1 gene was not amplified in the high avidity sera. Our findings suggest that IgG avidity alone may not be sufficient to discriminate recent from past *T. gondii* infection and should not be used as the sole confirmatory test in pregnant women with IgG and IgM *T. gondii* antibodies. IgG avidity testing in combination with PCR may be more reliable for distinguishing between high- and low-risk infection and decrease the frequency of unnecessary treatment of pregnant women.

INTRODUCTION

Toxoplasma gondii is an obligate intracellular parasite that infects all warm-blooded animals, including humans (Liu *et al.*, 2015). Felidae are the definitive hosts, harbouring the reproductive stage of the parasite, which is responsible for its transmission through passing oocysts in faeces. Humans and other animals become infected by direct ingestion of oocyst-contaminated food or water or by eating undercooked meat containing tissue cysts (Blader *et al.*, 2015).

Toxoplasmosis infection in humans, with a prevalence of 10 to 80% worldwide, usually results in subclinical or mild clinical manifestations including lymphadenopathy, fever, lymphocytosis, myalgia, or respiratory symptoms. The most important aspect of toxoplasmosis occurs with the first encounter of a pregnant woman with *T. gondii* infection, which can result in foetal congenital toxoplasmosis (Gangneux & Dardé, 2012). The rate of maternal/foetal transmission is dependent on gestation stage. During the first trimester, the rate of transmission is less than 10%, but can cause abortion or major defects such as hydrocephalus, macrocephaly, mental retardation, and cerebral calcifications. In the third trimester, the risk of placental transmission of the

parasite increases to 60-70% (Gangneux & Dardé, 2012; Moncada & Montoya, 2012) with foetal pathology usually manifested as an asymptomatic infection, although potentially leading to chorioretinitis (Paquet & Yudin, 2013). Therefore, identifying the time of an initial maternal infection during pregnancy is critical to reducing the risk of pathogenesis.

In contrast to acute infection, latent toxoplasmosis acquired prior to pregnancy does not lead to congenital infection. Thus, it is important to distinguish between active and past infection to minimize irreversible foetal injury. Laboratory diagnosis of toxoplasmosis is most commonly made by detection of IgM and IgG antibodies, although these tests cannot give precise information as to the time the infection was acquired. The avidity test is based on the fact that *Toxoplasma* IgG antibodies in an acute infection show weak binding to antigens (low avidity), while IgG antibodies from a chronic infection bind strongly (high avidity) (Press *et al.*, 2005). Research has suggested that the detection of *Toxoplasma* antibodies and evaluation of IgG avidity has high value in identifying infections incurred in the preceding four months (Petersen *et al.*, 2005; Press *et al.*, 2005). Direct detection of *T. gondii* DNA using PCR targeting the 35-fold repeated *T. gondii* B1 gene is commonly used for molecular diagnosis. The specificity of the B1 gene is 100%, showing no cross-reaction with other microorganisms (Burg *et al.*, 1989).

We evaluated *T. gondii* antibody IgG avidity by PCR of the *T. gondii* B1 gene for detection of acute *T. gondii* infection in pregnant women.

MATERIALS AND METHODS

Study group

From February 2013 to March 2015, all pregnant women referred to the Karaj medical laboratories were invited to participate in a cross-sectional study. Serum samples from 2120 pregnant women of various ages and at different gestation stages were included. Participants gave written informed consent. The study was approved by the Ethics Committee of Iran University of Medical Sciences under code number IR.IUMS.REC 92-03-31-24138.

Serological analysis

Enzyme linked fluorescence assay

The presence of *Toxoplasma* IgG and IgM antibodies was evaluated in all 2120 samples by enzyme linked fluorescence assay (ELFA) with the Vitek Immuno Diagnostic Assay System (VIDAS; BioMerieux, Marcy-lEtoile, France) as described by the manufacturer. The analysis was based on the enzyme immunoassay method with fluorescence detection using alkaline phosphatase-labelled monoclonal anti-human IgG or IgM antibodies. These enzyme-conjugated antibodies react with the substrate (4-methylumbelliferyl phosphate), and the relative fluorescence value of the solution is assessed and interpreted automatically (Prusa *et al.*, 2012). For IgG, ≥8 IU/ml was considered positive, 4-8 IU/ml was borderline, and <4 IU/ml was negative. For IgM, ≥0.65 IU/ml was positive, 0.55-0.65 IU/ml was borderline, and <0.55 IU/ml was negative.

Toxoplasma IgG Avidity

Toxoplasma IgG antibody avidity testing was conducted on the IgG- and IgM-positive samples by VIDAS according to manufacturer's instructions. The assay combines a two-step enzyme immunoassay sandwich method with final ELFA, using a dissociation agent such as urea. The IgG avidity is determined by the ratio of the sample treated with the dissociation agent to an untreated sample. The avidity index is interpreted as low (<0.2), borderline (0.2-0.3), or high (≥0.3) (Villard *et al.*, 2013).

Molecular analysis

DNA extraction

Toxoplasma IgG- and IgM-positive serum samples were submitted to DNA extraction procedures using the QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany) according to instructions. Extracted DNA was stored at -20°C until used in PCR.

Toxoplasma molecular detection

In *Toxoplasma* IgG- and IgM-positive serum, the 193 bp fragment of the 35-fold-repetitive B1 gene of *T. gondii* was detected by primers and PCR reaction according to Schwab & McDevitt (2003).

The PCR reaction mixture consisted of 25 µl Master Mix (Ampliqon III, Denmark), 2.5 µM each of primer, 10 µl of DNA template, and 10 µl distilled water to obtain a 50 µl reaction volume. Amplifications were performed with initial denaturation of 3 minutes at 95°C followed by 40 cycles of denaturation for 30 seconds at 95°C, annealing for 50 seconds at 55°C, extension for 20 seconds at 72°C, and a final extension of 5 minutes at 72°C (Schwab & McDevitt, 2003).

PCR of every tenth sample was run with the genomic DNA of *T. gondii* RH strain tachyzoites and distilled water as positive and negative control, respectively. PCR products were visualized using a transilluminator, after electrophoresis on 2% agarose gel (Figure 1).

RESULTS

Toxoplasma gondii IgG antibodies were detected in 1362 of 2120 (64.2%; 95% CI 62.2-66.3) serum samples, of which 40 (1.9%; 95% CI 1.4-2.6) were positive for both IgM and IgG antibodies by ELFA. Analysis of IgG- and IgM-positive samples revealed 15 with low IgG avidity, 20 with high IgG avidity, and five with borderline values. The B1 gene was amplified in 14 (35.0%; 95% CI 22.1-50.5) of the IgG/IgM positive samples. PCR analysis detected *T. gondii* DNA in all five IgM-positive samples with borderline-avidity and in nine of the fifteen IgM-positive samples (60%) with low-avidity antibodies (Figure 2). The B1 gene was not amplified in the serum samples showing high antibody avidity (Table 1). Thus 100% of latent infections identified by avidity testing were confirmed by PCR, but it was less reliable for acute infection.

DISCUSSION

Molecular evaluation revealed 35.0% acute *T. gondii* infection in pregnant women positive for both IgG and IgM. Our findings indicated that acute toxoplasmosis was not reliably identified by detection of *T. gondii*-specific IgM/IgG antibodies. In most infected patients, *T. gondii* IgM antibodies are detectable for two years following primary infection (Findal *et al.*, 2015).

Assessing increase in levels of *T. gondii*-specific IgG over time is also an unreliable approach (Findal *et al.*, 2015). Therefore, conventional serology testing is often inadequate to discriminate active from past infections (Montoya *et al.*, 2004; Tanyuksel *et al.*, 2004), although it is critical to screening for *T. gondii* IgG and IgM antibodies in high risk individuals for possible

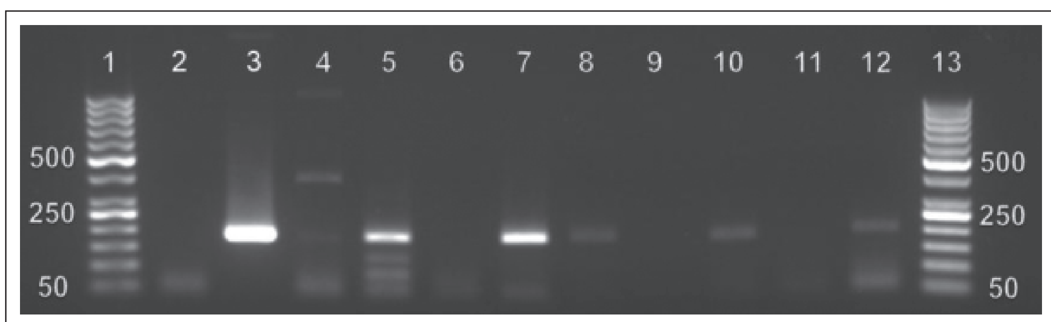

Figure 1. B1 gene amplification of *Toxoplasma gondii* from DNA extracted from serum samples of pregnant women. Line 1 and 13, 50 bp DNA ladder (Fermentas, St. Leon-Rot, Germany; Cat No SM0373); Line 2, negative control; Line 3, positive control, standard DNA of *T. gondii* RH strain). Lines 4, 5, 7, 8, 10, and 12 represent positive samples infected with *T. gondii*. Line 6, 9, and 11 represent negative samples uninfected with *T. gondii*.

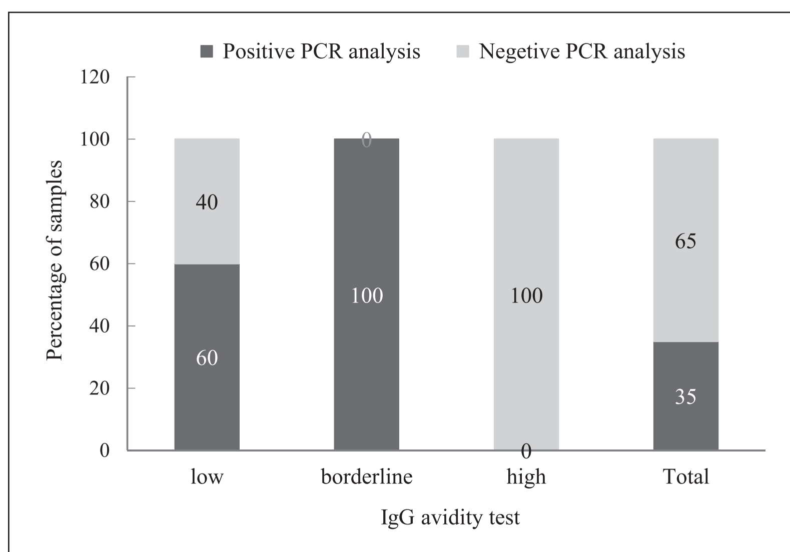

Figure 2. The IgG avidity value distribution of *T. gondii*-PCR positive in pregnant women.

Table 1. Comparison of IgG avidity test and PCR assay to confirm acute *Toxoplasma gondii* infection in pregnant women

IgG avidity		PCR analysis	
Rate	No	Positive	Negative
Low	15	9	6
Borderline	5	5	0
High	20	0	20
Total	40	14	26

referral for comprehensive diagnostic testing such as IgG avidity and molecular evaluation.

IgG avidity testing revealed 37.5% of IgM-positive women to exhibit low-avidity IgG antibodies, suggesting acute *T. gondii* infection. This finding is in contrast with previous studies suggesting that IgG avidity test is adequate for the differentiation of recently acquired from past infections (Press *et al*., 2005; Candolfi *et al*., 2007; Rahbari *et al*., 2012). Low-avidity is reported to be manifested for about one-year post-infection (Pelloux *et al*., 1998; Liesenfeld *et al*., 2001), thus the finding of low-avidity IgG antibodies is not sufficient to confirm a clinically significant recent infection. However, high-avidity IgG antibody levels have been shown reliable in ruling out recently acquired infection (Liesenfeld *et al*., 2001; Remington *et al*., 2004), hence we determined that 50% of the IgM-positive women in the study had acquired the infection prior to pregnancy. In this context, our findings agree with those obtained by (Liesenfeld *et al*., 2001; Remington *et al*., 2004) who suggested that IgG avidity testing to rule out acute infection be conducted within the first three to four months of gestation. A high-avidity result in the latter part of the second trimester or early third trimester cannot be interpreted as to time of initial infection (Liesenfeld *et al*., 2001; Remington *et al*., 2004). Therefore, a more inclusive diagnostic test to be applied to all *Toxoplasma* infected mothers would be of value.

Accumulated data have supported the use of PCR to identify *T. gondii* in the blood, amniotic fluid, cerebrospinal fluid, placental and brain tissues, aqueous humour, and bronchoalveolar lavage fluids for diagnosis of congenital and ocular toxoplasmosis and in immunocompromised individuals as the gold standard test (Lin *et al*., 2000; Halonen & Weiss, 2013). The presence of *T. gondii* DNA in maternal blood indicates a recent infection or overt parasitemia, which is clinically important (Chabbert *et al*., 2004; Slawska *et al*., 2005; Iqbal & Khalid, 2007).

Our findings showed all 20 IgM-positive women with high-avidity antibodies to be negative for *Toxoplasma* DNA, confirming that the IgG avidity test can be used to rule out recently acquired infection in early pregnancy. We found 60% of IgM-positive samples with low IgG avidity antibodies and all those with borderline avidity antibodies to be positive for *T. gondii* DNA. These results are consistent with those of Iqbal & Khalid (2007) and Bortoletti *et al.* (2013), who used nested-PCR analysis to detect *Toxoplasma* DNA and confirm acute infection. They showed that IgG avidity testing alone was not adequate for differentiating acute from chronic infection (Iqbal & Khalid, 2007; Bortoletti *et al.*, 2013). Previously, Yamada *et al.* (2011) found that combined data of IgG avidity in maternal blood and multiplex nested PCR in the amniotic fluid was useful for diagnosing congenital toxoplasmosis. Studies identifying *T. gondii* in blood samples of pregnant women by PCR analysis combined with IgG avidity test are scarce. Nevertheless, there have been noteworthy studies detecting *Toxoplasma* DNA in amniotic fluid during prenatal testing when maternal seroconversion occurs (Yamada *et al.*, 2011; Nishikawa *et al.*, 2009).

CONCLUSION

Our results suggest that IgG avidity alone may not be an accurate indicator of acute *T. gondii* infection and should not be used as the sole confirmatory test for pregnant women with IgG and IgM *T. gondii* antibodies. IgG avidity testing in combination with PCR or together with assessment of antigens or *T. gondii* DNA transcription (real time PCR) as a confirmatory test may be more reliable in distinguishing between high-risk and low-risk infections and decrease unnecessary treatment of pregnant women.

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