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Evaluation of the Plasma Level of Fibrinogen in the First Trimester in Mothers with Toxoplasmosis

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Abstract

Background and Objectives: Toxoplasmosis is a parasitic infectious disease. The occurrence of this disease brings about multiple complications resulting in abortion, death, or severe fetal complications. These complications may occur as a result of increased fibrinogen levels during pregnancy and increased serum factors associated with Toxoplasmosis. This study examined the plasma level of fibrinogen in the first trimester in mothers with acute toxoplasmosis for the first time.
Methods: In this study, 205 pregnant women who were referred to various medical centers of Tehran during 2014 - 2015 were studied. The bloods were examined for the levels of IgG avidity, anti - toxoplasma IgM and IgG, and fibrinogen levels. SPSS software v. 22.0 was used to study the results. Data regarding their demographic characteristics were collected by a questionnaire.
Results: Among a total of 205 patients, 41 cases had positive IgG titer, two had positive IgM titer (one low avidity), and five were borderline. Fibrinogen level was high in one case of positive IgM titer as well as low avidity and positive in 10 out of 41 cases with IgG titers.
Conclusions: The results of the present study shows that increased levels of fibrinogen in pregnant women during the first trimester of pregnancy is not significantly associated with toxoplasmosis.

Keywords: *Toxoplasma gondii*, IgG Avidity, Fibrinogen, IgM

1. Background

Toxoplasma gondii, an obligate, intracellular protozoan, is the disease agent of toxoplasmosis (1). Despite the wide distribution of toxoplasmosis in the world, the prevalence of toxoplasmosis with clinical symptoms is low compared to the infection rate (2, 3).

The most important methods of transmission include ingestion of spore oocytes from the soil, water, and food or bradyzoites existing in cystic tissue through the consumption of raw or undercooked meat and meat products (4). Placental transmission is another important transmission method of this parasite; among 1000 seronegative women infected with *Toxoplasma gondii* in pregnancy, five transmit the parasite to their fetus (5).

This parasitic infection is asymptomatic in immunocompetent individuals. Among the symptoms, lymphadenopathy is the most common symptom, which does not usually cause serious side effects and resolves without treatment. However, in individuals with immune system dysfunction, the disease is severe and disseminated and even causes death in some cases (2, 6). Infection with this parasite is of particular importance, especially for pregnant women. If a pregnant woman acquires this infection before or during pregnancy, the disease can be transmitted to the fetus and cause serious complications such as blindness and severe congenital neurological disorders in the fetus (7). The age of the fetus at the time of acquisition of the infection in the mother is one of the important factors affecting fetal health; the fetal infection risk up to 13 weeks of pregnancy is about 15%, after which the risk of infection increases and reaches 72% at 36th week of pregnancy (8, 9).

The most common method used for the diagnosis of this disease includes serologic tests such as ELISA, IFA, and IHA. In these tests, IgM is considered in the acute phase and IgG in the chronic phase of the disease (10, 11). One of the methods that are currently used is the Avidity Test. This method is based on binding affinity of immunoglobulins to the polyvalent antigens of *Toxoplasma gondii*. At early stages of the infection, the binding affinity of IgG to antigen is low. With the progression of the infection, affinity of antibody for *Toxoplasma gondii* antigen increases, which

indicates chronic infection (12, 13).

The incidence of this parasitic infection is of particular importance in pregnant women due to potential risks to the fetus. This study aims to investigate the relationship between toxoplasmosis and increased serum levels of fibrinogen in pregnant women. Fibrinogen is one of the most important positive acute phase proteins. It normally shows increased levels during pregnancy, which may increase toxoplasmosis. In pregnant women with normally increased levels of blood procoagulants, including fibrinogen, physiologically the anticoagulant levels are reduced in the blood during pregnancy and pregnant women have an overall weakened performance of fibrinolytic system. In these conditions, if the woman is infected with toxoplasmosis during a pregnancy, the excessive increase of this coagulative factor increases the risk of thromboembolism. If thrombosis occurs in the placental vessels, there is a risk of infarction of this area, which may lead to miscarriage. Thus, both mother and fetus are at risk of thromboembolism (14, 15). This study aimed to investigate the relationship between toxoplasmosis and increased plasma levels of fibrinogen in pregnant women.

2. Methods

2.1. Study Type and Population

This cross - sectional study was conducted on blood tests of randomly selected pregnant women who were referred for screening to four laboratories (Shohadaye ali abad - Yas - Armin - Dr.Hashemnejad) in different parts of Tehran from 2014 to 2015. The patients were asked to fill in a questionnaire on their demographic characteristics (age, gestational age and number of children, predisposing factors, use of drugs, several pregnancy, and history of underlying illness or any symptom of toxoplasmosis). The procedures of this study were also approved by the Ethical Committee of the Faculty of Medicine (Iran University of Medical Sciences, Code: IR.IUMS.FMD.REC.1393.3). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All tests were done duplicate. The Informed consent for participation in the study was obtained from participants. This study examined the plasma level of fibrinogen in the first trimester in mothers with toxoplasmosis for the first time. In this study, 205 pregnant women who referred to various medical centers of Tehran during 2014 - 2015 were studied. The sera were sitting in -20°C until use (less than 6 months). Titers of anti - *toxoplasma* IgG, IgM, and IgG avidity in the collected sera were analyzed by ELISA. The Clauss method was used to measure the amount of fibrinogen in the plasma samples (with taking 1.8 mL of blood on 0.2 mL of Trisodium citrate 3.8%). The ratio of 1 to 9 is important between anticoagulant and blood. The measurement of fibrinogen should be done up to 6 hours after the sampling. Half an hour before the test, remove all reagents from the refrigerator until reaching the laboratory temperature (20 - 25°C).

2.2. Titration of Antibody (Anti - Toxoplasma IgG and IgM) Using ELISA

In this study, the diagnostic kits "ToxoIgG - EUROIMMUN" and "ToxoIgM - EUROIMMUN" (Corporation Germany) were used for the titration of IgM and IgG. The procedure was performed according to the manufacturer's instructions. In summary, after adding samples to the wells and rinsing them, the conjugate enzyme was added; in the next step, the corresponding substrate was added. Finally, after adding the plug solution, they were read at a wavelength of 450 nm using an ELISA reader. After the calculations of anti - toxoplasma IgG, titers less than 8 IU/mL were considered negative, those greater than 11 were considered positive, and those between 8 and 11 were considered suspected or facultative. Anti-toxoplasma IgM titers were considered negative when less than 0.8 IU/mL, positive when more than 1.1, and titers of 0.8 - 1.1 were considered suspicious or facultative.

2.3. Anti - Toxoplasma IgG Avidity Titration Using ELISA

For this purpose, ToxoIgG Avidity - EUROIMMUN kit was used (Corporation Germany). The procedures were based on the manufacturer's instructions and after the relevant calculations, samples with RAI < 40% were considered antibodies indicated for low avidity, samples with RAI 40% - 60% suspicious, and samples with RAI > 60% with high avidity (1, 3).

2.4. Quantitative Measurement of the Amount of Plasma Fibrinogen by Clauss Coagulation Method

To measure fibrinogen, a Birefox kit made in England was used. In this method, after preparation of dilution of 1/10 from samples and addition of thrombin solution, clotting time was recorded in samples of patients and fibrinogen concentrations were calculated using standard charts and clotting time. Fibrinogen concentration was considered normal at 200 - 400 mg/dL (14, 15).

2.5. Statistical Analysis of the Results

The data were assayed by descriptive statistical methods. Test results and information collected by the questionnaire were analyzed with Microsoft Excel and SPSS v. 22.0 using Chi - square and one - way analysis of variance test at a significant level of less than 0.05.

3. Results

3.1. The Results of Titration of Anti - Toxoplasma IgG and IgM Using ELISA

The results of titration of anti - toxoplasma IgG in the serum of pregnant women were positive in 41 cases, negative in 162 samples, and suspected in two samples. The results of titration of anti - toxoplasma IgM showed two positive samples, 200 negative samples, and three suspected samples (Figure 1).

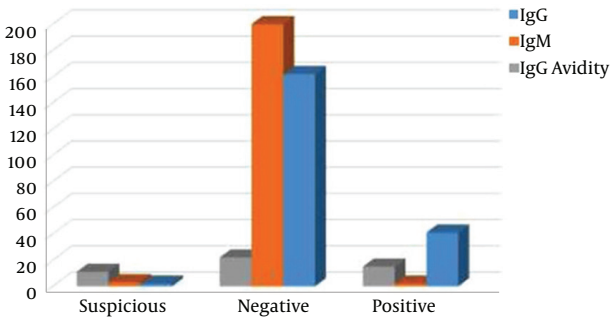

Figure 1. Analysis of the Results of IgG Avidity Test with Anti - toxoplasma IgG and IgM ($P < 0.05$)

3.2. The Results of IgG Avidity Test

In the studied population, IgG Avidity test was performed on 48 samples of mothers with positive or suspicious serum Anti - toxoplasma IgG and IgM. Of these, 15 samples had low avidity, 11 samples were suspicious, and 22 samples had high avidity (Figure 1). Analysis of the results of IgG Avidity test with anti - *toxoplasma* IgG and IgM was significant ($P < 0.05$).

3.3. Analysis of the Results of Fibrinogen Level and Anti - toxoplasma IgG and IgM Titers

Plasma fibrinogen concentration in the samples was between 20 - 740 mg per deciliter. Comparison of the mean values of fibrinogen concentration in the groups using ANOVA test showed no significant relationship between the groups $P > 0.05$.

The results showed that among the total of 205 patients, 41 had positive IgG titer, two had positive IgM titer (one low avidity), and five were suspicious. Fibrinogen level was higher than normal in one case with positive IgM titer and low avidity; in 10 cases, there was a total of 41 samples with positive IgG titers. Analysis of the results of fibrinogen level with anti - *toxoplasma* IgG and IgM titers was not significant (Figure 2).

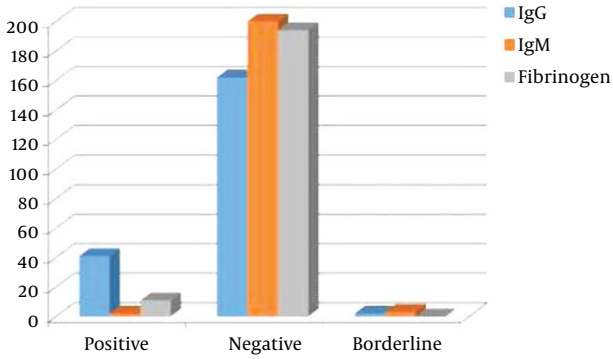

Figure 2. Analysis of the Results of Fibrinogen Level with Anti - toxoplasma IgG and IgM Titers ($P > 0.05$)

4. Discussion

All over the world, many researchers have reported cases of toxoplasmosis; we can say that more than a third of the world's population has been infected with this parasite (2). One of the transmission methods of toxoplasmosis is transmission from placenta to the embryo. The diagnosis of this disease during pregnancy is of great importance (13). Serious complications of congenital toxoplasmosis in the first trimester of pregnancy include abortion, CNS, and ocular disorders (16, 17). In case of toxoplasmosis in pregnant women, there is a risk of thromboembolism due to excessive increase in fibrinogen. If this complication occurs in placental vessels, infarction can occur in this area, which leads to miscarriage (18). Considering this issue, it is essential to know the fibrinogen level in pregnant women who are at risk of abortion. Given the importance of toxoplasmosis in pregnant women in the first trimester of pregnancy, the objective of this study was to investigate the prevalence of this parasitic infection and the relationship between toxoplasmosis and plasma levels of fibrinogen in a random sample of the study population. Classical methods for diagnosis of toxoplasmosis include anti - toxoplasma IgG and IgM titers. Considering the earlier production of IgM to IgG, this method provides the possibility to differentiate previous or current infection. One of the diagnostic problems of anti - toxoplasma IgM is the interpretation of serological diagnosis. Specificity of IgM (even if specific and sensitive) should be confirmed by another method as well. Most ELISA techniques can measure IgM antibody for months or years after infection (4). Therefore, IgM is not a reason of new infections, unless high titer increases approximately four - fold three weeks or one month after the initial sampling. Sometimes Rheumatoid Factor causes false positive IgM, which should be considered. In addition to this method, there are newer methods for the

detection of toxoplasmosis. One of these methods is the test to measure IgG avidity or affinity. In the present study, IgG avidity was tested for positive IgG and IgM samples. The analysis of the results shows a significant relationship in this group between mean IgG avidity and mean titers of anti - toxoplasma IgG, as well as IgM. Studies have evaluated the efficacy of IgG avidity in the diagnosis of acute toxoplasmosis infection during early pregnancy, such as the research conducted by Iqbal et al., which assessed a total of 224 women in the first trimester of pregnancy in Kuwait. Serologic screening showed that 119 patients (53.1%) were IgG positive and 31 patients (13.8%) were IgM positive. IgG Avidity and PCR tests were also done at the same time. In this study, 19 pregnant women with positive IgM had negative PCR, which is consistent with our study, and all cases had IgG with high affinity (19). The results of the present study were similar to that of Iqbal et al., and indicated that the IgG avidity method verifies anti - toxoplasma IgM antibody, which can have error in serological interpretation. Ghasemi et al., in 2014, studied the affinity of antibodies (IgG avidity) in the diagnosis of acute toxoplasmosis in pregnant women. The researchers proposed IgG avidity as a confirmatory method for positive IgM (20). Along with the present study and considering the serious complications of toxoplasmosis in the first trimester of pregnancy, Haiery and colleagues conducted a study in 2013 on 200 pregnant women in the first trimester of pregnancy and used ELISA IgG Avidity to differentiate acute or chronic phase of toxoplasmosis. A total of 20 cases with IgM positive had low avidity that showed acute phase of the disease (21). The current study titrated anti - toxoplasma IgG, IgM, IgG avidity, and -for the first time- the plasma levels of fibrinogen in the blood of pregnant women. Assessment of the plasma level of fibrinogen in pregnant women, especially in the first trimester of pregnancy, is of great importance in the prevention of complications in the mother and fetus. Based on this analysis, the results showed that the levels of anti - toxoplasma IgG and IgM had no significant relationship with fibrinogen levels. Very few studies have examined plasma levels of fibrinogen during pregnancy. One study by Yazdani and colleagues assessed the changes in plasma levels of fibrinogen and CRP during pregnancy, the relationship between these components, and the disease in mild and severe preeclampsia. The results showed no relationship between the plasma level of fibrinogen and CRP with mild and severe preeclampsia (22). In another study in 2007, Sevim and colleagues evaluated the increase in Hs - CRP in infection with *Toxoplasma gondii*. The results of this study also showed no positive correlation between *Toxoplasma* and Hs - CRP levels. This study was done on acute - phase proteins, which matched our results. Birjis - Tadri conducted a similar research in relation to Hs - CRP and the results were also not significant, which was consistent with our results (23). A study conducted on pigs showed that haptoglobins and CRP, which are acute - phase proteins, are variable during toxoplasmosis and showed no significant relationship between acute - phase proteins and toxoplasmosis. These changes had first an ascending and then a descending trend (24). In a study on toxoplasmosis in mice in 2001, Clark and colleagues showed that mouse trophoblasts secrete a factor named fibrinogen like protein 2 (FLP - 2). This prothrombotic factor converts fibrinogen into fibrin in response to pro - inflammatory cytokines, such as IFN - γ and TNF - α (25). Given that one of the transmission methods of toxoplasmosis is transmission from placenta to embryo, the diagnosis of this disease during pregnancy is of great importance. It is also essential to know the fibrinogen in pregnant women who are at a risk of abortion, as increased fibrinogen levels in pregnant women occurs physiologically. Given the importance of toxoplasmosis and high levels of fibrinogen in pregnant women, the present study, conducted for the first time, showed no significant relationship between toxoplasmosis and increased levels of fibrinogen. It is suggested that future studies investigate other acute phase proteins associated with toxoplasmosis in different communities (especially in those with impaired immune systems) and use PCR method beside IgG avidity for confirmation of acute toxoplasmosis. To get a more accurate data, studies should be done with a larger sample, which was out of time our study.

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Footnote

Conflicts of Interest: We declare no conflicts of interest.

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