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# *Toxoplasma gondii* specific IgG avidity assay: Role and implication in the confirmatory diagnosis of acute toxoplasmosis in seropositive pregnant women

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## Abstract

This study was undertaken to apply *Toxoplasma gondii* specific IgG avidity test in seropositive pregnant women to differentiate acute and past infection. *T. gondii* specific IgG avidity test was conducted in 39 seropositive pregnant women and their pregnancy outcomes were observed later on. Out of 39 *T. gondii* seropositive pregnant women 33 (84%) were only IgG positive and 6 (15.4%) were both IgG-IgM positive. All the IgG positive cases (100%) and 2(33.3%) IgG-IgM positive cases had high avidity antibodies and they gave birth to healthy babies. Rest of the 4 (66.7%) IgG-IgM positive women had low avidity and 50% of them had abortion and 50% gave birth to unhealthy babies. This reveals that the seropositive mothers having high IgG avidity had past infection and no risk of congenital transmission. Seropositive mothers having low IgG avidity had acute infection and so congenital transmission occurred. Presence of *T. gondii* specific IgG and IgM antibody does not indicate acute infection always. IgG-IgM positive pregnant women should be further evaluated by IgG avidity assay to confirm acute infection.

## Introduction

*Toxoplasma gondii* is an intracellular protozoan parasite of the phylum Apicomplexa, subclass coccidian.[1] Most immunocompetent individuals infected with this parasite remain asymptomatic or might experience nonspecific flue like symptoms including fever, headache, muscle pain, and lymphadenopathy.[2] However, primary infection during pregnancy lead to vertical transmission, followed by fetal infection resulting in grave consequences like abortion, intrauterine death, still birth and various types of congenital anomalies.[3] Rapid and early diagnosis of *T. gondii* infection during pregnancy can prevent aforesaid complications.

Clinical based diagnosis of toxoplasmosis in pregnant women is very difficult as they often remain asymptomatic or may have mild symptoms. Therefore, the diagnosis of acute *T. gondii* infection is most commonly made by serological methods based on the demonstration of a significant increase in specific IgG antibody levels or the presence of specific IgM antibodies in serum[4]

But, the greatest challenge is to confirm acute *Toxoplasma* (primary) infection and distinguish it from past (chronic) infection which is of utmost importance in evaluating the risk of fetal transmission, initiating antibiotics, and providing appropriate counseling.[5]

Though IgM positivity is a marker of acute infection in many infectious diseases, *T. gondii* specific IgM is not a true positive indicator of acute toxoplasmosis because of its sustained persistence for months or even years. This may result misdiagnosis and unnecessary interventions in pregnant women who were infected long ago and developed immunity. In this situation an assay measuring the antigen binding avidity of IgG antibodies presents a revolutionized serological technique for the diagnosis of toxoplasmosis in distinguishing between acute and past infection.[6,7]

This study was aimed to observe the utility of *T. gondii* specific IgG avidity test in differentiating acute and past infection among seropositive pregnant women for the assessment of the risk of mother-to-child transmission.

## Materials and Methods

The study was carried out from August 2013 to July 2014. Serum samples were collected from a total of 39 *T. gondii* seropositive pregnant women and subjected to IgG avidity test by ELISA. Later on pregnancy outcomes of these cases were observed and congenital transmission was confirmed by detecting *T. gondii* specific IgM antibody in cord blood collected at

the time of delivery by chemiluminescence.

### *IgG Avidity by ELISA*

This method is based on the strength of specific IgG binding to the multivalent *T.* antigen. This binding strength is called IgG avidity. In this assay, the hydrogen-bond disrupting agent, urea, is used to elute IgG from the immobilized antigen. As a result, the low affinity antibodies produced at an early stage of infection are separated from those with a higher binding affinity that reflect past immunity.

Microtiter strip wells coated with *Toxoplasma* antigen are incubated with diluted serum specimen (dual pipetting). After washing one well is incubated with avidity reagent and the corresponding well with washing buffer. In this step the low avidity antibodies are removed from the antigens whereas the high avidity ones are still bound to the specific antigens. Anti human IgG labeled with peroxidase is added. The immuncomplex is visualized with TMB to give a blue reaction product. Stop solution is added to stop the reaction and changing the color of the reaction product into yellow. Absorbance at 450 nm is read using an ELISA microwell plate reader.

## Results

A total of 39 *T. gondii* seropositive pregnant women were subjected to *T. gondii* specific IgG avidity assay and their pregnancy outcomes were observed. Among the 39 seropositive cases, 33 (84.6%) were only IgG positive and 6 (15.4%) were both IgG and IgM positive. No single IgM positive cases were found (Table I). Among the 39 seropositive patients, all the 33 only IgG positive cases had high IgG avidity. Out of 6 both IgG-IgM positive cases, 4 cases had low IgG avidity and 2 cases had high IgG avidity.

All the IgG positive mothers with high avidity gave birth to apparently healthy baby. 2 IgG-IgM positive mothers with high IgG avidity also gave birth to apparently healthy baby. Anti-toxoplasma IgM was negative in their cord blood. Out of 4 IgG-IgM positive mothers with low IgG avidity, 2 (50%) women underwent abortion and 2 (50%) gave birth to unhealthy babies with congenital anomalies; one gave birth to hydrocephalus baby and the other gave birth to a baby with jaundice and hepato-splenomegally. Cord blood of these two unhealthy babies were positive (100%) for anti *Toxoplasma* IgM.

**Table I**

**Pregnancy outcome of seropositive pregnant women**

| Antibody status of mothers | Avidity | | Outcome | | | IgM antibody in cord blood |
|---|---|---|---|---|---|---|
| | | | Apparently healthy baby | Abortion | Congenital anomalies | |
| IgG positive | High | 33 (100) | 33 (100) | 0 | 0 | 0 |
| IgG-IgM positive | High | 2 (33.3) | 2 (100) | 0 | 0 | 0 |
| | Low | 4 (66.7) | 0 | 2 (50) | 2 (50) | 2 (100) |

## Discussion

*T. gondii* infection during pregnancy is a serious threat to the fetus. In Bangladesh, *T. gondii* seropositive pregnant women was reported 11.8% to 24.3%.[8,9] In this study, 84.6% IgG positive and 15.4% IgG-IgM positive cases were found which is not negligible. The parasites can be transmitted to pregnant women through contaminated food and water. Reproductive wastages due to toxoplasmosis can only occur when a previously unexposed women acquires toxoplasmosis during pregnancy.

The risk of infection transmission and risk of severity of fetal demise are inversely correlated to gestational ages.[10] Maternal infections during the first trimester lead to gross fetal developmental anomalies, such as hydrocephalus, anencephaly, microcephaly, macrocephaly, spina bifida, etc. Infection during second trimester may lead to spontaneous abortions, stillbirths, intrauterine growth retardation and preterm deliveries. Conversely maternal infection in the third trimester often results in asymptomatic newborns. However, if not treated appropriately, these newborns might develop retinochoroiditis and neurological deficits like delayed motor skillness, feeding difficulty, hearing impairment, vision problems, seizures and mild to severe mental retardation during the first two decades of life in childhood or early adulthood[11]. Therefore, early and rapid diagnosis of toxoplasmosis is essential in pregnancy to prevent these fatal bad obstetric outcomes.

Toxoplasmosis is routinely diagnosed by the detection of anti-*Toxoplasma* IgM and IgG antibodies but it does not make a clear distinction between acute and past infection. In serological screening, the absence of IgG and IgM antibodies indicates no infection. Presence of only IgG antibody reveals past infection. Presence of only IgM antibody suggests acute infection. But presence of both IgG and IgM antibody suggests either acute infection or chronicity or past infection because of the sustained persistence of specific IgM antibodies for months or even years.[12]

Following acute infection, IgM antibody titres rise starting on day 5 and reach the maximum level at 1 to 2 months. In contrast, IgG antibodies are usually detectable within 1 to 2 weeks after acute infection, peak within 12 weeks to 6 months, and usually remain detectable throughout life. At this point, IgM antibodies decline more rapidly than IgG antibodies and eventually disappears. However, in many *Toxoplasma* infected cases, the IgM antibodies persist for years following acute infection.[12] It was also reported that IgM antibodies have been detected as long as 12 years after the acute infection.[13] Persistence of these IgM antibodies does not appear to have any clinical relevance. To overcome this problem *T. gondii* specific IgG avidity test has been recommended to confirm acute *T. gondii* infection in IgG-IgM positive cases in many studies.[6,7,14]

In the current study, IgG avidity assay by ELISA was conducted in 39 seropositive cases. Specific IgG of low avidity is an indicator of acute *T. gondii* infection and high IgG avidity indicates past infection or immunity.[7] In this study, it was observed that all 33 (100%) IgG positive (without IgM) cases had high avidity antibodies suggesting past infection and there is no risk of congenital transmission. On the other hand, among 6 (15.4%) IgG-IgM positive women 4 (66.7%) cases had low avidity IgG antibodies suggesting acute *T. gondii* infection and risk of congenital transmission is more in these cases. But rest of the 2 (33.3%) IgG-IgM positive women had high avidity antibodies suggesting that in these cases, the infection acquired in the past and they are immune to congenital transmission. Therefore, the presence of specific *T. gondii* IgM antibodies is not always an indication of a recent infection and these two cases can be considered in the chronic stage of infection.

In the follow-up, pregnancy outcomes of these patients also support the interpretation of IgG avidity test. All the IgG positive mothers and 33.3% IgG-IgM positive mothers with high avidity antibodies gave birth to healthy babies confirmed by the absence of anti-toxoplasma IgM in their cord blood. Rest of the 66.7% IgG-IgM positive mothers with low avidity antibodies had bad obstetric outcomes. 50% of them had abortion and 50% of them gave birth to *Toxoplasma* infected babies confirmed by the presence of IgM in their cord blood. One of them was hydrocephalus baby and the other had jaundice with hepatosplenomegally.

## Conclusion

Toxoplasmosis is not an uncommon disease in our country and serological screening for toxoplasmosis should become a routine diagnostic test in every pregnant women during antenatal check up in our country. Routine serological test solely cannot establish acute infection. So, IgG avidity test must be done after serologic screening. Presence of only IgG antibody indicates past infection and so IgG avidity assay is not required for these cases. If a pregnant woman is IgG-IgM positive, further evaluation should be done by IgG avidity assay for the assessment of fetal risk of transmission which is important for making decision regarding therapeutic intervention.

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