Assessment of toxoplasma IgG avidity test results in pregnant women

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Infection brought about by *Toxoplasma gondii* (*T. gondii*), toxoplasmosis, is transmitted to the fetus via the placenta. Infection may progress in the expectant mother totally asymptomatically. However, such infections are particularly significant because of undesired consequences for the fetus. Immunoglobulin G (IgG) avidity tests, which have come into use in recent years, make it possible to discriminate reliably between primary acute infections and reactivations, reinfections, or both, with a single serum sample. This discrimination has clinical value, particularly in pregnant women and immunosuppressive patients. Because appropriate use is not generally made of standard procedures and the time periods required by these in the immunoserological diagnosis of many infective illnesses, numerous difficulties are encountered in diagnosis and treatment. In general, the titration of different antibodies, and increases in the titer, are considered significant in immunoserological diagnosis. The determination of this requires a large amount of time. In the present study, IgG avidity tests, which have recently gained currency, will be taken into consideration and compared with classic methods in terms of optimal usage and early treatment.[1]

Sera from 879 women in the third trimester of pregnancy who attended the Obstetrics and Gynecology policlinic of the Dicle University Faculty of Medicine between 9 April 2002 and 9 May 2003 were evaluated for Toxoplasma IgG and IgM by the classic enzyme linked immunosorbent assay (ELISA) technique (Cobas Core II, Roche, USA). In patients determined to be positive for IgM, the same test was repeated the following week with new serum samples. Sera testing positive for both IgM and IgG and sera testing positive for IgG alone were stored at -20°C. Avidity tests (IgG avidity EIA Well; Radim, Italy) were performed with commercial kits. Results were interpreted as follows: avidity of greater than 30% was considered to be high, 20-30% was considered medium (gray region), and below 20% was considered low.

A total of 879 serum samples taken from pregnant women in their third trimester were evaluated for Toxoplasma IgM and IgG by the classic ELISA technique. Of these sera, 21 (3.1%) tested positive for Toxoplasma IgM and IgG, and 305 (45.0%) for IgG alone. In patients found positive for both Toxoplasma IgM and IgG, the ELISA test was repeated the following week with new serum samples, and the same results were obtained. All sera positive for both IgM and IgG, and as many of those positive for IgG alone as permitted by the number of kits, were analyzed by the IgG avidity test. In the evaluation of *T. gondii* avidity test results (**Table 1**), it was determined that 12 of the 21 patients testing positive for both IgM and IgG had a low avidity index (AI) (<20%), 6 had a medium AI (20-30%), and 3 had a high AI (>30%), and that only one of the patients testing positive for IgG alone had a low AI while the remaining 156 had high AIs.

While primary Toxoplasma infections occurring in the first trimester of pregnancy may lead to severe malformations, this risk is minimal or nonexistent in infections occurring through reinfection, reactivation, or both.

Hernanadez et al[2] used the Vidas system to confirm positive results for IgM with ELISA in 89 serum samples collected from 1999 to 2002 in 20 patients suspected of toxoplasmosis. The IgG avidity was determined by the Liaison system. It was found that the sera taken from all but 9 of 20 IgM-positive patients had high IgG avidity, demonstrating the importance of the IgG avidity in excluding acute toxoplasmosis.

Basiak et al[3] tested 58 women with nodular toxoplasmosis and 34 women diagnosed with asymptomatic *T. gondii* infection for *T. gondii* antibodies at different times after the onset of infection using immunofluorescence assay (IFA), ELISA IgM, ELISA IgA, and IgG avidity tests. They determined that IFA had 76.2% sensitivity and 31-67% specificity, and that IgG avidity had 100% sensitivity and 41.7% specificity. It was found that all methods evaluated had only a limited value in the

Table 1 - The total number of sera with *T. gondii* and IgG avidity results.

Immunoglobulin	AI%<20 n (%)	AI%20-30 n (%)	AI%>30 n (%)
IgM(+)IgG(+)	12 (100) (7.1)	6 (28.6) (14.3)	3 (14.3)
IgM(-)IgG(+)	159 (100) (92.9)	2 (1.3) (85.7)	144 (98.1)
Total	**180 (100) (92.2)**	**8 (4.4) 159 (88.3)**	**147**

EIA - enzyme immuno assay, AI - avidity index, IgG - immunoglobulin G, IgM - immunoglobulin M

differentiation of early and toxoplasmosis. The ELISA IgA had no advantage over IgG or IgM, IgG being the most specific and sensitive method in such differentiations.

Sobieszczanka[4] evaluated the sera of a total of 80 serum samples, 47 from patients suspected of having acute toxoplasmosis, 23 from pregnant women, and 10 from healthy blood donors. They used ELISA to test for IgM, IgA, and IgG antibodies and IgG avidity. Thirty-four (42.5%) of the 80 serum samples were found positive for IgM antibodies, of which 22 (64.7%) had low IgG avidity and IgA antibodies, confirming acute toxoplasmosis. The IgA antibodies were not present in the other 12 sera, and had high avidity despite the fact that 3 of these had low avidity and 9 had IgM. The 46 serum samples not testing positive for IgM or IgA antibodies were evaluated for avidity, and high-avidity IgG antibodies were found, an indication of chronic infection.

Liesenfeld et al[5] evaluated 125 serum samples from 125 pregnant women. It was found that 52 of 93 testing positive or suspicious on IgM had high-avidity antibodies, suggesting the possibility that they were infected before gestation. Spiramycin was given to 40 women to prevent congenital transmission, and as a result, high-avidity antibodies were found in 7 cases (17.5%).

If only the avidity test is used as a base in moderate or low avidity test results, especially ELISA IgM (-) results clearly indicating a chronic pattern, the results for these sera will be wrongly interpreted as conforming to a newly acquired infection. The IgG avidity test is beneficial in sera in which IgM results are doubtful on ELISA. The interpretation of uncertain results is difficult in all of the tests, and a new serum sample is generally required. Even so, it is impossible to interpret some samples definitively. Results suspicious for IgM may lead to unnecessary interventions such as Spiramycin usage or amniocentesis. It is recommended that such cases are followed with tests at intervals, that Spiramycin therapy is implemented, and that amniotic fluid is analyzed by polymerase chain reaction. If there are suspicious results on the ELISA IgM test in a woman in the 16th week of gestation, the IgG avidity test will yield a reliable result.[1]

Cozan et al[6] reported that the IgG avidity test may be misleading if used alone in sera with antibodies of low- or medium-avidity antibodies testing negative for IgM on ELISA. It has been determined that 40% of women testing negative on IgM ELISA have antibodies of low or medium avidity, and that most of these results are consistent with chronic infection. When the avidity test is used alone in such patients, there is the danger of an incorrect interpretation suggesting an acute infection.

Although the data present avidity tests as a supplementary confirmation method, they should not be the only test used in such cases because low- or medium-avidity antibodies may lead to an incorrect interpretation. It should be known that avidity tests are only for confirmation in the examination of pregnant women.[6]

In the present study, low avidity, an indication of acute infection, was found in 12 (57.1%) of 21 patients testing positive on ELISA for *T. gondii* IgM and IgG; medium avidity (the gray region) in 6 (28.6%); and high avidity an indication of a previous infection in 3 (14.3%). High avidity was determined in 156 (98.1%) of 159 serum samples testing positive on ELISA for *T. gondii* IgG alone, medium avidity in 2 (1.3%), and low avidity in 1 (0.6%). In interpreting these results, a hasty inference made from the low avidity values would be wrong, as it might lead to unnecessary anxiety for the pregnant woman and even needless abortion. It should be taken into consideration that avidity maturation takes time, and that the test should be repeated at intervals with new serum samples, and that other methods such as polymerase chain reaction analysis of amniotic fluid should be employed.

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