The Journal of Microbiology, September 2004, p.211-215
Vol. 42, No. 3

Performance of the Immunoglobulin G Avidity and Enzyme Immunoassay IgG/IgM Screening Tests for Differentiation of the Clinical Spectrum of Toxoplasmosis

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(Received June 3, 2004 / Accepted August 7, 2004)

Toxoplasmosis has been well known as an important human infection to consider especially in pregnant women. Although many serologic methods are available, the diagnosis of toxoplasmosis can be extremely difficult. The presence of increased levels of *Toxoplasma*-specific IgG antibodies indicates an infection, but it does not differentiate between a recent and past infection. The purpose of our study was to compare the performance of the ELISA *T. gondii* IgG/IgM test, a widely used enzyme-linked immunosorbent assay, to the ELISA IgG avidity method. One hundred and four serum samples (from 38 males and 66 females) were tested and evaluated from symptomatic patients (chorioretinitis, lymphadenopathy), and from women in their first trimester of pregnancy who were suspected of having toxoplasmosis. The high IgG avidity and ELISA IgG antibody levels were in agreement for 51 of the specimens (49.0%). Thirty-eight discrepant (borderline) results from the IgG avidity method were positive for IgM (3 specimens) and IgG (37 specimens). Interestingly, out of the eight serum samples that were positive for both IgG and IgM antibodies, two samples were low IgG avidity, and three samples were borderline. There was no statistically significant relation observed between the results of the IgG avidity method and the ELISA IgG test, and the IgG avidity method and ELISA IgM test (χ^2=1.987; p=0.370 and χ^2=2.152; p=0.341, respectively). The IgG avidity method was considered easy to perform and an acceptable approach for the differentiation of discrepant results (recent/chronic) and for the current detection of *T. gondii* antibodies. We concluded that the determination of IgG avidity is a helpful tool for the diagnosis of the ocular form of toxoplasmosis and it is a safe method for screening this disease in the first trimester of pregnancy.

Key words: *Toxoplasma gondii,* avidity, serology, diagnosis, ELISA

The overall laboratory evidence of an infection of *Toxoplasma gondii,* at a prevalence rate of 23% (Jones *et al.,* 2001), emphasizes the scope of toxoplasmosis in a community, and it explains the heavy burden of morbidity due to this parasitic disease. It was believed that congenital toxoplasmosis results from a primary infection acquired during pregnancy (Montoya, *et al.,* 2002), but not from the reactivation of a latent infection in immunocompetent pregnant women (Vogel *et al.,* 1996; Wong and Remington, 1994). In addition, it was believed that latent toxoplasmosis could reactivate and cause a congenital transmission of the parasite to infants who then become infected *in utero* (Kodjikian, *et al.,* 2004). Recently, it has been discovered that IgG avidity tests can provide confirmatory evidence of an acute infection and they can distinguish reactivations from primary infections with a single serum specimen. This is of particular value for pregnant and immunosupressed patients (Lappalainen *et al.,* 1995; Auer *et al.,* 2000; Marcolino *et al.,* 2000; Liesenfeld *et al.,* 2001; Prince and Wilson, 2001). The decision to offer a microbiology laboratory in a clinical setting depends on the availability of reliable, cost-effective, easy-to-perform, and rapid diagnostic tests. The aims of this present study were twofold: (i) to determine the prevalence of toxoplasmosis and (ii) to determine the performance of the IgG avidity method for the detection of anti*Toxoplasma* antibodies.

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This manuscript was presented in part at the 3rd Balkan Military Medicine Congress, Athens, Greece, October 6-10, 2002.

Materials and Methods

Study population

A total of 104 serum samples from patients were submit-

ted to the Gulhane Military Medical Academy (GMMA) Division of Medical Parasitology in Ankara, Turkey between September 1999 and December 2000. The sera, which consisted of the blood serum of patients with chorioretinitis (n=11), women in their first trimester of pregnancy (n=37), and patients with lymphadenopathy without a specific diagnosis (n=56), were tested routinely by our laboratory.

IgG/IgM ELISA.

IgG anti-*Toxoplasma* antibodies were determined by a commercially available enzyme-linked immunosorbent assay (ELISA) (*Toxoplasma gondii* IgG; Meddens Diagnostics, The Netherlands). Results were expressed in the intenational units per milliliter and were considered positive for titers of >10 IU/ml. Anti-*Toxoplasma* IgM antibody titers were assessed by the IgM ELISA test (Euroimmun *T. gondii* IgM; Germany).

IgG avidity assay.

The determination of *T. gondii* IgG antibody avidity was carried out using a commercial kit according to the manufacturer's instructions (*Toxoplasma* IgG avidity EIA, Italia). The ELISA *T. gondii* IgG test was run and the results were interpreted as suggested by the manufacturer. Serially diluted sera were briefly placed in microtiter wells coated with the *T. gondii* antigen. Plates were incubated for 1 h at 37°C and washed. After a dissociating reagent (urea solution in a buffer) was added, the horseradish peroxidase-conjugated anti-human IgG antibody was added to each well. The plates were then incubated, washed, and further incubated with a chromogenic substrate solution (Tetramethylbenzidine, TMB). This commercial test is based on the difference between the absorbance values due to antibody binding in the absence and presence of a urea solution. The optical density (O.D.) at 405 nm was measured with an automatic microplate spectrophotometer (Bio-Tek Instruments Inc., USA). The IgG avidity index (AI) was calculated as the ratio between the O.D. of the two wells [for the sample washed with the dissociating reagent (urea solution in a buffer) and the O.D. for the sample washed with a standard washing solution (PBS-Tween 20 buffer)], and then it was expressed as percent avidity. An AI of >30% was considered an indicator of IgG anti-*Toxoplasma* with high avidity, an AI of <20% indicated low avidity, and an AI between 21 and 30% suggested borderline avidity (grey zone), respectively (according to the manufacturer's instructions).

Statistical analysis.

The statistical analysis of the results was done with the SPSS for Windows (SPSS Inc., release 10.0, USA) statistical package. The Chi-Square test was also used for the evaluation of the significance of the relations observed between the tests. Pearson coefficients of the correlations were calculated. P values were set at $p \leq 0.05$.

Results

Comparative results of the ELISA IgG/IgM and IgG avidity test are demonstrated in Table 1. Of the 104 specimens evaluated by these tests, 100 were positive for IgG anti-*Toxoplasma* antibodies, of which 12 specimens had low avidity. Four of the 37 pregnant women in their first trimester had IgG low-avidity, but only two of these women were positive for IgM. Interestingly, two of the 11 chorioretinitis cases had low-avidity, but did not test positive with the IgM ELISA method. When 56 specimens of the patients with lymphadenopathy were tested by the IgG avidity test, six had low-avidity. Of these six specimens, two were positive by both the IgM and IgG ELISA test. Results of the serological tests, according to the clinical characteristics of the study population, are shown in Table 2. In the present study, the relations between both of the comparative results of the IgG avidity and IgM test, and of the IgG and IgM level were not significant from a statistical point of view ($\chi^2=2.152$; $p=0.341$ and $\chi^2=1.384$; $p=1.000$, respectively). Also, there was no statistically significant relation between the results of the IgG ELISA and IgG avidity test ($\chi^2=1.987$; $p=0.370$). The ELISA IgM test missed four specimens of the lymphadenopathy cases that had low-avidity. A similar performance was obtained in two chorioretinitis cases. The relations between the combinations of the IgG avidity and ELISA IgM assay, and of the IgG avidity and ELISA IgG assay were, however, not statistically significant ($p=0.406$ and $p=0.953$, respectively). The contingency

Table 1. Comparison of the results of the IgM ELISA, IgG ELISA and IgG avidity tests

IgG Avidity	IgM ELISA		IgG ELISA	
	Positive (n=8)	Negative (n=96)	Positive (n=100)	Negative* (n=4)
Low	2	10	12	0
Borderline	3	35	37	1
High	3	51	51	3

Table 2. Results of the serological tests according to the clinical characteristics of the study population

Clinical Manifestations	Gender		IgG Avidity			IgM ELISA		IgG ELISA		Mean age in years (Range)
	Male	Female	Low	Borderline	High	Positive	Negative	Positive	Negative*	
Chorioretinitis	10	1	2	1	8	–	11	11	–	39.7(20-72)
Pregnant women	–	37	4	14	19	2	35	35	2	26.8(18-38)
Lymphadenopathy	28	28	6	23	27	6	50	54	2	28.9(0-84)

*IgG ELISA and IgG avidity assay were run simultaneously.

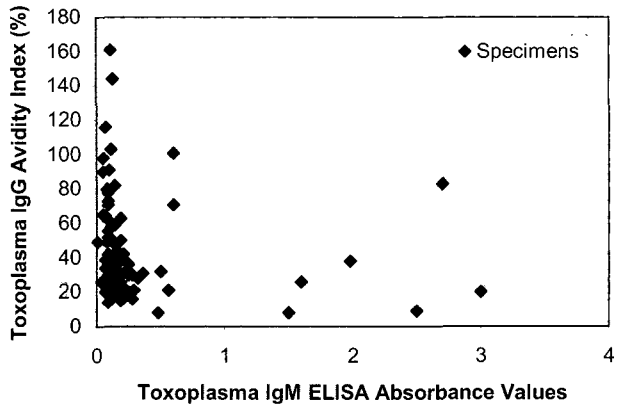

Fig. 1. IgG avidity index (%) and absorbance values of ELISA IgM tests for 104 patients suspected with toxoplasmosis.

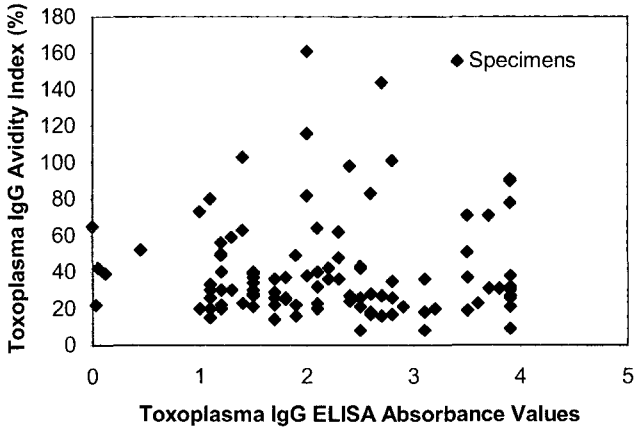

Fig. 2. IgG avidity index (%) and absorbance values of ELISA IgG tests for 104 patients suspected with toxoplasmosis.

coefficients between avidity and the IgG and IgM level were not statistically significant (φ=0.184; p=0.161 and φ=0.085; p=0.685, respectively). Fig. 1 compares the results of the IgM ELISA test with the IgG avidity test. Similarly, Fig. 2 compares the results of the IgG ELISA test with the IgG avidity test. The mean titers of *T. gondii*-specific IgM, IgG, and IgG avidity antibodies were 0.27±27.60, 2.20±0.51, and 40.47±1.02 in the serum samples, respectively.

Discussion

Measuring the avidity of a specific IgG antibody was demonstrated to be particularly useful for the purpose of this study. As a matter of fact, antibodies characterize the initial IgG antibody response to an infection with low avidity, in which binding to the specific antigen sites is easily dissociated (Lappalainen *et al.*, 1993; Liesenfeld *et al.*, 2001). The avidity of *Toxoplasma* specific IgG antibodies was best measured by dissociating the antibody from the antigen using a urea solution. The present application appears to be sufficiently simple technically to be used as a clinical diagnostic test for the maturation kinetics of avidity. Upon using the *Toxoplasma* IgG avidity EIA WELL kit, a low (<20% for the test) index was obtained for 12 of the 104-serum samples taken from the toxoplasmosis suspected patients. The mean avidity index of sera was 40.47%. On the other hand, the avidity of IgG, unlike its concentration, remained high once it matured. Because of this, a low avidity index would suggest a recent primary infection.

Infections with *T. gondii* during early pregnancy may frequently lead to many intrauterine malformations (Montoya and Remington, 2000). The detection of anti-toxoplasma antibodies by ELISA methods is commonly performed in many medical centers. The results of such tests are generally well accepted by clinicians because of their excellent sensitivities and specificities, the rapid availability of results, and the relatively low costs of the tests. It is important to understand that a single serologic test is not enough for the diagnosis of toxoplasmosis. The impact of toxoplasmosis on immunocompromised adults has been recognized, but an evaluation of the laboratory diagnostic methods for these high-risk patients has not been systematically examined. A laboratory diagnosis of acute toxoplasmosis is almost always based on either the seroconversion of IgG, or on the existence of positive anti-*Toxoplasma* IgM antibodies (Liesenfeld *et al.*, 1996). The diagnosis of a primary *Toxoplasma* infection in immunocompetent adults is accomplished by serologic methods. Anti-*Toxoplasma* specific immunoglobulin IgM is a sensitive indicator of an ongoing or recent infection (Pelloux *et al.*, 1998). Many studies have described *Toxoplasma* IgG avidity tests (Jenum *et al.*, 1997; Pelloux *et al.*, 1998; Paul, 1999) that are used to differentiate an acquired infection from a distant infection, because IgM antibodies may persist for months or even years after the primary infection (Marcolino *et al.*, 2000; Gras *et al.*, 2004).

In the present study, we retrospectively evaluated the use of different laboratory methods for the diagnosis of toxoplasmosis in symptomatic adults (chorioretinitis, lymphadenopathy, and women in the first trimester of pregnancy) that had been admitted to outpatient clinics. The data from these evaluations indicate that the ELISA IgG avidity test is an excellent method for the differentiation of an acute or primary reactive infection in individuals. Low- avidity IgG was determined in only two of four pregnant women that had the IgM and IgG antibodies for anti-*Toxoplasma*. The other two women who had low avidity IgG (negative for IgM) continued their pregnancies. No signs of toxoplasmosis were found in any of the fetuses, and no congenitally infected newborn was documented. When the IgG avidity results were compared with those of the IgM ELISA test in regard to the serum samples taken from subjects with lymphadenopathy, 16% of the IgM ELISA positive serum samples had high IgG avidity, thus, fundamentally ruling out a recent infection.

These differences were not statistically significant, but the overall performance of the ELISA IgG avidity testing was more informative than when only using a single serologic test for the outpatient group. The single avidity index may be diagnostic, but only when the level of the specific IgG antibody is taken into consideration. The reasons for the discrepancies between the two tests (IgG avidity and IgM) are almost uncertain; however, some reasons may include the differences in the antigen preparation and method, and in the selection of sera used to set up the cutoff between both the control positive and negative sera. The IgG avidity test also produced noticeably higher numbers of borderline (equivocal) results when compared with those of the IgM ELISA test.

Because IgM antibodies can be detected for many months or even years following the acute phase of an infection in some individuals, the presence of IgM antibodies is not always an indication of a recent infection (Marcolino *et al.,* 2000). On the other hand, the presence of specific *T. gondii* IgM antibodies in the chronic stage of an infection, and false-positive IgM positivity results can lead to and result in needless concern and a misdiagnosis (possibly affecting the decision to abort) particularly in pregnant women (Liesenfeld *et al.*, 1997; Montoya *et al.,* 2002). Since the U.S. Food and Drug Administration (FDA) has recommended that a solely positive IgM test result should undergo confirmatory testing, avidity specific *T. gondii* IgG tests have been presented to differentiate between recently acquired and distant infections (Montoya *et al.,* 2002).

Because most reports in the literature do not consider the overall precision of IgM antibodies detecting tests, the actual numbers of false-positive results might be underestimated (Liesenfeld *et al.,* 1997).

In the present study, our findings recommend that the avidity test should not be used as the only assenting test for patients, particularly pregnant women, suspected with IgG and / or IgM antibodies due to a possible misinterpretation of low- or borderline avidity antibody results.

As shown in Table 1, three samples (IgM positive) had high AI as the detecting test. So, the avidity test could not determine IgG antibodies with low avidity. This is probably due to the time interval of maturation of *T. gondii* IgG antibodies, which catch a parasite load so low as to be cleared by the immune system. After the appropriate time (for example 1 month later), AI might be found to have low anti-*T. gondii* IgG levels. Likewise, the 35 IgM negative samples were found to have borderline avidity. The other reason for the failure of the avidity test to detect IgG antibodies might well be that during a short *T. gondii* parasite exposure time, only the high avidity receptors will bind to the antigen and be activated to separate and distinguish. So, the approach by which a primary *T. gondii* infection may have an effect on antibody maturation can be related to the long perseverance of *T. gondii* in the blood. For an understanding of the importance of low avidity for the detection of early toxoplasmosis, blood samples should be serially collected from each sample at a different point in time.

In conclusion, the early detection of anti- *T. gondii* antibody avidity is a valuable and useful tool to use for identifying positive IgM, particularly in pregnant women, however, this was not significantly proven. Much more importantly, it is necessary not to forget that avidity tests are exclusively confirmatory tests used in the follow-up phase of patient care, and the data provided by these tests aids the diagnosis of toxoplasmosis in pregnant women (Montoya *et al.,* 2002).

Acknowledgement

We thank Yavuz Sanisoglu, Ph.D., from the Department of Biostatistics, GMMA for his assistance with statistical analysis.

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