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Detection of Toxoplasmosis in Pregnant Women's In Sabratha City of Using VIDS

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Abstract: Toxoplasmosis is usually asymptomatic, but can have severe consequences if It occurs in immunodeficient subject or fetuses. The diagnosis of toxoplasmosis during pregnancy is often based on maternal serological testing for IgM and IgG Anti- Toxoplasma antibodies. Persistence of IgM for long periods, posses' problems in distinguishing acute from chronic infection. The evaluation of specific IgG avidity enables more accurate dating, since avidity rises progressively during the course of infection. Test that measures immunoglobulin G (IgG) is used to determine if a person has been infected. If it is necessary to try to estimate the time of infection, which is of particular importance for pregnant women, a test which measures immunoglobulin M (IgM) is also used along with other tests such as an avidity test. Toxoplasma-specific IgG antibodies are detectable 1-3 weeks after infection and remain detectable for the life of the individual. Toxoplasma-specific IgM antibodies are also detectable 1-3 weeks after infection but generally decline to nil by one year after infection. The VIDAS® TOXO IgM (TXM) assay is intended for use on the instruments of the VIDAS family (VITEK® Immunodiagnostic Assay System) as an automated enzyme-linked fluorescent immunoassay (ELFA) for the presumptive qualitative detection of anti-Toxoplasma gondii IgM antibodies in human serum. Seventy-six (76) women in the first 16 weeks of pregnancy were tested for VIDAS IgM, IgG antibodies and VIDAS Toxo-IgG avidity. Low avidity antibodies were demonstrated in 2 (33.3%) of 6 sera positive with IgM assay and 4 (12.12%) of sera positive with IgG assay. Low avidity was also detected in 2 (3.27%) of 61 sera negative with IgM. The low avidity suggesting a recent infection, while high avidity in 3 (50%) of the 6 positive IgM and 24 (72.72%) of 33 positive IgG indicating that the Infection acquired in the distant past. These findings highlight the value of VIDAS IgG avidity when used in combination with the VIDAS IgM and IgG assays to provide a confirmatory evidence of an acute infection with a single serum specimen for pregnant women.

Keywords: Toxoplasmosis, IgM and IgG Anti- Toxoplasma antibodies, VIDAS IgG, VIDAS IgM

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INTRODUCTION

Toxoplasmas gondii has a worldwide distribution and one of the most prevalent Infectious agents in human. Acute infection is usually asymptomatic. However, it was believed that congenital toxoplasmosis results from a primary infection acquired during pregnancy [1], but not from the reactivation of a latent infection in immunocompetent pregnant women [2], whereas Kodjikian *et al*., [3], believed that latent Toxoplasma could reactivate and cause a congenital transmission of the parasite to fetuses.

Detection of *Toxoplasma gondii* specific antibodies is the most common approach used to identify toxoplasmosis.

Unfortunately, classic serological approaches are usually not useful for distinguishing recent from past toxoplasmosis. The tendency of specific IgM to persist for a long time even at high level has been verified in several studies [4, 5]. It has been discovered that IgG avidity tests can provide confirmatory evidence of an acute infection and they can distinguish reactivation from primary infections with a single serum specimen for pregnant and immunosuppressed patients [6, 7]. Thus, IgG produced within the first few months following primary infection exhibits low avidity, whereas IgG produced several months or years later exhibits high avidity [8].

A high IgG avidity test result with the more recently developed VIDAS method; is available commercially in Europe and has been stated to rule out that infection occurred during the prior 4 months, Thereby, extending the value of avidity testing to the fourth month of gestation [9].

The aim of this study is to determine the performance of IgG avidity method for the detection of anti-Toxoplasma antibodies in pregnant women during the first 16 weeks of gestation.

MATERIAL AND METHODS

Study Group

Sera samples of 76 women in the first 16 weeks of gestation were studied. The sera were submitted to outpatient clinics, between August 2022 and January 2023. The assay was carried out at the Central Health Laboratory in Libya/city Sabratha.

VIDAS Toxoplasma IgM and IgG Assay

The sera had been tested with VIDAS IgM and IgG tests. They are automated quantitative tests in human serum or plasma using the ELFA technique (Enzyme linked fluorescent assay). The assay principle combines a twostep enzyme immunoassay sandwich method with a final fluorescent detection. The solid phase receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready to use and predispensed in the sealed reagent strips.

All of the assay steps are performed automatically by the instrument after a sample dilution step. The reaction medium is cycled in and out of the solid phase receptacle (SPR) several times. Measurements of IgG and IgM antibodies were performed and interpreted according to the direction of the manufacturer of VIDAS Toxo IgM and IgG kit: bioMerieux, Marcy-I'Etoil, France). Once the assay is completed, results are analyzed automatically by the computer. Fluorescence is measured twice in the reagent strips reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before solid phase receptacle (SPR) is introduced in to the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The relative fluorescence value is calculated by subtracting the background reading from the final result.

The following table is used in the interpretation of IgM and IgG titer.

Titer (IU/ml)	Interpretation
<4	Negative
4 ≤ titer < 8	Equivocal
≥ 8	Positive

Equivocal samples should be retested. If the interpretation remains equivocal, a new sample must be collected.

VIDAS IgG Avidity

After initial detection of anti- Toxoplasma IgM and IgG, all samples were tested with IgG avidity assay. The assay combines a twostep enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The SPR serves as the solid phase as well as the pipetting device for the assay. VIDAS IgG avidity uses a dual strip comprising one reference strip and one test strip. The sample to be tested, after dilution, is dispensed into both sample wells of the dual strips.

Measurement of Toxoplasma IgG avidity was performed and interpreted according to the directions of the manufacturer with (VIDAS Toxo-IgG Avidity Kit, bioMerieux, Marcy- I'Etoil, France). This test is also performed by the fully automated VIDAS machine which automatically executes the calculation and interpretation of results. The index numbers below are used in the discrimination between the cases.

High avidity IgG is a strong indication of a primary infection dating back more than 4 months. Low avidity IgG may see in acute infection with *T. gondii* [9].

Table 2: used in the interpretation of IgM and IgG titer

Avidity	Interpretation
Index < 0.200	Low avidity IgG
0.200 ≤ index < 0.300	Borderline avidity
Index ≥ 0.300	High avidity IgG

RESULTS

Comparative results of the VIDAS IgG, IgM and VIDAS IgG avidity test are demonstrated in Table 1 and 2 .From the seventy-six specimens evaluated by these tests, six were positive for Toxoplasma Specific IgM antibodies and 33 for IgG antibodies. Two of the six (33.3%) IgM positive and four of the 33 (12.12%) IgG positive cases had low IgG avidity suggesting an active Toxoplasma infection. Also two of equivocal IgM (22.2%) and three of equivocal IgG (75%) showed low avidity test results. They are also indicating an active infection, whereas three of IgM positive (50%), twenty-four of IgG positive (72.72%) and seven of equivocal IgM showed high avidity IgG antibodies, these indicating that the infection was acquired in the distant past.

Interestingly, two specimens from the 61 (3.27%) women with IgM negative showed low IgG avidity suggesting an acute infection. While 52 (85.24%) of the same group showed high IgG avidity. All the women with suspected acute Toxoplasma infection should have prescribed to them spiramycin throughout their pregnancy.

Table 1: Comparison of VIDAS IgM and VIDAS IgG avidity test results in pregnant women.

IgG Avidity	Number of specimen with VIDAS IgM		
	Positive n=6	Equivocal n=9	Negative n=61
Low	2(33.3%)	2(22.2%)	2(3.27%)
Borderline	1(16.7%)	0	7(11.47%)
High	3(50%)	7(77.8%)	52(85.24%)

Table 2: Comparison of the VIDAS IgG and VIDAS IgG avidity test results in pregnant women.

IgG Avidity	Number of specimen with VIDAS IgM		
	Positive n=33	Equivocal n=4	Negative n=39
Low	4(12.12%)	3(75%)	Not done
Borderline	5(15.15%)	1(25%)	Not done
High	24(72.72%)	0	Not done

DISCUSSION

Detection of *T. gondii* during early stage of pregnancy is so important to avoid intrauterine malformations. The common method used in detection of Toxoplasma antibodies is through performing ELISA test. This test is based on either the seroconversion of IgG or on the existence of positive Toxoplasma IgM antibodies [10]. The avidity specific *T. gondii* IgG test can differentiate between the recently acquired and distant infection [1]. The data from the present evaluation that have been carried out on 76 pregnant women, indicated that VIDAS IgG avidity test is an excellent method to differentiate between cases, where similar results have been reported in previous study [11]. Low avidity IgG (< 0.200) was determined only on two of six and four of 33 pregnant women they had the IgM and IgG antibodies respectively. Also, low avidity IgG was determined in two and three out of nine and four pregnant women they had equivocal IgM and IgG antibodies respectively. All these indicated acute Toxoplasma infection while the remaining other pregnant women with high avidity have chronic Toxoplasma infection.

The VIDAS IgG avidity test was useful in sera with equivocal results with the VIDAS IgM and IgG tests. Equivocal results are often difficult to interpret and usually required a follow up samples and even then, in some instance uncertainty interpretation remain, similar results have been reported in previous study [1]. With the avidity technique, it is able to show that among women presenting with Toxoplasma IgM at the time of pregnancy, a high proportion had not actually been infected recently. Therefore, the role of subsequent IgG avidity measurements is to confirm or to dispute the IgM diagnosis.

When maternal primary infection is diagnosed, either by IgG seroconversion or by IgM positively combined with low avidity of IgG, the mother should be referred without delay to obstetric consultation and amniotic fluid sampling for polymerase chain reaction (PCR) which is useful for verification of fetal infection.

The positioning of the avidity assay behind the IgM and IgG tests is in order to identify each subject with conventional markers of infection. Also, because the avidity test is dependent on the presence of specific IgG, the paucity of the IgG very early after infection favors IgM examination in the first sample.

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