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The role of Avidity Test in the Diagnosis of Acute and Chronic Infection with Toxoplasma Gondii

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ABSTRACT
Background: *Toxoplasma gondii* has a worldwide distribution and is one of the most prevalent infectious agents in humans. However, primary infection during pregnancy
constitutes a great diagnostic challenge, by predisposing the offspring to the risk of congenital toxoplasmosis.
Objective: To detect the Toxoplasma IgG antibodies developed at the early stage of infection in pregnant women. **Methods**: 80 pregnant women, who were in the 2nd to 4th month of their pregnancy, enrolled in this study. Anti-toxoplasma IgG, IgM and IgG avidity were evaluated by ELISA method. **Results**:The patients were categorized into three groups as follows: Group A, 2 cases; IgG+,IgM+, 2.5%; group B, 3 cases; IgM+, IgG-, 3.75%; and group C, 75 cases; IgM -, IgG +,75(93.75%). 2.5% of the pregnant women had positive IgG and IgM among which 6.25% had low avidity which revealed an active infection in the pregnant women. In the current study, 75(93.75) of pregnant women had positive IgG and negative IgM, all of which had high avidity, which is an indication that in our population the level of toxoplasmosis infection is high and most women have had contacts with this parasite before pregnancy. **Conclusion:** In this study, the low avidity test was 6.25% showing that the occurrence of toxoplasmosis infection is still a serious issue. Observation of 75(93.75%) high avidity among group B suggests that either IgM has a high half-life or there is a false positive IgM as a result of rheumatologic disorders. Therefore, avidity test is important to differentiate between past and acute infection and because the titer of IgG remain high for long time.
Keywords: Toxoplasmosis, Avidity test, acute and chronic infection.

Introduction

*Toxoplasma gondii*is one of the most prevalent infectious agents in humans *and has* a worldwide distribution [Remington,1995]. In immunocompetent subjects, acute infection is usuallyasymptomatic or characterized by mild, nonspecific clinical symptoms (e.g., cold or light case of the flu), and spontaneous recovery is the rule. [Montoya,2004] However, in pregnant women, acute infection by *T. gondii* may result in congenital disease, causingabortion or severe damages to the fetus at birth or later in life[Alvarado-Esquivel,2002] and/or sequelae that may be prevented or reduced by earlytreatment [Pinon,2001; Villena,1998]. Toxoplasmic chorioretinitis can be seen incongenitally or prenatally acquired disease as a result of acuteinfection or reactivation [Montoya,2004].
Although many serologic methods are available, diagnosis of early Toxoplasmosismay be extremely difficult [Yasodhara,2001]. Evaluation of IgG, IgA, and IgM to diagnose the unconfirmed toxoplasmosis is confronted with some problems [Jenum,1997]. Determination of IgG is valuable, however in early stages, it turns out to be negative after a few weeks, and it would become positive. On the other hand, serum IgM remains positive for several months [Joynson,1990].
Avidity is the binding force of the antibody (serum specimen) with the corresponding antigen, at the first time; the avidity test was conducted using denaturalization technique to diagnose congenital Rubellosis [Yasodhara,2001]. Recently, several tests for avidity of toxoplasma IgG antibodieshave been introduced to help discriminate between recently acquired and distant infection [Hedman,1989;Pelloux,1998].In the initial steps of Toxoplasmosis infection IgG avidity is low but the avidity of IgG due to the previous infections is very high[Candolfi,2007].The early diagnosis and treatment of toxoplasmosisduring pregnancy may protect the embryo from infection and consequent damage [Koskiniemi,1989].Our goal from The *Toxoplasma gondii* IgG avidity test is intended to differentiate between past and acute infection and because the titer of IgG remain high for long time.

Materials and Methods

Patients. Eighty pregnant women who were in the first Trimester of their pregnancy period and were at risk of Toxoplasmosis were included in this study. These women referred to the Special Clinic in Al- Kut city from January 2015 to the end of March 2015.
They were later referred to the Medical Diagnostic Laboratory to diagnose the activeness of the infection.
ELISA Test. The levels of anti-toxoplasma IgG and IgM were measured at the beginning of pregnancy according to manufacturer's instruction (Vircell Microbiology

Company). The anti-*Toxoplasma* IgG, IgM ELISAs were performed as described by the producers.

Avidity Test. There are different methods that used to performed the Avidity test these differences in the dilution method or differences in the type, quality or quantity of the *Toxoplasma* antigen used in the different assays might explain the observed divergences.in this study the Avidity test was performed by the method of(Liesenfeld *et al*. 2001).. using Euroimmun kit requires a 1:100 dilution to be prepared independently of the actual *Toxoplasma* specific IgG level of the examined sera were added to micro plates coated with Toxoplasma antigen. In the second step, concentrated (8Molarity) urea solution was added to the antigen-antibody complex. After washing the excess antibody, labeled anti-IgG antibody was added to the test microplates. After 30 minute of incubation and re-washing, substrate solution was added and in the final step the reaction was stopped by adding sulfuric acid. The optical density (OD) was measured at 450 nm.

Statical Analysis: The data were analyzed by descriptive statistical methods. Computation of diagnostic values was performed using SPSS version14 (IBM, USA

RESULTS AND DISCUSSION

The results of the analysis of 80 serum samples from pregnant women during the first 4 months of pregnancy had(93.75%) positive IgG and negative IgM as in Table (2) . The results of the avidity tests were categorized into three groups. Group A, 2 cases; (2.5%) IgM +IgG + ;group B, 3 cases;(3.75%) IgM + IgG - ; and group C, 75 cases;(93.75%) IgM – IgG +. the number of sera classified as having low and high avidity as in Table (2)

Table (1) prevalence of Toxoplasma Abs in the study groups.

Antibodies	No. Positive	No. Negative	Total
IgM	5(6.25%)	75(93.75%)	80 100%
IgG	77(96.25%)	3(3.75%)	80 100%

P value < 0.05

Table (2) The results of the avidity tests were analyzed according to these three groups .

Avidity	Group A IgM (+)IgG (+)	Greoup B IgM (+)/ IgG (-)	GreoupC IgM (–)/ IgG (+)	Total
High	0	0	75	75(93.75%)
Low	2	3	0	5(6.25%)
Total	2(2.5%)	3(3.75%)	75(93.75%)	80 100%

P value <0.05

The diagnosis of toxoplasmosis during pregnancy is often based on maternal serological testing for IgM and IgG anti- Toxoplasma antibodies. Since pregnant women are not usually referred to screening tests before pregnancy, The evaluation of specific IgG avidity enables more accurating[Joynson,1990]. The Traditional serological techniques have some limitations in evaluating the duration of *Toxoplasma gondii* infection in pregnant women therefore the avidity test used to diagnose the active Toxoplasma infection during the first trimester of pregnancy[Liesenfeld,2001].

the current study, 75(93.75) case of the pregnant women had positive IgG and negative IgM and all had high avidity test results indicating that in our society the level of Toxoplasma infection is high and the women have contact with this parasite before pregnancy[Pelloux,1998].

The *Toxoplasma* specific IgG avidity test is a method that can generally differentiate the recent and more distant infection in a single serum sample[Liesenfeld,2001; Liesenfeld,2001].The presence of high avidity IgG antibodies can be used to rule out recently acquired infection[Horvjth,2005].

In the first 2 to 4 months of pregnancy,(2.5%)of the pregnant women had positive IgG and IgM and (6.25%) had a low avidity test which reveals the presence of the active infection in such pregnant women. T. gondii-specific IgM remains detectable as long as 2 years following primary infection there for cannot be reliably identified by detection of T. gondii[Hedman,1989].

Active Toxoplasma infection in(5)case of pregnant women in our study would be considered a warning for the health care management system of the society, because of the birth of disabled newborns with congenital blindness, microcephalia and mentally

handicapped, who place a big economic burden on the country and the society[Candolfi,2007].On the other hand, the infection can be diagnosed and treated very promptly after a positive

avidity test[Alvarado-Esquivel,2002].The high avidity result can only say that the infection has occurred four or more months before, but cannot say precisely the clinical stage of the infection[Koskiniemi,1989].

At the present time, data suggest that the avidity test represents a valuable additional confirmatory method[Liesenfeld,2001; Horvjth,2007].

The low avidity antibodies can persist for many months after the acute infection in

immunocompromised patients and in patients with anti-*T. gondii* chemotherapy. In these cases, we cannot use the low avidity result for the diagnosis of recently acquired infection [Montoya,2002;Cozon,1998].A number of researchers found the different ratios of avidity titers; for instance, in one study, the avidity lower than 20% was reported as low avidity [Jenum,1997].another researchers reported a low avidity below 30% [Yasodhara,2001; Joynson,1990].while anothers reported one below 40% [Montoya,2001;, Horvjth,2005].

Observation of (93.75%) high avidity among group A suggests that either IgM has a high half-life or there is a false positive IgM as a result of rheumatologic disorders[Hedman,1989].The determination of IgG antibody avidity is an additional analysis to the classic serology in regard to the status of a Toxoplasma gondii infection[Alvarado-Esquivel,2002].Measurement of T. gondii-specific IgG avidity has proven to be a powerful tool for distinguishing recent from past toxoplasmosis

Therefore, avidity test is of prime importance and it is possible to treat the infection if a differential diagnosis of the active infection of Toxoplasma is performed on time.

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