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Evaluation of IgG Avidity for Diagnosis of Acute *Toxoplasma gondii* Compared to Nested PCR in Pregnant Women in First Trimester

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Abstract

Background

Toxoplasma gondii (T. gondii) is intracellular protozoa. Infection with T. gondii can result in a serious sequel to the fetus if infection occurs in the first trimester of pregnancy. Laboratory diagnosis is the main method of diagnosis.

Aim

The aim of the present study was to detect recent infection with Toxoplasma gondii in cohort pregnant Egyptian women in the first trimester by serological detection of concrete IgM, IgG and IgG avidity test. The results of serological tests were compared by nested PCR method.

Materials and Methods

One hundred and twenty pregnant women in the first trimester of pregnancy were included in the study. Blood samples were obtained and subjected to serological studies for specific immunoglobulins M (IgM), G (IgG) and avidity IgG for T. gondii. Suspected results were confirmed by nested polymerase chain reaction for detection of T. gondii DNA.

Results

Positive IgG for Toxoplasma gondii was (31.7%) and positive IgM was (18.3%). IgG avidity results showed that low avidity IgG and high avidity IgG represented 31.7% for each of positive IgG for Toxoplasma while intermediate avidity represented 36.8%, Table 1.
Twenty two pregnant women were positive for IgM, however, only 7 of them

(31.2%) were associated with low avidity IgG suggesting recent infection. Seven positive IgM results were associated with high IgG avidity (31.2%) and 8 positive IgM samples were associated with boarder line IgG avidity results (36.4%). Among pregnant women with negative IgM, isolated, low avidity IgG was found in five patients (5.1%), boarder line IgG avidity in 6 patients (6.1%) and high IgG avidity in 5 patients (5.1%). We studied 26 pregnant women with nested PCR to confirm serological test results to detect recent Toxplasma infection. Among women with positive IgM with boarder line avidity IgG only 2 were positive by PCR while 8 (80%) were negative. On the other hand three women with isolated low avidity IgG were positive by PCR (60%) and 2 were negative. All women with positive IgM and high avidity IgG were negative by PCR and all women with isolated boarder line IgG avidity were negative also by PCR. According to this table, the sensitivity of IgG avidity test was 100 95%CI: 30.48-100, specificity 77.8% (95%CI: 40.06% - 96.53%) with a positive predictive value 60% (95% CI: 15.40 % - 93.51%), and negative predictive value 100% (95% CI: 58.93% - 100.00%).

We can conclude that confirmatory testing for recent *Toxoplasma* infection with the combined use of IgG avidity with IgM antibody test in pregnant women during the first trimester has the potential to decrease the need molecular method for diagnosis and even the need for follow up samples for detection of diagnostic rising titer with delay of acute Toxoplasma diagnosis. The rapid and accurate diagnosis leads to appropriate therapeutic intervention in adequate time.

Keywords: Toxoplasma gondii, IgG,IgG avidity, igM,PCR

Introduction

Toxoplasma gondii (T. gondii) is an obligate intracellular protozoan parasite. It causes toxoplasmosis. It is a worldwide chronic infection affecting around one third of the world's human population [1]. The infection may pass unnoticed or may be presented with non-categorical symptoms, cervical lymphadenopathy or ocular disease [2] and formation of cysts that may remain in latent form in many organs [3].

Among affected populations, pregnant women have a great risk due to the effect on the pregnancy outcomes [4-6]. Though these infections are conventionally either asymptomatic or associated with self-inhibited symptoms in adults like fever, malaise, and lymphadenopathy, infections in pregnant women can cause earnest health quandaries in the fetus if the parasites are transmitted such as congenital toxoplasmosis and cause rigorous sequelae in the infant affecting mainly central nervous system leading to mental retardation, optical incapacitation, and epilepsy.

The most frequent challenge encountered is the diagnosis of recent infection acquired during early gestation. It was believed that congenital toxoplasmosis

results from a primary infection acquired during pregnancy [7], but not from the reactivation of a latent infection in immunocompetent pregnant women [8].

Laboratory diagnosis of Toxoplasma relies on the detection of anti-Toxoplasma concrete immunoglobulins G, M, A by enzyme-linked immunosorbent (ELISA) assays methods. In acute infection, categorical immunoglobulins M (IgM) and categorical immunoglobulin G (IgG) conventionally elevate within 1 to a fortnight of infection [9]. IgM antibodies have been reported to persist for up to 18 months after infection and even for years [10,11]. So, the presence of IgM antibodies is not always a denotement of a recent infection and its presence in the chronic stage of an infection can result in dispensable procedural risks of diagnosis and side effects of treatment, and may even lead to termination of a non-infected pregnancy. [7,11].

The presence of elevated levels of Toxoplasma categorical IgG antibody denotes infection has occurred, but does not distinguish between recent infection and antecedent one. A negative IgM with a positive IgG result betokens infected at least 1 year entirely.

Acute toxoplasmosis is diagnosed infrequently by detecting the parasite in body fluids, tissue, or secretions by histologic demonstration of the parasite and/or its antigens by immunoperoxidase stain, or by isolation of the organism [1,12,13]. Molecular techniques for detection of Toxoplasma DNA, though categorical and sensitive, albeit, not available in each laboratory with desideratum of categorical equipments and facilities.

Recently, avidity tests that measure the force between the binding capacity of the composed IgG and categorical antigens have been claimed to differentiate recently engendered IgG in acute infection from that perdurable IgG, Low avidity IgG is conventionally present in recent infection.

It has been suggested that the amalgamation of a sensitive test for Toxoplasma concrete IgM antibodies and quantification of the avidity of IgG antibodies to T. gondii had the highest predictive value with regard to the time of infection [14, 15].

Precedent studies from Egypt revealed high rates of positive serological tests for T. gondii between pregnant and nonpregnant women [16]. However, we could not find a report about avidity IgG test results.

The aim of the present study was to detect recent infection with Toxoplasma gondii in cohort pregnant Egyptian women in the first trimester by serological detection of concrete IgM, IgG and IgG avidity test. The results of serological tests were compared by nested PCR method.

Materials and Methods

This study was performed at the Mansoura Faculty of Medicine, Egypt, between June 2013 and March 2013 on consecutive pregnant women attending out patient clinic of gynecology and obstetric for antenatal care. Blood samples were obtained and sera were seperated from 120 pregnant women in their first trimester of pregnancy and were screened for anti-*Toxoplasma* IgG, IgM and IgG avidity antibodies by ELISA (Equipar S.r.L Via Volonterio, 36A 21047 Saronno-Aly). The screening tests were essentially performed following manufacturers' instructions. Later Patients with positive IgM with boarder line avidity or high IgG avidity results and patients with isolated positive IgG with low avidity were subjected to further study by nested PCR for detection of Toxoplasma gondii DNA on Buffy coat obtained from heparinized whole blood samples.

All women participating in this study gave their informed consent. The Ethical Committee of Mansoura Faculty of Medicine, University, approved the study.

Nested-PCR for Toxoplasma gondii

The PCR assay was done on a selected number of specimens. Buffy coat was prepared from the peripheral blood. Buffy coat samples were lysed in 2 vols TNN lysis buffer (0.5% Tween 20, 0.5% Nonidet P-40, 10 mm NaOH) with 10 mM Tris and 250 μg proteinase K ml^{-1} for 5–15 h. The DNA was extracted by the phenol/chloroform method. The final pellet was resuspended in 25 μl TE buffer (10 mM Tris, 1 mM EDTA, pH 7.2) and stored at −70 °C until used.

The nested-PCR amplifications were performed on all DNA samples to amplify a fragment of the B1 gene as described earlier with slight modifications [17]. Briefly, the primers used in the first round of the PCR (inner primers) were 5′-GGAACTGCATCCGTTCATGAG-3′ and 5′-TCTTTAAAGCGTTCGTGGTC-3′, which correspond to nucleotides 694–714 and 887–868, respectively. The primers used in the second round (outer primers) were 5′-TGCATAGGTTGCAGTCACTG-3′ and 5′-GGCGACCAATGTGCGAATAGACC-3′, which correspond to nucleotides 757–776 and 853–831, respectively. Five microlitres of template DNA was added to a final volume of 50 μl PCR mixture consisting of 5 μl 10× PCR buffer (50 mM Tris/HCl, pH 9.1, 3.5 mM $MgCl_2$), 8 μl 1.25 mM deoxynucleoside triphosphates, 0.5 μl *Taq* DNA polymerase (5 units μl^{-1}) and 1.5 μl (20 pmol) of each of the outer primers. The amplification was performed in the GeneAmp 9700 PCR System (Applied Biosystems). The cycling conditions for both PCRs were 95 °C for 5 min, followed by 30 cycles at 94 °C for 30 s, 55 °C for 90 s and 72 °C for 1 min, and a final extension at 72 °C for 10 min.

Five microlitres of the first-round PCR product was used as a template for the second-round PCR in a total volume of 50 µl under the same conditions as in the first round, using the inner primers. The PCR product was analyzed on a 1% agarose gel stained with ethidium bromide. The PCR amplification is expected to yield a product of 213 bp for the positive reaction [18].

Results and Discussion

The study included 120 consecutive pregnant women attending obstetric and gynecology outpatient clinic for antenatal care. They were in the first trimester of pregnancy with mean± SD duration of pregnancy 15± 1.5 weeks. Their mean age was 26.4± 5.6 years with mean± SD gravidity 3.2± 1.9 and mean SD parity 1.15± 1.9. Positive IgG for *Toxoplasma gondii* was (31.7%) and positive IgM was (18.3%). IgG avidity results showed that low avidity IgG and high avidity IgG represented 31.7% for each of positive IgG for Toxoplasma while intermediate avidity represented 36.8%, Table 1.

Table 1. Clinical and serological results for Toxoplasmosis in pregnant women (n=120)

Age	**26.4± 5.4**
Duration of pregnancy (mean± SD) weeks	15 ± 1.5
Gravidity (mean± SD)	3.2± 1.9
Parity (mean± SD)	1.15± 0 .9
IgM for Toxoplasma (No.-%)	22 (18.3%)
IgG for Toxoplasma (No.-%)	38 (31.7%)
Low IgG avidity (No. -%)	12(31.6%)
Intermediate IgG avidity (No.-%)	14 (36.8%)
High IgG avidity (No.-%)	12(31.6%)

Twenty two pregnant women were positive for IgM, however, only 7 of them (31.2%) were associated with low avidity IgG suggesting recent infection. Seven positive IgM results were associated with high IgG avidity (31.2%) and 8 positive IgM samples were associated with boarder line IgG avidity results (36.4%). Among pregnant women with negative IgM, isolated, low avidity IgG was found in five patients (5.1%), boarder line IgG avidity in 6 patients (6.1%) and high IgG avidity in 5 patients (5.1%), Table 2.

Table 2. Results of IgM for Toxoplasma regarding IgG avidity results

	IgM **Positive Negative** **(n=22) (n=98)**
Low IgG avidity	7 (31.2%) 5(5.1%)
Boarder line IgG avidity	8 (36.4%) 6(6.1%)
High IgG avidity	7 (31.2) 5(5.1%)

We studied 26 pregnant women with nested PCR to confirm serological test results to detect recent Toxplasma infection. Among women with positive IgM with boarder line avidity IgG only 2 were positive by PCR while 8 (80%) were negative. On the other hand three women with isolated low avidity IgG were positive by PCR (60%) and 2 were negative. All women with positive IgM and high avidity IgG were negative by PCR and all women with isolated boarder line IgG avidity were negative also by PCR, Table 3.

Table 3. Comparison of serological tests with PCR for Toxoplasma DNA (n=26)

	PCR **Positive Negative** **(n=8) (n=18)**
IgM Positive+Boarder line avidity (n=8)	2 (20%) 6 (80%)
IgM Positive +High avidity IgG (n=7)	0 (0%) 7 (100%)
IgM negative+Low IgG avidity (n=5)	3 (60%) 2 (40%)
IgM negative+ Boarder line IgG avidity (n=6)	0 (0%) 6 (100%)

According to this table, the sensitivity of IgG avidity test was 100 95%CI: 30.48-100, specificity 77.8% (95%CI: 40.06 % - 96.53%) with a positive predictive value 60% (95% CI: 15.40 % - 93.51 %), and negative predictive value 100% (95% CI: 58.93 % - 100.00 %).

Toxoplasma gondii infection during the first trimester of pregnancy leads to serious sequels for the fetus, resulting in congenital malformations or abortion. Clinical diagnosis is not valid as the infection usually asymptomatic. Laboratory diagnosis is the cornerstone for such condition. Previously laboratories depend mainly on detection of IgM, which proven to have many limitations.

In the present study determination of IgG avidity by ELISA had proven to be sensitive and specific diagnostic tool in association with detection of IgM.

In the present study IgM for Toxoplasma gondii was positive in 18.3%, while IgG was positive in 31.7% among pregnant women.

Results differ in studies according to the used serological method for screening and according to the geographical locations of the study. In Egypt, a recent study reported the prevalence of IgG to be 72.1% and 2.8% for IgM [19]. The difference in rates can be attributed to the difference of the number of included subjects in the study. On the other hand, we could not find study about the avidity IgG as a diagnostic tool for recent infection.

Routine serological diagnosis of toxoplasmosis provides a rapid screening tool for diagnosis of *Toxoplasma gondii*. However, there are limited sensitivity and specificity of serological diagnosis depending on the test used.

In this study, 22 (18.3%) women in the first trimester of pregnancy had *Toxoplasma* specific IgM antibodies, suggesting an acute infection that requires prompt therapeutic intervention. Moreover, 38 (31.7%) women had positive IgG. Usually, detection of specific IgM for *Toxoplasma* is claimed to be a sensitive indicator of recent infection. Nevertheless, false positive IgM antibody test results have been reported previously [20]. In such cases, the diagnosis of primary infection with *T. gondii* in first trimester of pregnancy can be accurately defined by determination of IgG avidity test for *Toxoplasma*, which has the capacity to differentiate between recent and previous infections.

On avidity testing, only 7 of 22 (31.2%) IgM positive women had low avidity IgG antibodies indicating a recent *T. gondii* infection in these women. It's worth noticing that a similar number of women 7 (31.2%) with positive IgM had high-avidity antibodies suggesting that the infection was acquired before gestation. The apparent ability in detecting infection status by IgM serology and avidity tests may be due to the fact that IgM antibodies can persist for months or even years following the acute phase of an infection in some individuals; thus the presence of IgM antibodies is not always an indication of a recent infection [21,22]. The presence of specific IgM for *T. gondii* in the c; tonic infection as observed in 31.2% of the IgM-positive cases in this study that can lead to apparently a misdiagnosis especially in women in early pregnancy.

In sera with low or borderline avidity antibodies and negative IgM antibodies, IgG avidity test was potentially misleading, if used alone. In this study, two IgM-negative women with low avidity antibodies were confirmed negative for *Toxoplasma* DNA on PCR analysis. Similar results have been reported in previous studies [18, 22]. It was previously reported high-avidity antibodies in 74.8% of the IgM-positive serum samples from pregnant women during the first 16 weeks of gestation [22]. It is known that the maturation of the IgG response varies considerably between individuals and thus low or borderline avidity antibodies may persist for months up to more than 1 year [23, 24].

Previous studies reported a lower mean IgG avidity index of 0.2 in pregnant women after 5 months of infection [25]. If an avidity test result is used without the presence of IgM this will lead to misinterpretation as an acute infection.

The sensitivity of IgG avidity test was 100 95%CI: 30.48-100, specificity 77.8% (95%CI: 40.06 % - 96.53%) with a positive predictive value 60% (95% CI: 15.40 % - 93.51 %), and negative predictive value 100% (95% CI: 58.93 % - 100.00 %).

Our findings validate that the avidity test represents a confirmatory method, most useful if low avidity antibodies are detected in IgM-positive women and also in IgM positive women with high avidity antibodies.

Previous reports have found that PCR can predict the presence of *T. gondii* DNA in blood specimens [26, 27]. Depending on this finding, the presence of *Toxoplasma* DNA in the maternal blood usually give index on the recent infection and the presence of parasitaemia. The clearance time for *Toxoplasma* DNA from the blood of patients was estimated to be 5.5–13 weeks previously in patients with acute toxoplasmic lymphadenopathy [28].

In this study, all the 7 IgM-positive women with high-avidity antibodies and 6 of the 8 IgM positive women with borderline avidity antibodies were negative for *Toxoplasma* DNA on PCR analysis, confirming the high sensitivity and specificity of the avidity test for detecting recent *Toxoplasma* infection in early pregnancy.

Depending solely on detecting *Toxoplasma* specific IgM antibodies and/or by detecting a threefold increase in IgG antibodies in follow up samples in pregnant women during the first trimester may result in unnecessary interventions in pregnant women or delay in diagnosis of such stressing condition.

Using nested-PCR analysis to detect *Toxoplasma* DNA to confirm the recent infection appears as a useful adjuvant diagnostic tool. So, with the use of combined IgM and avidity IgG tests, the use of PCR can be applied only to boarder line results identified previously. The use of PCR as a diagnostic method can be applied to pregnant women with IgM antibodies with isolated presence of borderline-avidity antibody results or presence of isolated low or boarder line avidity IgG.

We can conclude that confirmatory testing for recent *Toxoplasma* infection with the combined use of IgG avidity with IgM antibody test in pregnant women during the first trimester has the potential to decrease the need molecular method for diagnosis and even the need for follow up samples for detection of diagnostic rising titer with delay of acute Toxoplasma diagnosis. The rapid and accurate diagnosis leads to appropriate therapeutic intervention in adequate time.

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