Comparative study between nested semi-quantitative PCR technique and serological tests in diagnosis of toxoplasmosis during pregnancy and the role of IL-12 in toxoplasmosis

A study in AL-Madinah, Saudi Arabia

Fawzia A Habib¹, Lobna M Saber², Amany A Abd El-Aal³, Hala F Gawad⁴, Asmaa A Abd El-Aal⁵ and Lamia A El-Hosseiny⁶

¹. Department of Obstetrics and Gynecology, ³. Department of Parasitology & ⁴. Department of Physiology, Faculty of Medicine, Taibah University, Saudi Arabia, ². Department of Biochemistry, Faculty of Medicine, Al-Azhar University, Cairo, Egypt, ⁵. Department of Clinical pathology, Faculty of Medicine, Cairo University, Cairo, Egypt, ⁶. Department of Biomedical Technology, National Research Center, Cairo, Egypt

ABSTRACT

Background: Difficulties in interpretation of serological tests carried out to detect primary maternal infection with toxoplasmosis during pregnancy implies more and more frequent use of the Polymerase Chain Reaction (PCR).

Aim - Methods: To evaluate the results of serological tests, the nested semi-quantitative PCR method was applied in 168 pregnant women, for the presence of B1 gene of Toxoplasma gondii. The presence of IgG and IgM Toxoplasma gondii antibodies was detected by the indirect haemagglutination (IHA) method. In addition, a trial was done to evaluate the role of IL-12 (Th-1 cytokine) in toxoplasmosis in 40 pregnant out of our 168 cases in which Toxoplasma gene was demonstrated in 20 blood samples of them. The serum level of IL-12 was estimated using ELISA technique.

Results: The study revealed significant differences between the results of IHA and PCR. Toxoplasma gondii genetic material in blood was found in 68 (40.5%) samples. IgG was detected in 28 (16.7%) of these PCR-positive cases, positive IgM was found in 8 out of these 28 samples. On the other hand, 100 (59.5%) cases were PCR-negative; 44 (26.1%) of them were serologically positive and the remaining 56 (33.3%) cases were serologically negative. No correlation was estimated between the sero-reactivity and the serum level of IL-12. On the other hand, a positive correlation was estimated between the results of IL-12 and the presence of Toxoplasma gene in patient's blood. The 20 gene positive samples obtained higher level of IL-12 irrespective to their serum Toxoplasma immunoglobulin level. Moreover, the concentration of the gene positive samples were significantly higher than the gene negative group P<0.01.

Conclusions: The present study highlights the need for a confirmatory test in addition to serology to detect primary acute toxoplasmosis in pregnant women. Nested (semi-quantitative) PCR amplification of the B1 gene of T. gondii using whole blood is a rapid, sensitive and specific diagnostic procedure for the diagnosis of T. gondii infection in adult females before or during pregnancy. The significant rise in the IL-12 that was shown in the present study with recently infected cases suggested the possible use of this cytokine as an indicator or marker to diagnose recent infection of Toxoplasma gondii in the absence of specific Toxoplasma antibodies.

Keywords: toxoplasmosis, pregnancy, Polymerase Chain Reaction, serological tests, diagnosis.

INTRODUCTION

Toxoplasmosis is caused by infection with Toxoplasma gondii, a single-cell protozoan that belongs to the family Coccidia. T. gondii infection is found in 30%-50% of the human population worldwide. Although adult-acquired toxoplasmosis is usually mild to asymptomatic, the disease can be severe in the immunocompromised subjects.

When acute T. gondii infection is suspected in a pregnant woman, the diagnosis should be pursued. Early diagnosis of toxoplasmosis in pregnant women allows early intervention and prevention of congenital disorders. Toxoplasmosis is usually diagnosed on the basis of antibody detection. Indirect haemagglutination test (IHA), 1 enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody test among the serological tests used to diagnose such infection. In acute infection with toxoplasmosis, IgG and IgM antibody levels generally raise within one to two weeks of infection.

The most frequent challenge encountered by physicians all over the world is determination of acute infection in pregnant women. Women who acquired the infection prior to pregnancy are essentially not at risk of delivering an infected infant, unless the woman is immunosuppressed.
is not common to conduct a systematic screening of pregnant women to detect seroconversion during gestation. Thus, a single serum sample from each woman is submitted for evaluation, and from this sample the physician hopes to detect whether the patient has recently been infected, thereby placing the fetus at risk.

The high prevalence and life long persistence of Toxoplasma immunoglobulin G (IgG) antibodies among healthy individuals in many geographical areas and varies on what proportion of T. gondii infections are attributable to undercooked meat exposure or to cat feces, soil, or water exposure, makes IgG data not reliable as a proxy indicator for recent infections. Another problem is the frequent lack of reliability when IgM, IgA, or IgE Toxoplasma antibody test results are used in an attempt to discriminate between recent and more distant infection. This often leads to incorrect information being provided by the laboratories to the physicians as well as by the physicians to their patients. Moreover, serological tests to follow up treatment of toxoplasmosis are not capable of evaluating the efficacy of the treatment administered. It is desirable to detect the pathogen directly. Therefore, a major effort has been made towards improving the ability to diagnose recently acquired infection in the pregnant women. Among these, is the PCR technique that has been consistently used to detect DNA of T. gondii in various biological samples.

On the other hand, during acute infection of toxoplasmosis, rapid growth of the parasite is continued by a vigorous IFN-dependent, cell-mediated immune response. While, in the chronic infection, the parasite is differentiated into slow-growing cysts that are capable of persisting for the entire life of the host. Primary infections acquired during pregnancy or fulminate re-emergence of chronic infections, are capable of causing severe birth defects, including hydrocephaly, calcification, neurological defects, and chorioretinitis, which may be recurrent.

Previous studies have shown that infection with T. gondii drives a potent Th1 response that is necessary to control the infection through induction of IL-12, which drives production of IFN-γ by NK cells and T cells, such cytokines are reported to be essential to control parasite replication and prevent death due to toxoplasmosis in the murine system. Moreover, on human polymorph nuclear leukocytes in response to Toxoplasma gondii antigens, it has been reported that they produce IL-12, TNF-α, and the chemokines macrophage-inflammatory protein (MIP) -1 alpha and MIP -1 beta.

In this study, a trial was made at testing the utility of a nested, semi-quantitative PCR assay to detect recent Toxoplasma infections in pregnant women with either positive or negative serological results. In addition, this work has aimed at evaluating the role of IL-12 (Th1 cytokine) in the diagnosis of the disease.

MATERIAL AND METHODS

STUDY POPULATION

In total 168 cases, were selected out of pregnant women attending the Obstetrics and Gynecology outpatient clinic of the Maternity and Children Hospital, and the Medical section, Fema le branch, Taibah University in Al-Madinah, Saudi Arabia for the first routine checking. All of them underwent clinical and ultrasonographic examinations. Pregnant women that were receiving antibiotic therapy, residents out side Al- Madinah city and those with history of abnormal pregnancies due to organic causes were excluded from this study.

BLOOD SAMPLES

Two blood samples, 2ml each, were collected from each woman. As a routine, a blood sample is usually taken at the first antenatal health care visit, confirming pregnancy, which is usually between the eighth and thirteenth week of gestation. Serum was separated from one of the samples and stored in aliquots at -20 °C until used to test for anti-Toxoplasma antibodies, using an indirect haemagglutination test (IHA). The other blood sample was collected on EDTA, transferred to the laboratory and stored at -20°C until processed to be used in PCR technique. The serum level of IL-12 was estimated using ELISA and compared with Toxoplasma immunoglobulin seroreactivity and the presence of the Toxoplasma gene.

STUDY GROUPS

Classification of the patients was done according to the results of indirect hemagglutination test (IHA) as follows:

Group I: positive cases for anti-Toxoplasma IgM.
Group II: positive cases for anti-Toxoplasma IgG.
Group III: serologically negative cases for anti-Toxoplasma antibodies.

IgM AND IgG DETECTION USING IHA TEST

The indirect haemagglutination test [Toxoceil-IHA, Biokit, Spain] was performed according to the manufacturer’s instructions. Each serum sample was diluted from 1/16 up to 1/128. Any serum with a titer of 1/128 was further diluted up to 1/8192. Sera showing nonspecific agglutination were absorbed and retested. The absorbent reagent comprising stabilized and unsensitized sheep cells at a cellular concentration of 10% and was resuspended in a pH 7.2 buffer solution with stabilizing agent, provided within the kit. Absorption of nonspecific agglutinins was done by mixing the undiluted test serum with the absorbent reagent and incubating at room temperature for one hour followed by centrifugation. The supernatant was obtained to be further diluted and tested qualitatively and quantitatively as discussed above. A positive and a negative control serum and an antigen control was included in each batch of tests. Generally, titers higher than 1/32 were considered positive.

Nested or semi-quantitative Polymerase chain reaction (According to El-Awady et al, 2000).

POSITIVE CONTROL

T. gondii (P-strain) tachyzoites, kindly provided by the Department of Parasitology, Research Institute of Ophthalmology, Giza, Egypt were used for evaluation of Nested PCR sensitivity. Tachyzoites were collected by peritoneal lavage of infected mice. The number of T. gondii tachyzoites in suspension was determined by counting on a haemocytometer prior to DNA extraction. Extraction of the positive control DNA was performed by phenol/chloroform extraction methods. Serial dilutions of this positive
control were prepared and sensitivity of the test was estimated according to the lowest number of tachyzoites to be amplified.

NEGATIVE CONTROL
The PCR mixture without DNA and with DNAse-free water was used as negative control to monitor for cross-contaminations.

DNA EXTRACTION OF THE BLOOD SAMPLES
DNA was isolated from blood samples using a commercial purification system (Wizard Genomic DNA Purification Kit; Promega, Madison, WI) following the manufacturer’s instructions for DNA purification from blood. Final pellets were resuspended in 30 µL of TE buffer (10 mM Tris, 1 mM EDTA, pH 7.2) and stored at –70°C until used.

DNA AMPLIFICATION
Nested primer sets were used for amplifying fragments of the B1 gene, which is present in 35 copies and is conserved in the T. gondii genome, as described by El-Awady et al (2000). The outer primers were from bases 171 to 190 (5’-CGT GGT GTC GTC GT-3’) and from bases 602 to 583 (5’-GCA AAA CAG CGG CAG GTCT-3’) producing an amplified product of 432 bp. Inner primers were from bases 180 to 196 (5’-CCG CCT CTT CTC GT-3’) and from bases 392 to 372 (5’-GTT GGG GGG GAC CTC TCT-3’) producing an amplified product of 213. The first 50 µL PCR reaction mixture contained outer primers at a final concentration of 50 pmol each, 20 mmol/L dNTPs and 1.25 U recombinant Taq DNA polymerase in 1 X PCR reaction buffer (50 mmol/L KCl and 10 mmol/L tris-HCl, 1.5 mmol/L MgCl2, 0.1% triton X 100) [DynAzymeTM]. PCR amplification was performed for 2 minutes at 94 °C for one cycle, followed by 30 cycles using denaturation at 94 °C for 1 minute, annealing for 2 minutes at 57 °C and extension for 3 minutes at 72 °C. The nested PCR reaction was performed using 5 µl of the first PCR reaction product in a mixture containing the inner primers at final concentration of 50 pmol each, 20 mmol/L dNTPs, 1.25 U recombinant Taq DNA polymerase in 1 X PCR reaction buffer. Amplification was carried out at 94 °C for 2 minutes (one cycle), then followed by 35 cycles each for denaturation at 94 °C for 1 minute, annealing at 58 °C for 1 minute and extension at 72 °C for 1 minute. The run was terminated with a final extension at 72 °C for 10 minutes.

DNA VISUALIZATION
The amplification products were detected by gel electrophoresis using 3% agarose gel in 1 X tris-borate-EDTA buffer. DNA bands were visualized using 0.5% ethidium bromide in the presence of ultraviolet light.

DETERMINATION OF SERUM IL-12
Analysis was performed by quantitative sandwich Enzyme Linked-Immune-Sorbent Assay (ELISA), using commercially available kit (IL12) ELISA Kit), Diaclone research, (URS) – France. A monoclonal antibody specific for IL12 was coated onto the wells of the microtitre strips. Standards and samples were pipetted into the wells and any IL12 present was bound by the immobilized

Table 1. Results of PCR performed for diagnosis of toxoplasmosis in sera of 168 cases in relation to sero-reactivity.

<table>
<thead>
<tr>
<th>Sero-reactivity</th>
<th>Number of cases</th>
<th>PCR +ve</th>
<th>PCR -ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM+ ve cases</td>
<td>12 (4.8%)</td>
<td>8 (4.8%)</td>
<td>4 (2.4%)</td>
</tr>
<tr>
<td>(are IgG+ ve also)</td>
<td>N=72</td>
<td>28 (16.7%)</td>
<td>44 (26.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>N=72</td>
<td>28 (16.7%)</td>
<td>44 (26.2%)</td>
</tr>
<tr>
<td>Number of negative cases</td>
<td>N= 96 (57.2%)</td>
<td>40 (23.8%)</td>
<td>56 (33.3%)</td>
</tr>
<tr>
<td>Total number of cases</td>
<td>= 168 (100%)</td>
<td>68(40.5%)</td>
<td>100(59.5%)</td>
</tr>
</tbody>
</table>
antibody. During the first incubation, the IL12 antigen and a biotinylated monoclonal antibody, specific for IL12 monoclonal anti-IL-12 (p40), was simultaneously incubated. After washing, the enzyme (streptavidin - peroxydase) was added. After incubation and washing to remove all the unbound enzyme, a substrate solution which is acting on the bound enzyme was added to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of IL12 present in the samples. The enzyme - substrate reaction was stopped by quickly pipetting 100 µL of 1.8 N sulfuric acid into each well to completely and uniformly inactivate the enzyme. Result must be red immediately after the addition of sulfuric acid. The intensity of the color was measured on a spectrophotometer using 450 nm. The amount of IL12 in each sample was determined by extrapolating OD values to IL12 concentrations using the standard curve.

CONTROL GROUP
Serum samples were collected from 25 healthy pregnant women (serologically negative and PCR negative) to act as control group.

STATISTICAL ANALYSIS
Data were analyzed with standard program of SPSS, Echo Soft corporation, USA, 1995 statistical package. Data were analyzed with the chi-square to evaluate the possible differences between the study groups. Student’s t test was applied to the data conforming to normal distribution. For all tests a probability < 0.05 was considered significant.

RESULTS
None of the 168 pregnant women enrolled in this study had apparent symptoms of toxoplasmosis during their pregnancy. The subsequent categorization of the cases into three groups was done according to the results of IHA as follows:

Group I: 12 positive cases for anti-Toxoplasma IgM.
Group II: 72 positive cases for anti-Toxoplasma IgG.
Group III: 96 serologically negative cases for anti-Toxoplasma antibodies.

Figure 2. Sensitivity assessment of the PCR technique with different Toxoplasma gondii DNA concentrations. 1st and 2nd PCR products were visualized by agarose gel electrophoresis stained with ethidium bromide. Lane 1: 1st PCR product of purified DNA of 1 trophozoite. Lane 2: 2nd PCR product of the purified DNA of 1 trophozoite. Lane 3: 1st PCR product of purified DNA of 5 trophozoites. Lane 4: 2nd PCR product of the purified DNA of 5 trophozoites. Lane 5: 1st PCR product of purified DNA of 10 trophozoites. Lane 6: 2nd PCR product of the purified DNA of 10 trophozoites. Lane 7: DNA molecular weight marker (ϕX 174 Hae III).

Figure 3. PCR product of 1st (lane 2) and 2nd (lane 3) positive blood samples of T.gondii extracted by microwave method. Lane 1 is a negative control, and M is DNA molecular weight marker (ϕX 174 Hae III).
The results of the IHA and PCR are summarized in table 1, and figure 1.

Regarding the results of IgM detecting IHA and IgG detecting IHA in relation to PCR, only 8 samples with IgM-positive results showed positive PCR. As for IgG antibodies, 72 out of 168 cases were IHA-positive, these included the 12 cases that were IgM-positive. The titers of the positive IHA results were ranging between 1/64 and 1/512.

The sensitivity of the nested PCR, was evaluated according to the number of T. gondii tachyzoites in the positive control sample that was collected by peritoneal lavage of infected mice and counted on a haemocytometer prior to DNA extraction. Nested PCR was sensitive enough to detect and amplify one Toxoplasma trophozoite (fig.2). The outer and the inner primers were reacted successfully obtaining amplified DNA fragments of Toxoplasma gondii of 432 pb and 213 pb (fig.3). Applying this sensitivity on the study group, 68 (40.5%) out of the 168 pregnant women were PCR positive (Table 1). In total 28 of these PCR positive cases were sero-positive; these included 20 that were positive only for IgG antibodies and 8 samples that were positive for both IgM and IgG antibodies. On the other hand, 100 (59.5%) cases were PCR-ve, out of which 44 were serologically positive, whereas the remaining 56 cases were serologically negative.

The data presented in table 1 demonstrate that there was no agreement between the results of IHA and PCR (40 sera negative by IHA testing were PCR positive).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Control)</td>
<td>PCR negative</td>
</tr>
<tr>
<td>N = 15</td>
<td>N = 20</td>
</tr>
<tr>
<td>Means ± S.D.</td>
<td>Means ± S.D.</td>
</tr>
<tr>
<td>sero-ve/ PCR -ve</td>
<td>sero-ve/ PCR +ve</td>
</tr>
<tr>
<td>N = 15</td>
<td>N = 20</td>
</tr>
<tr>
<td>IL-12 Concentration</td>
<td>173.35±7.21</td>
</tr>
<tr>
<td>(pg/mL)</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>P value for t test</td>
<td>NS</td>
</tr>
</tbody>
</table>

P<0.05 significant (S). P<0.001 significant (HS). p>0.05 non –significant (NS)
Moreover, there was no significant correlation between the semi-quantitative values of 68 positive PCR and the serological results (p>0.05). Also, no correlation was noticed between the positive PCR results and the titers of IHA (p= 0.05).

According to the results in this study, the presence of both specific antibodies and/or Toxoplasma DNA in the 68 cases table 1 indicate that T. gondii infection might have occurred during the current pregnancy, as the serum samples were collected within the first 20 weeks of gestation. On the other hand, negative test results in both (IgM) IHA and PCR exclude recent Toxoplasma infection in 100 pregnant women included in the study.

On the other hand, in the present study a trial was done to evaluate the role of IL-12 in toxoplasmosis in 40 pregnant out of our 168 cases in which, Toxoplasma gene was demonstrated in 20 blood samples of them, while the other 20 blood samples was free of Toxoplasma gene.

The 40 samples were chosen according to the results of PCR (20 PCR positive and 20 PCR negative). The results represented in table number 2 and figure 4, demonstrate that, there was no correlation between the sero-reactivity and the serum level of IL-12 (p> 0.05). On the other hand, an agreement was observed between the results of IL-12 and the presence of Toxoplasma gene in patient’s blood (fig 4&5). The gene positive group obtained higher concentration of IL-12. (mean± SD = 233.80± 20.48) in comparison with gene negative group and control group, (mean± SD = 167.36± 23.87 and 173.35± 7.21) respectively p< 0.001).

No correlation was found between the sero-reactivity and the serum level of IL-12, twelve sero-negative samples for Toxoplasma immunoglobulin showed high level with ELISA IL-12, while 6 sero-positive samples obtained low level of ELISA IL-12 (table 2 - figures 4 & 5).

Also, a positive correlation was present between IL-12 and the presence of Toxoplasma gene in patient’s blood (table 2). All the 20 gene positive samples obtained higher level of IL-12 irrespective to their serum Toxoplasma immunoglobulin level (12 of them were sero negative and 8 were sero positive). Twelve out of the 20 gene positive group are sero-negative showed higher level (mean±SD

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**Figure 5:** Means concentration of IL-12(pg/mL) in PCR positive and PCR negative cases in comparison to control group using IL-12p40 ELISA

*** = significantly different from control group at p<0.001

**Figure 6.** The relationship between the semi-quantitative PCR value results and the serological results using indirect haemagglutination test (IHA).
culture, which is insensitive and time-consuming.1 In gene negative groups, samples showed no statistical difference from control group (mean±SD = 167.12 ± 23.87). Moreover, there was a significant difference in the concentration of IL-12 between the two groups (fig. 5), the concentration readings of the gene positive samples were statistically higher than the gene negative group P < 0.001.

N.B: Semi quantitative PCR values related to figure number 1 and 2: High +ve PCR: 1.5 x 104 (GE/ml Blood), Moderate +ve PCR: 1.5 x 103 (GE/ml Blood) and Low +ve PCR: 1.5 x 102 (GE/ml Blood).

DISCUSSION

The proportion of women at risk of acquiring the infection during pregnancy in many countries, including Saudi Arabia, is not well known. Acute infection with Toxoplasma during pregnancy may lead to severe complications, if not fatal infection of the fetus. Therefore, emphasis is made on early diagnosis of the infection to allow proper management and prevent complications.20 The diagnosis is routinely based on serological tests, however, this may be inadequate because antibody production either fails or is significantly delayed.7

The ideal situation for the diagnosis of acute T. gondii infection in pregnancy involves collection of serum sample at the very beginning of pregnancy - or preferably before conception - which is usually not possible.21 Moreover, in most countries, routine testing for antibodies to Toxoplasma in pregnancy is performed only in suspected cases. The test or the combination of tests chosen to be performed in such an antenatal screening should ideally be able to determine if the infection occurred after conception.21 The first serum sample is usually taken at the first antenatal health care visit confirming pregnancy, which is usually between the eighth and thirteenth weeks of gestation. Thus, if seroconversion is the only accepted criterion for the diagnosis of T. gondii infection, then the first gestational weeks accounting for 25–30% of the total duration of the pregnancy will not be covered by the serologic test aimed at the detection of the infection.20

PCR has been consistently used to detect DNA of T. gondii in various biological samples and has shown high sensitivity in the diagnosis. PCR has been shown to be a potentially powerful diagnostic method compared with culture, which is insensitive and time-consuming.1 Therefore, PCR was used in this study in comparison with the routinely used serologically test (IHA) in most of our laboratories, in a trial to assess their reliability to detect acute Toxoplasma infection during pregnancy. In the present work, the sensitivity of PCR for purified T. gondii DNA has been found to be very high as it was able to detect and amplify a single tachyzoite DNA. This attributed to the fact that, the B1 gene contains 30-35 copies of repetitive sequences in every T. gondii trophozoite.1 Therefore, this reported sensitivity, combined with the high specificity offered by the PCR led us to search for the presence of the T. gondii genome in maternal blood in cases of pregnancy included in the present study, thus concluding the presence of active toxoplasmosis. In this study, a simple and rapid procedure for the isolation of DNA from maternal blood by microwave oven was used, where extraction was completed in as little as 15 minutes. The method had been used by Cheyrou et al13 to isolate DNA of hepatitis B virus from serum samples, where the DNA was reproducibly used for PCR amplification.

The clearance time for Toxoplasma DNA from the blood of patients with acute toxoplasmosis was estimated to be 5.5–13 weeks.25 Based on this, the presence of Toxoplasma DNA in the maternal blood most probably indicates a recent infection or an apparent parasitaemia. This suggests that the 68 positive cases (40.5%) in this study by PCR whether positive or negative by IHA were recent infections. The negative results obtained by both the PCR and serology in 56 cases in this study (table 1) ruled out an infection in these women. Out of the remaining 44 cases, included in the present study, 2 of them showed no evidence of infection by PCR, inspite of the presence of anti-Toxoplasma IgG and IgM antibodies. The positive IHA findings could be attributed to the presence of a long-standing immunity to toxoplasmosis or a cross-reactive antibody.26 Anti-toxoplasma IgG antibodies have been reported to persist for a long time, up to years.26 False-positive IgM antibody test results have been reported by other investigators.5,7 In addition, even true positive results must be interpreted with caution.7 Because anti-Toxoplasma IgM antibodies commonly persist well beyond 6 months, positive results are very poorly predictive of infections acquired within the previous 2 to 3 months.26,28 Actually, specific IgM antibodies can be detected in both, the acute and chronic phases of toxoplasmosis, which imposes the need for confirmatory evidence in the case of acute infection.1,20

In total 40 of the cases included in the present study had Toxoplasma DNA in their blood samples, while no antibodies were detected in their sera. After infection, T. gondii DNA appears earlier than the immunological response.1 El-Awady MK et al noticed that in some of their positive Toxoplasma patients, specific anti-Toxoplasma IgM and IgG antibodies were not detected until after treatment. Some times proved acute Toxoplasma infections failed to be detected serologically as reported by many researchers.22,26 Conversely, some women were falsely identified by serological means as being infected, and thus were subjected to unnecessary antim parasitic treatment and even diagnostic amniocentesis.7 These reports emphasize the importance of confirmatory testing after serology.

The relatively high number of positive cases, (68 cases or 40.5% of the study population) of acute toxoplasmosis obtained in this study may be attributed to the fact that in Saudi Arabia, consumption of lamb is greater than that of beef. These animals are usually reared outdoors, which put them at greater risk of environmental exposure than animals reared indoors such as poultry, thus increasing their risk of acquiring T. gondii infection.21 Even during reactivation of the disease, specific IgG antibody titres do not constantly increase and a change in the titre or the presence of IgG is not helpful.20 In the present study, such
a change was noticed in our cases with relatively low titre and in the serologically negative cases, patients that proved to be acutely infected by Toxoplasma as detected by PCR. On the other hand, no agreement was detected between serological results and PCR results. Moreover even in serologically positive cases, no correlation was noticed between the positive PCR results and the titers of IHA. Also, no agreement could be established between the semi-quantitative values of the positive PCR and the serological results (fig. 1 and 6).

Our results significantly correlate IL-12 with the recent Toxoplasma infection as detected by gene amplifying method (PCR) and support the results of other investigators. They recorded after experimental studies that, this cytokine might have an important role in active or/and recent Toxoplasma infection and concluded that, IL-12 is released early in infection and is absolutely essential to control both acute and chronic infections. The significant rise in the IL-12 ELISA concentration in recently infected cases (gene positive, serologically negative group) suggested the possible use of this cytokine as an indicator or marker to suspect recent infection of Toxoplasma gondii in the absence of specific Toxoplasma antibodies.

On the other hand, lethality due to Toxoplasma infection was studied by Mordue et al in 2001, working on murine animal model, inoculated with high virulent Toxoplasma initial inoculums. Unexpectedly, lethal infections were associated with over induction of inflammatory cytokines rather than an insufficient immune response. Lethality was associated with excessive levels of Th1 cytokines in the serum. The findings of Mordue et al indicate that acute virulence in T. gondii is associated with over stimulation of Th1 cytokines with excessive levels of (Th1 cytokines) IL-18, IFN-, and IL-12, thus over induction of Th1 cytokines may be an important general mediator of pathology in toxoplasmosis. It should be taken in consideration that, the elevated level of cytokines during active toxoplasmosis resemble systemic infection with gram-negative bacterial pathogen in that, both stimulate high levels IFN to control infection, however, IFN released in Toxoplasma infection relies primarily on IL-12, while IL-18 is required for induction of an inflammatory pathway that results in control of bacterial infection. In contrast, infection of mice with a low dose of parasites resulted in an immune response characterized by moderate levels of IFN-, IL-12, and TNF- and leading to eventual control of parasite replication and minimal tissue pathology which and paradoxically are also required for protection.

The previous data call for the importance of this cytokine to follow up Toxoplasma infection during pregnancy, especially for the non immune, high risk group that harbouring the Toxoplasma gene in their blood with absence of the specific Toxoplasma antibodies in their serum (12 cases in our study). Therefore, it could be concluded that, although Th1 cytokines are essential for parasite control, this response must be tightly regulated to prevent lethal immunopathology. Further studies are needed to elucidate the mechanism of immune damage during acute toxoplasmosis could have important implications for the treatment of parasitic infections.

In conclusion, this study highlights the need for a confirmatory test in addition to serology to detect acute toxoplasmosis in pregnant women. Nested (semi- quantitative) PCR amplification of the B1 gene of T. gondii using whole blood is a rapid, sensitive and specific diagnostic procedure and considered a valuable tool for establishing the diagnosis of T. gondii infection in adult females before or during pregnancy, the latter being very important. The serological immune profile is heterogeneous, and may be delayed or even fail to be represented. This makes the search for antibodies an unreliable method for diagnosis. On the other hand, a single PCR-positive sample collected in the first half of pregnancy could confirm a recent infection, even in the presence of serologic results that are difficult to be interpreted due to the lack of sequential follow-up during pregnancy.

On the other hand, the significant rise in the IL-12 that was recorded in the present study with recently infected cases with toxoplasmosis, suggested the possible use of this cytokine as an indicator to suggest recent infection of Toxoplasma gondii in the absence of specific Toxoplasma antibodies. Further studies will elucidate the mechanism of immune damage during acute toxoplasmosis, which might have important implications for the treatment of this parasitic infection.

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Conflict of interest: None.

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