

**Toxoplasmosis: Detection of Serum immunoglobulin by ELISA and placenta DNA by PCR ; a comparative study**  
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**Toxoplasmosis: Detection of Serum Immunoglobulin by ELISA and Placenta DNA by PCR; A Comparative Study**

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**Abstract**

The prenatal diagnosis of congenital toxoplasmosis is important to prevent unnecessary termination of pregnancy. Thus, there is a need for application of laboratory test that is sensitive and specific for the diagnosis of toxoplasmosis. The objective of the present study was to check the validity of determination of serum specific IgG and IgM anti-toxoplasma antibodies in comparison to PCR method for detecting toxoplasmosis DNA in placenta samples. Twenty one women suffered from abortion were included in this study. Their ages ranged from 20 -35 years. Cases of abortion due to other causes were excluded. Sera and placentae were collected from all participants. Specific IgM and IgG anti-toxoplasma antibodies were determined using capture ELISA technique according to the manufacturer's instructions. Nested primer sets were used for amplifying fragments of the B1 gen. Specific IgG anti-toxoplasmosis antibodies were detected by ELISA in 17 (77.2%) women out of 21 while specific IgM anti-toxoplasmosis antibodies were detected in 8 (36.3) women out of the 21 examined sample. However, PCR detected toxoplasmosis DNA in 20 (95.4%) out of 21 placental samples.

Nested PCR amplification of the B1 gene of *T. gondii* is a rapid, sensitive and specific diagnostic procedure and considered a valuable tool for the diagnosis of *T. gondii* infection in adults females. Detection of specific IgG and IgM anti-toxoplasma antibodies in serum of women with abortion was with higher specificity. However, their sensitivities were 85% and 40% for serum IgG and IgM respectively.

**Keywords :** *Toxoplasma gondii* , ELISA .PCR.

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**داء المقوسات: الكشف عن مصل الغلوبولين المناعي بواسطة تقنية اليزا والحمض النووي للمشيمة  
بواسطة طريقة PCR ; دراسة مقارنة**

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**الخلاصة**

يعتبر التشخيص المبكر لداء المقوسات الولادي من الفحوصات المهمة وذلك لمنع حدوث ولادات لاجنة مصابة بداء المقوسات الولادي . ولهذا السبب ظهرت هناك حاجة ملحة لايجاد فحوصات مختبرية حساسة ودقيقة لتشخيص داء المقوسات. وهدفت الدراسة الحالية لاجراء مقارنة بين طريقتين مهمتين في تشخيص داء المقوسات وهي 1- تحديد الكلوبولينات المناعية IgM و IgG في المصل 2- طريقة PCR لتحديد مدى دقة هذين الفحصين في التشخيص المختبري . تمت في الدراسة الحالية اقناع احدى وعشرون سيدة يعانين من الاجهاض للمشاركة في الاختبارات اعلاه تراوحت اعمارهن بين 20- 35 سنة وتم استبعاد حالات الاجهاض التي كانت لأسباب اخرى . جمعت عينات المصل والمشيمة منهن وتم قياس IgM و IgG باستخدام تقنية الاليزا ELISA . واستخدمت مجموعة البادئات لتضخيم اجزاء من جين B1 . اظهرت نتائج الدراسة الحالية ان 17 سيدة كانت موجبة في الكلوبولين المناعي IgG وبنسبة (77.2%) بينما كانت 8 سيدات موجبة في الكلوبولين المناعي IgM وبنسبة (36.3%) . واطهرت نتائج الـ PCR في المشيمة الاصابة بداء المقوسات في 20 سيدة وبنسبة (95%) . نستنتج ان وجود الكلوبولينات المناعية الخاصة بداء المقوسات في مصل النساء المجهضات كانت ذات خصوصية عالية . الا ان حساسيتها للاختبار كانت 85% و 40% للـ IgM و IgG على التوالي.

**الكلمات المفتاحية:-** داء المقوسات ، اليزا، PCR

**Introduction**

Toxoplasmosis is caused by the obligate intracellular parasite, *Toxoplasma gondii*: It is estimated that at least 500 million people worldwide are infected with *T. gondii* Cats are the final hosts of this parasite . Sexual reproduction takes place only in the primary host, leading to excretion of infective oocysts that can be consumed by humans while gardening or by the consumption of insufficiently washed fruits or vegetables . In an alternative pathway, humans can be infected by consumption of insufficiently cooked infected meat. A further source of infection is placental transmission of *T. gondii* from an acutely infected mother to her unborn fetus (congenital toxoplasmosis). Congenital toxoplasmosis may cause abortion and serious damage to fetus, with severe neurological disorders and Therefore an accurate diagnosis of toxoplasmosis during pregnancy and early treatment is crucial [1].

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Human infection may occur either by the congenital or acquired routes. Congenital toxoplasmosis is caused by transplacental transmission of *T. gondii* tachyzoites, whereas acquired infection results from ingestion of food, soil, or water contaminated with *Toxoplasma gondii* cysts or oocysts [2].

The reliability of the detection of *T. gondii* DNA in amniotic fluids (AF) or blood is of utmost importance. Real-time PCR assays have recently emerged as a dramatic improvement in the reliability of PCR assays. Since the reaction tubes need not be opened after amplification, avoiding potential contamination of the environment with amplicons, the risk of positive results are dramatically reduced. Quantitative PCR also provides additional data to direct the choice of specific treatments [3].

Real – time PCR also provides the opportunity to compare the sensitivity of different DNA targets. Several studies report a relatively high level of false negative results in prenatal diagnosis or huge differences between immunocompromised patients using current intermittent presence of the parasite in the specimens tested, or by poor performance of the PCR assay[4]. The use of more repetitive but not polymorphic DNA targets may help to resolve this issue [5].

The detection of the parasite DNA by PCR has considerably improved diagnosis, particularly the prenatal diagnosis of congenital disease [6] the PCR for identifying toxoplasmosis remains unsatisfactory for the following reasons. (i) only in-house PCR assays are available and they are associated with lack of standardization and variations of efficiency [4]. (ii) for most toxoplasmosis infections, the diagnostic sensitivity of this molecular method remains low, e.g., 50 to 80% for prenatal diagnosis and (iii) the technical specificity is not high for many assays due to the presence of misleading spurious amplification products, implying the need for an additional step for confirmation of the identity of the PCR product[7]

Among the many factors influencing the outcome, the choice of the DNA target and primers is generally considered essential. Few DNA target loci have been described for *Toxoplasma* PCR, but more than 25 different primer pairs have been used in different assays, most of them targeting the repetitive 35-copy –number B1 gene [5, 6]. Thus, this study was conducted to check the validity of determination of serum specific IgG and IgM anti-toxoplasma antibodies in comparison to PCR method.

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**Participants and Methods**

Twenty one women suffered from abortion were included in this study and their ages ranged from 20-35 years. Abortive cases due to other causes were excluded. Sera and placental samples were collected from all participants in the study. A weight of 300 g of placenta were ground, trypsinized, filtered and washed three times with normal saline. Aliquits of 1ml placental samples were lysed in 3 volumes of TNN lysis buffer (0.5% Tween 20 , 0.5% Nonidet P-40, 10 mM NaOH) with 10 m M Tris and 320 µg of proteinase K/ ml for 3 to 16 h. The DNA was extracted by simplified phenol-chloroform method. Following the centrifugation and washing steps, total DNA was eluted from the spin columns with 100 µl of elution buffer, and aliquots were directly transferred to a PCR reaction [7] . The research protocol was approved by the Tikrit University from 1/10/20132 to1/2/2014 . Informed written constant were taken from all participants in the study.

**IgM and IgG detection**

Specific IgM and IgG anti- toxoplasma antibodies in serum were determined using capture ELISA technique (ETI-TIOXOK-M) reverse, Sorin Biomedica , France) following the manufacturer's instructions.

**Polymerase chain reaction (PCR)**

Nested primer sets were used for amplifying fragments of the B1 gen. The outer primers are from bases 171 to 190 (5'-CCG TTG GTT CCG CCT CCT TC-3') and bases 602 to 583 (5' GCA AAA CAG CGG CAG CGT CT-3') producing an amplified product of 432 bp . Inner primers are from bases 180 to 196 ( 5'-CCG CCT CCT TCG TCC GTC GT-3') and from bases 392 to 372 (5'- GTG GGG GCG GAC CTC TCT TG-3') producing an amplified product of bp [8] .The first 50 Ml PCR reaction mixture contained outer primers at a final concentration of 50 poml each, 20 mmol /L d NTPs and 1.25 U recombinant tag DNA polymerase in 1× PCR reaction buffer (50 mmol/L KCL and 10 mmol / L tris -HCL , 1.5 mmol/L MgCL<sub>2</sub> , 0.1 % triton ×100) (DynAzyme TM). PCR amplification was performed 2 minutes at 94 °C for 1 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 5µl of the first PCR reaction in a mixture containing the inner primes at final concentration of 50 pmol each, 20 mmol /L dNTPs. 1.25U recombinant taq DNA polymerase in 1× PCR reaction buffer. Amplification was carried out at 94 °C for 2

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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 . The amplification products were detected by gel electrophoresis using 3% agarose gel in 1× tris – borate – EDTA buffer. DNA band were visualized using 0.5% ethidium bromide in the presence of ultraviolet light [8, 9]

### Results

Some examination using ELISA shows that 17 (77.2%) women out of 21 were with detectable increase in the specific IgG titer . In addition specific IgM antibodies were with detected in only 8 (36.3%) women out 21 study population . However , toxoplasma DNA was detected in 20 (95.4) placental samples out of the 21 tested samples by using PCR .The validity of the ELISA test for IgG and IgM indicated a high specificity (100%) for both antibodies . However, their sensitivities were 85% for specific IgG and 40% for IgM antibodies. In addition, the positive predictive value for specific IgG was 100% , while its negative predictive value was 25% . Concerning IgM the positive predictive value was 100% , while the negative predictive value was 8%. (Table 1)

**Table 1. Validity of ELISA test for detection of serum IgG and IgM- anti-toxoplasma antibodies in comparison to the detection of placental DNA by PCR technique .**

Variable	IgM	IgG	PCR
Frequency of positivity	36.3%	77.2%	95.4%
Sensitivity	40%	85%	Reference test
Specificity	100%	100%	Reference test
Positive predictive value	100%	100%	Reference test
Negative predictive value	25%	8%	Reference test

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### **Discussion**

The prenatal diagnosis of congenital toxoplasmosis is important to prevent unnecessary termination of pregnancy. Very early infection of the mother (within two weeks of the last menstrual period) possess little or no risk to the fetus, which is important since when first seen, most participants are at least two months pregnant. A positive screening test (for IgG and IgM ) could be interpreted as showing a very low risk if the titer of IgG remained stable in two specimens obtained three weeks apart and run in parallel, with the first sample obtained before the 10<sup>th</sup> week of pregnancy . In this situation, a consistently high IgG titer indicates that the infection was most probably acquired more than two months earlier. Although it is impossible to conclude that the risk is absolutely zero, since cases of congenital toxoplasmosis due to maternal infection before pregnancy have been described, that possibility dose not seen to warrant further prenatal diagnostic testing. Several studies have described the results obtained with the PCR in the diagnosis of toxoplasmosis using P30 and B1 gene targets or a segment of the 18S ribosomal DNA. [10].

In the present study , the sensitivity of PCR detection of *T. gondii* DNA was high enough to detect approximately the DNA of one trophozoite. However, *T. gondii* DNA was detected in 20 (95.45%) participants out of 21 clinically diagnosed as having toxoplasmosis . Also anti-toxoplasma IgG antibody was detected in the sera of 17 patients (36.3%) . PCR has been consistently used to detect DNA of *T. gondii* in various biological samples and has shown high sensitivity (single tachyzoite ) in the diagnosis [11] . PCR has been shown to be a potentially powerful diagnostic method compared with culture, which is insensitive and time-consuming [12]. The potential of PCR to diagnose active toxoplasmosis is of great help in immunocompromised patients , especially when serological techniques failed . The sensitivity of PCR for *T. gondii* DNA has been found to be very high because the B1 gen contains 30-35 copies of repetitive sequences in every *T. gondii* trophozoite [11,12].

The presence of anti-toxoplasma IgM antibody in the chronic stage of infection has been reported , especially in pregnant women . Therefore, confirmatory tests are needed either by additional tests or by demonstration of a significant rise in antibody titers in serial serum samples obtained at a minimum of 3-weeks intervals.

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Moreover, specific IgG antibody titers do not constantly increase during reactivation of the disease, but a change in the titer or the presence of IgG is not helpful [10].

In the present study such a change was noticed in some patients, which increased the difficulty in defining the efficacy of the treatment. The immune-diagnosis of the infection with *T. gondii* has been studied by various research groups around the world. For anti-*T. gondii* IgG detection, the "gold standard" method used until now has been the Sabin-Feldman test [13]. In addition, various methods such as commercially available IFA and ELISA, employing whole parasites or extracts derived from tachyzoites have been used to detect IgM and IgG antibodies against *T. gondii*. However, there are problems with the detection of parasite-specific IgM and the precise identification of the acute toxoplasmosis; specific sensitivity may be too weak to detect low IgM antibody levels, or the assay may detect IgM antibodies that persist for a long period after the primary infection [14]. This is a serious matter, since women that acquired infection early before pregnancy can be diagnosed as primarily infected during gestation, resulting in unnecessary chemotherapy [7, 11]. It has already been reported that the main targets for *T. gondii*-specific IgM in sera from patients with acute toxoplasmosis are simple method was previously developed to enrich or purify these membrane compounds and test

them as antigens in an indirect ELISA [15]. In the present study, detection of specific IgG antibodies by ELISA was with high sensitivity and specificity. However, the test was with high positive predictive value but with lower negative predictive value. In conclusion, nested PCR amplification of the B1 gene of *T. gondii* is a rapid, sensitive and specific diagnostic procedure and considered a valuable tool for the diagnosis of *T. gondii* infection in adults females. Diagnosis of *T. gondii* infection during pregnancy is very important. Serological immune profile is heterogeneous, and may be delayed or fail to be represented. This makes it an unreliable method for diagnosis and / or treatment follow-up.

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