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Diagnostic study for Toxoplasmosis by Serological test and PCR technique in Kirkuk province

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Abstract

Background: Severe Toxoplasma gondii infection in early pregnancy brings the risk of transferring the infection to the unborn baby with serious abnormality. The aim of identifying toxoplasmosis in early stage is to recognize mother-to-infant transmission for early cure to avoid undesirable complication. Using serological test to determine anti-Toxoplasma specific IgG and IgM antibodies possibly may not identify new or even previous infection. However, many molecular techniques are now being extensively used through the world among these methods polymerase chain reaction PCR technique emerged as the most widely accepted method for directly detection infectious agents. Thus, the goal in this study is to compare immunoassay by indirect (IgM and IgG) detection diagnostic technique and conventional PCR as molecular assay in diagnosis of *T. gondii* in whole blood of aborted females in Kirkuk province-Iraq.

Methods: Here we applied indirect routine serological diagnosis test for detection toxoplasma specific IgM and IgG antibodies in females blood with spontaneous abortion and compare the efficiency of this method with PCR technique to detect the pathogenic protozoan *T. gondii* based on identification of (the B1 gene) as a target. Eighty-one females were incorporated in this study with a history of recurrent spontaneous abortions. All of these cases were limited to only females in the reproductive age (19-44 years). There are several serological diagnostic test kits available in Iraqi markets; however, the sensitivity and effectiveness of these commercial kits are not examined by most of the laboratories before they are obtained. Therefore, blood samples from female patients with single or repeated abortion were tested for detection specific anti-Toxoplasma antibodies using commercially available (Medical-Biozec) kit in Kirkuk province and compare that with the results are obtained from conventional PCR assay.

Results: The results showed that the conventional-PCR analysis is remarkably more sensitive than serological test, with a relatively high proportion of the positive samples for PCR compared with the serological diagnosis. The serological test results revealed that anti-*T. gondii* antibodies were detected in 14.81% of aborted women, 3.7% of them were positive for IgM, while 11.11% were positive for IgG. On the contrary, conventional PCR analysis found that 44.44% of aborted women showed positive result for B1 gene of *T. gondii*. Despite this, most of positive serology-aborted women exposed positive result for B1 gene of *T. gondii*, except two cases include false-positive IgM results.

The conventional-PCR confirmed 29.62% more positive samples than serology test.

In conclusion: Using PCR technique in diagnosis of active toxoplasmosis in congenital toxoplasmosis cases is necessary especially when serological techniques almost fails. In this study, we applied for the first time a conventional PCR assay based on *T. gondii* B1 gene detection in aborted women as a diagnostic technique in Kirkuk province. The developed PCR was valuable and sensitive technique to make the diagnosis of *T. gondii* infection or toxoplasmosis in women with recurrent abortions, which permits an early diagnosis of most cases and should be recommended.

Introduction

Toxoplasmosis is a zoonotic protozoa disease caused by the intracellular parasite *Toxoplasma gondii*. Nicolle and Manceaux identified this obligate intracellular protozoan parasite in 1908 in a North African rodent [1].Toxoplasma is a parasite with diverse distribution existing in different hot and humid areas. This parasite has ability to grow in wide-ranging vertebrate hosts. The various types of serological tests have proved that this disease is a major health problem in many parts of the world.

In general, T. gondii infections are asymptomatic and self-limiting especially among healthy immunocompetent hosts. However, in immunocopromised patients and pregnant women severe disease occurs. Moreover, the parasite may develop acute infection and severe complication during pregnancy that can cause a variety of other signs of fetal damage [2]. Although the majority of infants appear to be healthy at birth, considerable long-term sequelae may become noticeable only months or years later. Congenital toxoplasmosis can cause fetal death or severe ocular sequelae and neurological complication, such as hydrocephalus, microcephalus, or blindness[3]. Due to the risk of T. gondii congenital infections in the unborn child and its consequence in the newborn, the diagnosis of toxoplasmosis should be done early and accurately.

Still today, the main diagnostic techniques for the toxoplasmosis are serological techniques. However, serological methods have many limitations to diagnose this disease since the specific anti-*T. gondii* antibodies are not produced at early stage of parasitemia [4]. For that reason, using serological methods sometime could produce untrusted results in pregnant women leading to undetected miscarriage or congenital toxoplasmosis [5].

Generally, in the patients with severe and chronic toxoplasma the infection stage and strain of the parasite can be determined by IgG, IgM, IgE and IgA antibodies [6].

There are many other diagnosis techniques but the molecular methods such as PCR amplification have been recognized as excellent and sensitive technique to diagnose congenital toxoplasmosis in immunodeficient patients in much reduced time [5]. Most researchers have used B1 gene for recognition *T. gondii* in various biological samples [3]. The PCR technique can identify tachyzoites in amniotic fluid

(AF), placenta, cord blood [7, 8]. Recent studies have shown that testing amniotic fluid using PCR technique is valuable for detection fetal *T. gondii* infection [9].

In this study, we verified the efficacy of a PCR assay to detect Toxoplasma infections in blood samples of women with recurrent abortion in Kirkuk province and whether it can be used as a confirmatory diagnostic test comparing with the serological testing for specific anti-*T. gondii* IgM and IgG antibodies

Materials and Methods

Patients

The study was conducted at the Obstetrics and Gynecology department of the hospitals and private clinics in Kirkuk province- Iraq, over a 6-months period between 1st of March to December 30th 2016. The study group comprised 81 women aged between 19- 44 years and medium age of 30years, who had spontaneous abortion and whom referred with a physician report for TORCH tests to decide the final diagnosis of pregnancy loss or miscarriage.

Different information about the patients were collected according to questionnaire sheet before the collection of blood samples. Three ml of venous blood was collected from redial vein from each patient and kept in sterile tubes with anticoagulant in deep freezing conditions until DNA extraction and amplification by using PCR method.

Detection of anti – Toxoplasma IgG and IgM Test by commercially available kits (Medical-Biozec kit):

The kit for toxoplasma antibody detection test (Medical-Biozec kit) was obtained from purchase sources (Biozek- Medical B.V.Vissenstraat 32, 7324AL Apeldoorn- Netherlands). The one-step test can be performed using whole Blood/ Serum/ Plasma. The test should be performed immediately after specimen collection (whole blood was used in this study): Prior DNA extraction the samples were subjected for anti-Toxoplasma IgG and IgM antibodies detection. The whole blood of all cases were tested for existence of specific anti-*T. gondii* IgM and IgG antibodies via (Medical-Biozec kit) according to the manufacturer's instructions.

Isolation of genomic DNA from whole blood:

There are numerous techniques with different commercially presented kits that are used for DNA purification from blood. However, Bartlett and White [10] method is one of the routinely protocol used for both scientific research and clinical purposes and it is also inexpensive and robust. Therefore, this protocol for genomic DNA extraction from whole blood were followed in the current study.

<u>Materials</u>

This method includes standard substance that can be obtained from any suppliers:-

Reagent A:

Red blood cell lysis: 10 ml of 1M Tris-HCL, 0.47 g $MgCL_2$, 109.4 g of sucrose, and 10 ml of Triton X-100 were added to 800 mL of distilled water. The pH of the solution was adjusted to 8.0, then made up to 1 L with distilled water and sterilized by autoclave at 121°C for 15 min.

Reagent B: Cell lysis buffer: 400 mL of 1 M Tris-HCL (pH 7.6) was mixed with 120 ml of 0.5 M EDTA (pH 8.0) and 8.76g of NaCL. The pH of the mixtures was adjusted to 8.0 before it made up to I L with distilled water. Next, the mixtures were sterilised using autoclave at 121°C for 15 min, and then 10gm of sodium dodecyl sulphate was added to the sterile solution in each tube.

Methods

DNA extraction

The frozen whole blood samples were defrosted and mixed well. 300 µL from each sample was put in a 2mL sterile glass tube and 900 µl of R.B.Cs lyses buffer (reagent A) was added and mixed well for 5 min at room temperature, then the tubes were centrifuged at (4000 rpm) for 20 min at room temperature. The supernatant was discarded from the top of each tube, taking care not to disrupt the cell pellet; residual wetness was removed by upsetting the tube and spoiling slightly onto tissue paper. For all tubes, 650 µl of cell lysis buffer (reagent B) was added and mixed gently until the suspension become clear. To resuspend the cell extract, the tubes were mixed gently and 100 µL of 5 M sodium perchlorate was added for each tubes and mixed well by inverting them several times. Subsequently, the tubes were placed in water bath for 60 min at 65°C with gently inverting few times during incubation. Following that, the tubes were cooled at room temperature before adding 3 mL of ice-cold chloroform and mixing on a rotating mixer for 35 min and finally the tubes were centrifuged at 4000 rpm for 20 minutes. The solutions supernatant from each tube was moved into a clean sterile stopper glass tube while the palette was discarded.

For DNA precipitation and purification, 5 μ L of chilled absolute ethanol was added to each tube and mixed well until the cotton-like clump of DNA precipitate was begun to form at this point. Then formed DNA was washed twice using 2 mL of chilled 70% ethanol and the tubes were centrifuged at (4000 rpm) for 10 min. Followed that, the supernatant was decanted carefully and the white precipitate allowed to air-dry by reversing them on a paper towel (usually about 5–10 min). Finally, the DNA precipitate was liquefied in a suitable volume of distilled water and

stored at 4°C for future use. The DNA purity and concentration was measured using a NanoDropTM spectrophotometer.

PCR amplification of Toxoplasma

The conventional PCR was targeted *T. gondii* B1 gene in all DNA, which existed in 35 copies and well preserved among all of the tested strains. In accordance with Van de Ven et al. [3] method, the primers used in this study were on B1 gene (133 bp), where the (forward) primer is 5' TTGCAT AGGTTGCAGTCACT-3' and the (reverse) primer is 5' TCTTTAAAGCG TTCGTGGTC-3'. This gene is about 35-fold repetitive, and its function is unknown.

PCR assay was used according to Al-Sanjary and Hussein [11]. The amplification reaction mixture final volume was 25 μ L which contained 1 U of Taq DNA polymerase, KCl 30 µM, 10 µM Tris-HCl (pH 9), MgCl2 1.5 µM, deoxynucleoside triphosphate at a concentration of 250 µM, 10 pmol primer, and 50 ng of genomic DNA. Amplifications were carried out with thermal cycler, the reaction's programme consisted of one denaturation step at 94°C for 5 min, followed by 40 cycles consisting of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1.5 min. A final elongation step at 72°C was achieved for 10 min. Amplification products were visualized in 1.5 % agarose, using gel electrophoresis stained by Ethidium bromide. A Ladder of 100 bp DNA was used as a size marker in the gels.

Results

Eighty-one females who have had spontaneous abortions (mean age 30.53 ± 6.34 years) from Kirkuk city were enrolled for this study. They all had abortion early on or later into their pregnancy with single or recurrent abortion. All 81 women were screened for active Toxoplasma infection by investigation of the existence of specific IgM and IgG anti-*T. gondii* antibodies using Medical-Biozec kit. It had been found that the positive IgG only detected in 9 women (11.11%), and IgM only in 3 (3.7%) cases, these findings revealed an overall of 12(14.81%) positive toxoplasmosis and 69(85.18%) negative toxoplasmosis women (table 1.).

The serology test finding for IgM and IgG anti-T. gondii antibodies of examined whole blood was subsequently subjected to PCR. Almost all of the positive anti-T. gondii antibodies cases showed B1 gene of T. gondii except 2(46%) of blood samples revealed false- positive anti-Toxoplasma IgM result. Positive samples were detected by presenting a 133 bp band of B1 gene amplification test on 1.5% agarose gel (Fig 1). PCR analysis was positive in 36 (44.44%) cases of the total DNA samples, which is relatively, higher proportion compared with serological diagnosis by only 12 (14.81%) cases. The conventional-PCR confirmed 24(29.62%) more positive samples.



Figure 1. Amplification of 133 bp from Toxoplasma gondii DNA in the blood of aborted women. Lane M, molecular weight marker (100 bp ladder), Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13 (positive samples). Running conditions: agarose gel (1.5%), 5 v/cm for 2 h, stained with ethidium bromide

Table 1: Distribution of studied groups according to	the
anti-Tavanlasma- antihadias	

anti-Toxopiasma- antibodies									
Test	Positive		Negative		Total				
	samples		samples						
	No.	%	No.	%					
Serology test									
IgG	9	11.1	72	88.9	81				
IgM	3	3.7	78	96.3	81				
Total	12	14.8	69	85.1	81				
PRC test	36	44.4	45	55.6	81				

Regarding the relation between Toxoplasma infection and the age of patients, a positive connection was found between the age of aborted women and the positivity of PCR (table 2). This means that infection with *T. gondii* is more frequent in females above 26 years. Women in the 26-37 year age range revealed (22/81) positive cases than among women under 25 years of age, as it can be seen in table 2.

 Table 2: Distribution of Toxoplasma infection according to age using PCR technique

Age groups (year)	PCR	Positive mples	PCR Negative (+) samples		
() ••••)	No.	%	No.	%	
19-25	6	7.4	15	18.5	
26-31	11	13.5	10	12.3	
32-37	11	13.5	14	17.2	
38-44	9	11.1	6	7.4	

Discussion

T. gondii infection can be diagnosed by different methods such as indirect serologic tests, direct molecular method by amplification of specific nucleic acid sequences such as polymerase chain reaction (PCR), immunoperoxidase stain method which histologically demonstrate the parasite and/or its antigens, or by isolation of the parasite from different infected tissue [12]. In particularly, PCR technique for diagnosis of *T. gondii* DNA in body fluids and

tissues has been effectively used to identify congenital toxoplasmosis. PCR was first developed for diagnosis of congenital toxoplasmosis in amniotic fluid [13] and the superiority of this technique in diagnosis belong to its sensitivity to recognize the parasite DNA even at very low-density infections [14].

The accurate number of women at risk of *T. gondii* infection during pregnancy in many countries including Iraq is not well known and in particular, the distribution of Toxoplasma infection in Kirkuk province has not been well documented. However, several previous serological studies found different prevalence rates of toxoplasmosis in the Kirkuki-women population [15-17]. The variances in the previous serology results might be attributed to the differences in the diagnosis method, source and type of Toxoplasma kits, and times of sample collection and processing [15].

In spite of the large number of commercial kits to detect the existence of anti-*T. gondii* antibodies in infected people, these tests often have lower sensitivity or specificity comparing with other methods, and the reported results can be wrong or misinterpreted. Therefore, we compared the accuracy of *T. gondii* diagnosis between two different techniques, Medical-Biozec kit, which is commercially available kit, and PCR technique.

In the present study, the rate of *T. gondii* infection using PCR technique was 3 times higher in Kirkuki aborted women compared to those with serology test by Medical-Biozec kit since the incidence of anti-Toxoplasma antibodies in the aborted women was 12(14.81%), while PCR test positive results was 36(44.44%). *T. gondii* DNA was detected in 10/12 of women with spontaneous abortions who were positive for anti-*T. gondii* antibodies 9/9 IgG and 1/3 IgM with two cases include false-positive IgM results. Although 29.63% of aborted women showed evidence of infection by PCR technique, anti-*T. gondii* antibodies IgG or IgM were not detected in the same infected patients by Medical-Biozec kit. This result evaluated the accuracy and specificity of PCR analysis for detecting Toxoplasma infection in aborted women blood.

The existence of specific *T. gondii* IgM antibodies in blood samples of aborted female were 3 (3.7%) cases, but just 1 (1.2%) of IgM-positive case of the infection was confirmed due to the presence of the parasite, especially PCR analysis demonstrated the existence of the parasite DNA in the same patient blood. IgM anti-T. gondii antibodies are usually refer to acute infection and considered a sensitive indicator to presence of new or recent infection. Unfortunately, during T. gondii infection the IgM can be seen approximately 14 days post-infection and decline to undetectable levels after several months to more than a year later [18]. On the other hand, 2 (2.46%) of blood samples revealed false- positive anti- T. gondii IgM result since PCR technique did not detect the parasite DNA in these cases. Even though positive IgM result indicate current infection, it may also be false positive reaction [19]. IgM test results are hard to interpret, and the consistency of test kits is mainly depend upon other factors. IgM antibodies may persist for several months after an active or acute infection (up to 1 year). Therefore isolated IgM positive result is not an absolute value for current infection, as IgM can be "residual" or a false positive that might be caused by cross-reactivity or nonspecific reactivity [19, 20].

Toxoplasma DNA was also found in 9 (11.11%) of abortive women with positive IgG which indicates that the infection is chronic toxoplasmosis. It is known that women with inactive toxoplasmosis (positive IgG) present irregular parasitemia with low parasitic burden, but this does not distinguish between a recent infection or ancient infection [21].

In the contrary, Toxoplasma DNA was detected in Toxoplasma-seronegative 24(29.63%) aborted women by PCR technique. This result might indicate to the early phase of infection prior to antibody titers are at detectable levels at the time of serology test leading to negative serology result. The other explanation is that the serology test may be ineffective to detect T. gondii infection in special immunodeficient patients because the titers of specific anti-Toxoplasma antibodies may not increase in this kind of patient. Based on this, the existence of small number of released parasite from the infected tissue leads to presence small amount of Toxoplasma DNA in the peripheral blood that possibly can be recognized only by PCR technique. In addition, PCR technique just demands a small amount of DNA for amplification as initial template or target sequence, therefore Toxoplasma infection can be recognized even if the parasite DNA that were mixed with

patient whole blood was in a very small quantity in the clinical samples.

The sensitivity of B 1 gene amplification by PCR technique may be due to the repetitive nature of the B 1 gene, of which 25-50 copies are present in the genome of *T.gondii* and the amplification of a single specific fragment of this gene is sufficient to give a positive result [22, 23]. The PCR looks to be the most sensitive procedure in recognition *T. gondii* infection and it has been showed success in detection and amplification of *T. gondii* B 1 gene in ocular fluids and retinal sections [24].

In addition, the result of Toxoplasmosis serological test is low reliable because sometime it is hard to be interpreted and the constancy of diagnostic test kits is variable [12]. The most common problem with the diagnosis kits is the failure to detect low titers of antibody. In addition, the other problem of some commercial serological kits for IgG/IgM anti-Toxoplasma antibodies is high rates of unacceptable false-positive test results or even negative results because of the low specificity of the diagnostic kits [25].

A positive result of serological test is only indicative to the contact with the toxoplasma antigen, while direct *T. gondii* DNA detection in blood or other clinical samples using molecular technique such as PCR technique absolutely approves the parasite existence in the samples. Therefore, used PCR technique in this study offers a specific and sensitive process for diagnosis *T. gondii* in clinical samples. The conclusion of this study is in good agreement with earlier reports that PCR technique is advised over serologic techniques for toxoplasmosis diagnosis [26].

Relating to age, women above 26 years showed considerably high incidence of toxoplasmosis than under 25 Years of age. Overall, the frequency of antibody seropositivity of T. gondii in our study was almost one in every two aborted women above 26 years of age. The result of our study does not mean that older age female is a risk factor for T. gondii infection, but it proposes that the older females are more susceptible to the parasite than younger females. This can be explained by longer exposing to the causing agent by older person than younger one resulting in constant level of anti-Toxoplasma antibodies in the serum for years [27, 28]. Moreover, increased number of high-risk pregnancies because of delayed childbearing has also continued to rise in recent years [29].

Conclusion

Serological detection of specific anti-Toxoplasma immunoglobulin is mainly serological technique to detect *T. gondii* infection in Kirkuk province, and this method vary in sensitivity and specificity depending on commercially available serologic kits which may fail to detect infection especially in some immunocompromised patients. For the first time in Kirkuk province, we performed convential PCR technique to detect toxoplasmic DNA in whole blood samples of female patients with spontaneous abortions. The results described in this study showed that the PCR technique is rapid and reliable technique **References**

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دراسة تشخيصية لداء المقوسات الكوندي Toxoplasma gondii باستخدام التشخيص السريري وتقنية ال PCR في محافظة كركوك

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الملخص

الإصابة بداء المقوسات Toxoplasma gondii في فترة الحمل المبكر تؤدي الى نقل الإصابة الى الجنين والتي تؤدي الى حدوث مضاعفات خطيرة. الهدف من التشخيص المبكر لداء المقوسات هو لمعالجة الام الحامل في الفترة المبكرة للاصابة ومنع انتقال العدوى من الام الى الجنين. المحاولات التشخيصية المصلية للكشف عن الإصابة المضادة IgG و IgG ضد المقوسة الكوندية احتمال تفشل في الكشف عن الإصابة الحديثة او حتى عن الاصابات السابقة في المرأة المصابة بالعدوى. في الوقت الحاضر الدراسات الجزيئية الحديثة والتي تستخدم حاليا في التشخيص المختبري للعصابة من التشخيصية المصابة بالعدوى. في الوقت الحاضر الدراسات الجزيئية الحديثة والتي تستخدم حاليا في التشخيص المختبري للعديث من الاصابة ومنع انتقال العدوى من الام الى الحديثة او حتى عن الاصابات السابقة في المرأة المصابة بالعدوى. في الوقت الحاضر الدراسات الجزيئية الحديثة والتي تستخدم حاليا في التشخيص المختبري للعديد من الامراض ومن ضمنها تقنية التفاعل المتسلسل للبوليميريز PCR اظهرت وحققت نجاحا للكشف عن الإصابة من بين مختلف المختبري للعديد من الامراض ومن ضمنها تقنية التفاعل المتسلسل للبوليميريز PCR اظهرت وحققت نجاحا للكشف عن الإصابة من بين مختلف التقنيات الجزيئية. لذلك الهدف من الدراسة الحالية هو المقارنة بين الطريقة المصلية التقليدية (الكشف عن اضداد IgG و IgM و IgM و التفنية الجزيئية الجزيئية. لذلك الهدف من الدراسة الحالية هو المقارنة بين الطريقة المصلية التقليدية (الكشف عن اضداد IgG و IgM و

في هذه الدراسة تم مقارنة نتائج التشخيص المصلي لتقليدي في الكشف عن الاضداد ضد طغيلي المقوسات في دم النساء المصابات بالاجهاض المتكرر ومقارنة جودة النتائج مع تقنية التفاعل المتسلسل للبوليميربز PCR اعتمادا على الكشف الجين B1 الموجود في الطفيلي.

تم أخذ عينات الدم من 81 سيدة حامل مصابة بالاجهاض (اجهاض واحد او متكرر) ممن يشتبه بإصابتهن بداء المقوسات من مختلف مستشفيات والعيادات الطبية الخاصة في مدينة كركوك . جميع الحالات كانت مقتصرة على النساء في عمر الانجاب 44-19 سنة. هنالك عدة أنواع من الطرق التشخيصية Kits المتوفرة في الأسواق العراقية لتشخيص داء المقوسات لكن معظم هذه الاختبارات لم يتم تقييم جودتها ودقتها في الكشف عن الإصابة. لذلك تم مقارنة نتائج الكشف عن الاجسام المضادة باستخدام Kit (Medical-Biozec) التجاري المتوفرفي مدينة كركوك مع تقنية التفاعل المتسلسل للبوليميريز PCR.

تبين من النتائج بان الطريقة التشخيصية الجزيئية PCRكاكثر حسياسة و دقة للكشف عن الطفيلي من الطريقة المصلية. في الطريقة المصلية تم الكشف عن وجود الاجسام المضادة لطفيلي داء المقوسات في دم %14.81 من النساء المجهضات، منها %3.7 كانت تعود ل نوع IgM و 11.1% تعود لنوع IgG. لكن باستخدام تقنية PCR على نفس العينات تبين بأن %44.44 من المجموع الكلي للعينات كان تحتوي على جين Ig مما يؤكد على الإصابة بداء المقوسات. جميع العينات الموجبة للتشخيص المصلي اظهرت نتائج إيجابية للتشخيص الجزيئي PCR عدا عينتين اظهرت نتيجة سلبية للتشخيص الجزيئ. التشخيص الموجبة للتشخيص المصلي اظهرت نتائج إيجابية للتشخيص الجزيئي 29.62 عدا عنائل عنه عن الإصابة بداء المقوسات. جميع العينات الموجبة للتشخيص المصلي اظهرت نتائج إيجابية للتشخيص الجزيئي 29.62 عن المهرت نتيجة سلبية للتشخيص الجزيئ. التشخيص الموجبة للتشغيص المصلي عنه في الكشف عن الطفيلي داء المقوسات بزيادة عن المجموع للكلي لعدد العينات الموجبة للتشخيص المصلي. نذلك يمكن ان نستنتج بان التشخيص الجزيئ ضروري للكشف عن داء المقوسات بالأخص عندما تفشل التشخيص المصلي للكشف عن الطفيلي تحت ظروف الإجهاض المتكرر للمرأة الحامل. في هذه الدراسة استخدمنا لأول مرة التقنية الجزيئية PCR وذلك لتشخيص داء المقوسات في النساء المعرضات للاجهاض المتكرر للمرأة الحامل. في هذه الدراسة استخدمنا لأول مرة التقنية الجزيئية PCR وذلك لتشخيص داء المقوسات في النساء المعرضات للاجهاض في مدينة كركوك. وهذه التقنية اثبتت فعاليتها ودقتها في التقنية الجزيئية PCR وذلك لتشخيص داء المقوسات في النساء المعرضات للاجهاض وي وذلك لمنع الإجباض المرض للجنين مؤدية الى التشخيص والتي تتيح الفرصة للكشف المبكر عن وجود الطفيلي وعلاج المرأة الحامل وذلك لمنع الإجباض المرض للجنين مؤدية الى