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Comparison Between the Efficiency of nested PCR Analysis and IgG Avidity Test in Detection Of Acute *Toxoplasma gondii* Infection in Early Pregnancy in Ramadi city.

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ABSTRACT

Background: Primary maternal infection with toxoplasmosis during gestation and its transmission to the fetus continue to be the cause of tragic yet preventable disease in offspring. ***jective***: This study was aimed to Comparison between efficiency of these two methods(nested PCR and IgG avidity test) in detection of acute toxoplasmosis in pregnant women early in the first trimester is of utmost importance in order to offer them early therapy or other interventions to prevent congenital infection of fetuses. ***Methods:*** One hundred sixteen blood samples were collected from pregnant women at different ages, and in the first trimester of pregnancy. These women have history of habitual abortion, intrauterine fetal death and congenital anomalies of the fetus. Blood samples were tested for specific anti-*Toxoplasma* IgG avidity test and detection of *B1* gene of *T. gondii* by nPCR. ***Results:*** In this study, it was found out that the rate of Toxoplasmosis by nested PCR 48 cases (41.4%),while by T. IgG avidity test was 76cases(65.5%) (high avidity 54.3% and low avidity11.2%). The detection rate by nested PCR was significantly higher than by T. IgG avidity test. ***Conclusion***: PCR technique is more sensitive and specific than T.IgG avidity tests.

Introduction:

Toxoplasma gondii is a zoonotic protozoa with worldwide distribution that infects humans and a wide range of mammalians and birds [(1)]. *Toxoplasma gondii* is the only species in the genus and, hence, is simply referred to as“Toxoplasma” Although most infections in humans are asymptomatic, severe complications may occur after congenital Toxoplasma infection such as abortion, stillbirth, mortality and hydrocephalus in newborns or retinochoroidal lesions leading to chronic ocular disease as well as lymphadenopathy , retinitis or encephalitis in immunocompromised persons due to postnatally acquired infection[(2)].

The parasite is known to cause congenital diseases and abortion in both of humans and livestock. Maternal toxoplasmosis during early pregnancy may lead to death of fetus [(3)]. Acute primary maternal toxoplasmosis if acquired during the first trimester of pregnancy can cause significant morbidity and mortality in developing fetuses [(4)].

Infection typically occurs through ingestion, either of parasitic cysts present in undercooked meat or oocysts shed by infected domestic cats. Less common routes of transmission include organ transplantation and blood transfusion [(5)]. The diagnosis of infection by T. gondii is carried out by the detection of specific anti-Toxoplasma immunoglobulin (IgM and IgG) and to discriminate chronic from reactivated

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infection, IgG avidity is also determined with VIDAS instrument bioMerieux, France),moreover, the diagnosis of toxoplasmosis using tissue samples, blood, and urine is done detecting T. gondii DNA by a Real-Time PCR Fluorescence Resonance Energy Transfer (FRET), targeting T. gondii 529 bp repeated Region [6].

The most often used target for PCR detection is the 35 fold repetitive B1 gene (Burg et al., 1989). PCR is the only method that can detect T. gondii organisms in low numbers (10 organisms per ml) and can detect a partly destroyed parasite [7]. There for in this study we select two advance methods to compare between more efficiency of them in the detection of early infection for successful treatment.

Methods:

Study group:

The cases were collected during the period from 1st December 2012 to the end October 2013,in the Al- Ramadi maternity and pediatric hospital, They were included 120 women, who had spontaneous abortion; whom referred with a physician report for TORCH tests to determine the final diagnosis of pregnancy loss. Relevant information about the cases and the possible risk factors were recorded for each woman. Any woman undergoing therapy against the disease

was excluded from the study.

Imunological test:

The sera of all cases were tested for the presence of specific IgG anti- Toxoplasma antibodies via IgG avidity test kits) biomerieu Company, Spain according to the manufacture's instructions.

Isolation of genomic DNA from whole blood:

DNA was extracted from the whole blood samples of the study groups using a commercial purification system) Wizard Genomic DNA purification kit Promega) following the manufacture's instruction for DNA purification from blood. Purified DNA molecules were resuspended in 100 µl of DNA rehydration solution (TE buffer 10) mM Tris-HCL, 1mM EDTA, pH=8). and stored at -80○C, after estimation of DNA concentration and purity thereafter they were separated by agarose gel electrophoresis.

Amplification and detection of Tgondii DNA by Nested PCR (nPCR assay)

According to Burge et al. (1989). [8] method, nested PCR was performed for all DNA samples to amplify a fragment of B1 gene by two steps with different primer pairs as follows:

First round:

The primers used in the first round correspond to nucleotides 694-714 and 887-868 with the sequences: 5'- GGAACTGCATCCGTTCATGAG-3' and 5'-TCTTTAAAGCGTTCGTGGTC-3' (Promega, USA) respectively. These primers used to amplify 193bp of B1 gene. 3µl of DNA template were added to a final reaction volume equal to 25µl consisting of 2.5µl PCR buffer (10 mMTris-HCL, 50 mM KCL), 1µl MgCl2, 1µldNTPs mix, 2µl for each primer, 0.25µlTaq polymerase (5000 U/ml) and13.25µl of H2O (nuclease free). Negative control reaction in each experiment was set up containing all components of the reaction except template DNA

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.

Second round

Nested reaction was performed to amplify 96 bp of B1 gene by using the primers which correspond to nucleotides757-776 and 853-831 with the sequences5'-TGCATAGGTTGCAGTCACTG-3',5'-GGCGACCAATCTGCGAATACACC-3'respectively. Three micro liters of the first round products were used as a template for the second round PCR with the same components of the master mix in a total volume of 25µl. Negative controls of sterile water were included in the nested reaction. The cycling condition for nPCR were 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 1min, and a final extension at 72°C for 10min.PCR product was analyzed on 2% agarose gel at 5 v/cm for 2hours and stained with novel juce

Statistical analysis:

Data were analysed with chi-square and P value ≤ 0.05 was considered statistically significant.

Results:

Table (1), shows the detection rate of toxoplasmosis in pregnant women, by nested PCR analysis and T. IgG avidity test. in this study, it was found out that the rate of Toxoplasmosis by nested PCR 48 cases (41.4%),while by T. IgG avidity test was 76cases(65.5%) (high avidity 54.3% and low avidity11.2%). The detection rate by nested PCR was significantly higher than by T. IgG avidity test.

Table (1): Comparison between the positivity of toxoplasmosis using nested PCR analysis and T. IgG avidity test

Total No.	nested PCR		T. IgG avidity test	
116	No.	%	No.	%
	48	41.37	76	65.51

Comparison between the two diagnostic tests:

Comparison between IgG avidity test and nested-PCR assay to confirm ongoing or recent *Toxoplasma* infection in the selected group of pregnant women. Toxoplasmosis positivity in both testes are 35(46%),and negative in both testes 27(68%).while Toxoplasmosis positivity in IgG avidity test and negative in nested PCR are 41(54%), the toxoplasma negative in IgG avidity test while positive in nested PCR are 13(33%), the sensitivity and specificity for T. IgG avidity test as follow:Sensitivity: 72% , Specificity: 39%

Table (2): Comparative between the results of nested PCR analysis and T . IgG avidity test.

			Nested PCR Analysis		Total
			positive	negative	
avidity test group	positive	Count	35.00	41.00	76.00
		%	0.46	0.54	1.00
	negative	Count	13.00	27.00	40.00
		%	0.33	0.68	1.00
Total		Count	48.00	68.00	116.00
		%	0.41	0.59	1.00

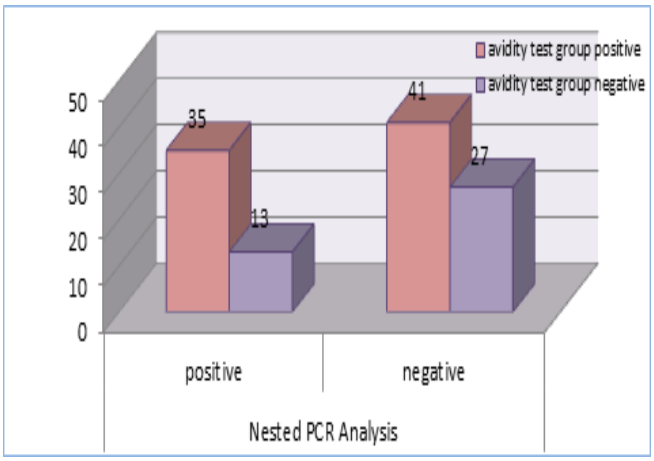

Figure (1): Comparative between the results of nested PCR analysis and T . IgG avidity test.

Amplification of B1 gene of *Toxoplasma gondii* DNA from the blood:

Amplification of B1 gene target by polymerase chain reaction and by agarose gel electrophoresis 2%, showed that, out of 116 specimens of toxoplasma48 (41%) were positive for B1 gene while 68 (59%) were negative.

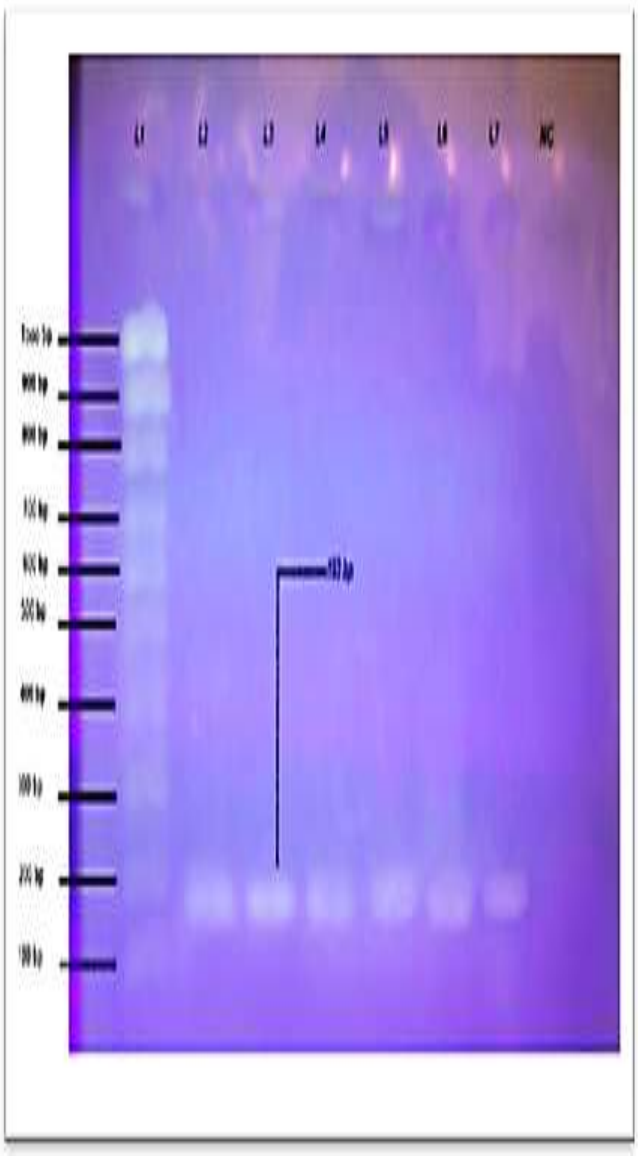

Figure (2): Amplification of B1 gene of Toxoplasma gondii DNA from the blood of the infected women. Lane-1, molecular weight marker (100 bp ladder), Lanes-NC negative control, Lanes 1-7 positive samples at 193bp in the first round of nested PCR, Running conditions: Agarose gel (2%), 5 v/cm for 1hrs, stained with novel juice.

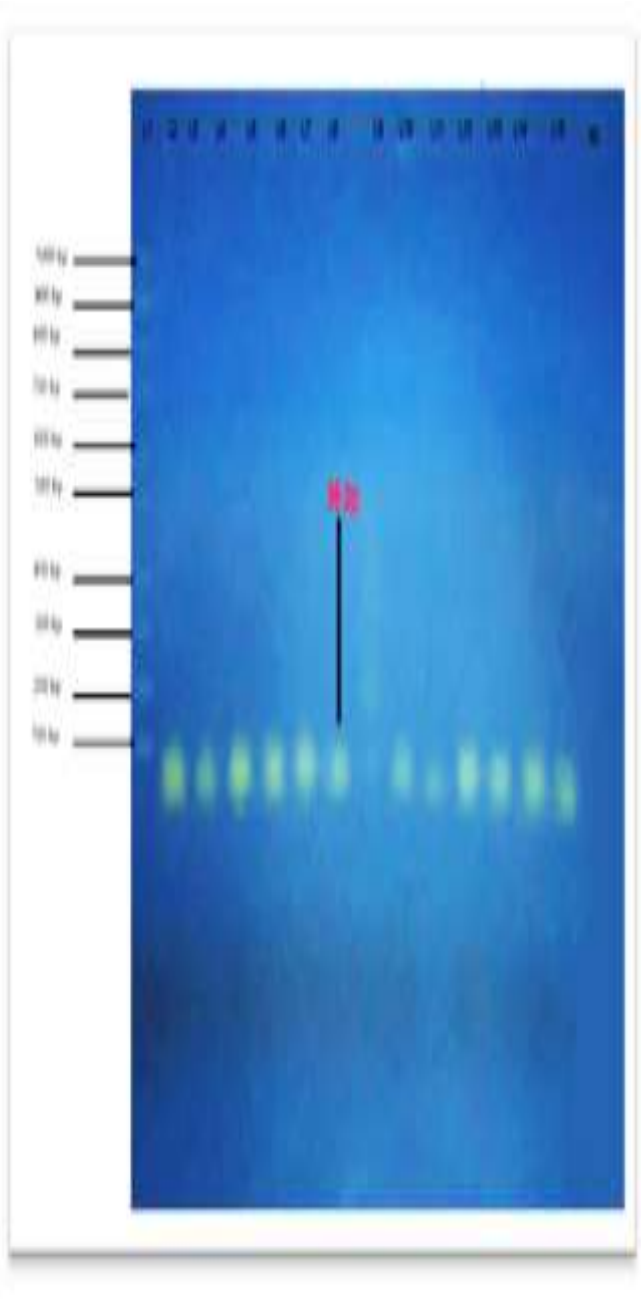

Figure (3): Amplification of B1 gene of Toxoplasma gondii DNA from the blood of the infected women. Lane-1, molecular weight marker (100 bp ladder), Lanes-NC negative control, Lanes 1-15 positive samples at 96bp in the second round of n-PCR, Running conditions: Agarose gel (2%), 5 v/cm for 1 hour, stained with novel juice.

Discussion:

Toxoplasmosis can cause significant morbidity and mortality rate in the developing fetuses and in immunecompromised patients. For both conditions, a rapid and accurate diagnosis is required in order to start the relatively efficient antiparasitic treatment [(9)]. Serological diagnosis of toxoplasmosis

provides high sensitivity, but specificity varies depending on the test used [10]. Diagnosis of maternal infection is by serology testing IgG & IgM. Since IgM can remain positive for several months after the primary infection, this test may not provide useful information to document recently infection during pregnancy.

The study shows that positivity by nested PCR 48 cases (41.4%) while T.IgG avidity test was 76% {high avidity 63cases was (54.3%)} suggesting past infection and low avidity{13 cases was (11.2%)} suggesting an acute infection warranting appropriate therapeutic intervention and invalid 40 cases was (34.5%) suggesting do not detectable antibody originally , in T. IgG avidity test the rate of sensitivity and specificity was low (72%, 32% respectively) this means that the false positive and negative was high rate. Compared with nested PCR dependent the specificity of B1 gene is 100%. The B1 specific gene probe used for the detection of *T. gondii* [11].

The results of this study showed that the detection rate for Toxoplasmsis by nested PCR was significantly more than by T. IgG avidity test, where The rate by in PCR 48 cases (79%) while in IgG avidity test was 35 cases (46%)from a total of 116, these results are consistent with the study that observed by Hideto in japan [12].

The study disagree with those observed by Iqbal in Kuwait [13]. This result can be explained by several studies that have reported that, PCR could detect parasitaemia a few weeks prior to the appearance of any clinical signs or symptoms [14]. PCR is highly sensitive and specific because of single trophozoite can be detected in a clinical sample [15].

In the present study true positivity by nPCR 48 cases (79%) while the same samples by IgG avidity test 35 cases were (46%) this suggested that the presence of both specific antibodies and DNA indicate the possibility that Toxoplasma gondii infection occurred during the current pregnancy especially if the serum sample was collected within the first 20 weeks of gestation.

In the present study, the target user to investigate was B1 gene..the specificity of B1 gene is 100%. The B1 specific gene probe used for the detection of T. gondii does not cross-react with other microorganisms found in immunocompromised patients such as Sarcocystis spp., Neospora spp., Plasmodium spp., Aspergillus spp., Candida spp., and Cryptococcus spp [16]. The low percentage of Sensitivity and Specificity for T. IgG avidity test may explain that it is rare in woman who got toxoplasmosis before getting pregnant will pass the infection to her fetus, because she will have built up immunity to the infection. It can occur through, if a pregnant woman who's had a previous infection becomes immunocompromised and her infection is reactivated[17].

Although immunological testing has been one of the major diagnostic for toxoplasmosis, it has many limitations, it may fail to detect specific anti Toxoplasma IgM or IgG during the active phase of infection, because these antibodies may not be produced until after several weeks of parasitemia . Furthermore, the test may fail to detect T. gondii infection in certain immunocompromised patients due to the fact that the titers of specific anti Toxoplasma antibodies may fail to rise in this type of patient. Indeed detection of T. gondii DNA using nested PCR minimizes the problems faced when using serodiagnostic assays and facilitates diagnosis in difficult cases [18].

Generally, a negative PCR result indicates that there was no *T.* gondii DNA in the sample. also, it can indicate that the PCR was inhibited and malfunctioned. PCR failure may have a variety of causes, e.g. instrument failure, errors during preparation of the reaction mixture, degraded or non functional reagents, organic solvents or other PCR inhibitory substances still present in the DNA extract. an amplification control is usually used to identify such false negative results [19].

While in the second test, which depends on the immunoglobuline may be the same person who does not produces immunoglobuline for the reasons mentioned in the above therefore loses Specificity ,and Increases the error rate .

Conclusions:

- PCR technique is more sensitive and specific than T.IgG avidity tests and.
- Blood specimen is a safe and non-invasive method for early detection of toxoplasmosis in pregnant women.

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مقارنة كفاءة اختباري nested PCR وIgG avidity test في الكشف عن داء المقوسات الكوندية في مرحلة مبكرة من الحمل في مدينة الرمادي

سعيد عراك تركي سراب فوزي العاني رجاء جهاد الحديثي

الخلاصة

الاساس : العدوى الأولية للأمهات مع داء المقوسات أثناء الحمل وانتقاله إلى الجنين لا تزال قضية مرض يمكن الوقاية منها ومن المأساوية في النسل. الهدف: هدفت هذه الدراسة إلى المقارنة بين كفاءة هاتين الطريقتين (PCR وIgG avidity test) في الكشف عن داء المقوسات الحاد في النساء الحوامل في وقت مبكر من الحمل في الأشهر الثلاثة الأولى أمر في غاية الأهمية من أجل ان تقدم لهم العلاج المبكر أو التدخلات الأخرى لمنع العدوى الخلقية للأجنة. الطرق: تم جمع مائة و ستة عشر عينات دم من النساء الحوامل في أعمار مختلفة، وفي الأشهر الثلاثة الأولى من الحمل. هذه المرأة لديها تاريخ من الإجهاض المعتاد، وفاة الجنين داخل الرحم والتشوهات الخلقية للجنين. تم اختبار عينات الدم لمحددة للكشف عن داء المقوسات الكوندية لل IgG avidity test والكشف عن الجين B1 من المقوسات الغوندية بواسطة nPCR. النتائج: في هذه الدراسة، فقد وجد أن نسبة من داء المقوسات بواسطة PCR n 48 حالة (41.4٪)، في حين كان من 76عينه. 65.5) IgG avidity test (54.3٪) (٪ عالية ومنخفضة avidity11.2٪) . كان معدل الكشف عن طريق PCR أعلى بكثير من قبل IgG avidity test الاستنتاج: تقنية PCR هي أكثر حساسية وتحديدا من T.IgG avidity test