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Validity of B1 Gene in Diagnosis of Prenatal and Postnatal infection with *Toxoplasma gondii*

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Abstract :

Toxoplasma gondii is one of the most frequent parasite all over the world. Most patients are asymptomatic, except in immunosuppressed individuals and pregnant women , which can be seriously injured. Prenatal diagnosis should be made rapidly in view of the fact that treatment of the mother can reduce fetal sequelae.

The present study was used Rapid test as a serological method to detected IgG and IgM antibodies titers and the Nested polymerase chain reaction technique (nPCR) in 74 samples of blood were collected from pregnant women whom with history of abortion. The aborted cases were 33 out of 74, while 41cases were reach birth, 15 cases were early birth (29-36 week) period and 26 cases were reach birth with normal pregnancy period (37-42 week).

The result of Rapid test reveled that IgM and IgG were positive in pregnant women 29/ 74 and 47/74 respectively , while the nested polymerase chain reaction technique (nPCR) result showed 36/74.

The Nested polymerase chain reaction technique (nPCR) was also done on the (41) newborn babies. The total number of newborn babies with congenital anomalies were 16 and the positive result of (nPCR) in newborn showed 17. The validity of B1 gene in prenatally toxoplasmosis showed the sensitivity, specificity, positive productive value and negative productive value were (91%, 81% ,78% and 100%) respectively, while the sensitivity, specificity, positive productive value and negative productive value in postnatally were (88%, 83%, 94% and 96%) respectively.

The aim of study was the early finding congenital toxoplasmosis of maternofetal transmission, for early treatment to avoid unwanted sequelae using nPCR technique in support of early diagnosis *T. gongii* in the prenatal of congenitally and to evaluate these results to the postnatal infection.

Introduction:

Toxoplasma gondii is an intestinal obligate intracellular protozoan that parasitizes members of the cat family as definitive hosts and has a wide range of intermediate hosts, infection is common in many warm-blooded animals, including humans (1).

Infection acquired during pregnancy may cause severe damage to the fetus (2). Congenital toxoplasmosis, is worldwide infection, can differ from asymptomatic to serious illness, to avoided neonate infection can be attained by information of the pregnant, management of pregnant women and already early treatment of the infected newborns to reduce the widen rates and clinical manifestations (3). Detecting *T.gondii* DNA in the body fluid is a valuable diagnostic method for the prenatal diagnosis of congenital toxoplasmosis(4 and 5), earlier diagnosis is made and the better ending because it is safer, dependable and the consequences are more quickly obtained than the conventional diagnostic method (6 and 7).

T.gondii infection can be diagnosed indirectly with serological methods and directly by Polymerase Chain reaction (PCR), hybridization, isolation, and histology (8). Whereas indirect serological methods are generally used in immunocompetent patients, perfect diagnosis in immunocompromised people is mostly undertaken by direct detection of the protozoan (1). Congenital toxoplasmosis is the result of transmission through placenta of *T.gondii* tachyziote from an highly infected mother (9). Congenitally infection occur when the parasite pass through placenta

and the classical signs and symptoms, such as hydrocephalus, retinochoroiditis and cerebral calcifications or they can be asymptomatic and develop during childhood or adult life (10).

The diagnosis of *T.gondii* by detection specific anti-*Toxoplasma* immunoglobulin (IgM and IgG) and to differentiate acute from reactivated infection ,the presence of IgG antibodies indicates past infection, but the level of IgG antibody reactivity does not indicate when that infection occurred (11). Testing for maternal IgM antibodies is the most common method used worldwide in the effort to determine if and when a pregnant woman has experienced acute infection with toxoplasmosis (11, 12 and 13). A negative IgM will rule out the presence of acute infection, but a positive result requires careful understanding (11).

Material and Methods:

Samples collection:

Seventy four (74) blood samples were collected from women in whom with recurrent abortion. A routine serological screening program performed used immunochromatographic strip to detected IgG and IgM titers in the Maternity and Childhood Teaching Hospital and in Al-Dewanyia province.

Forty one (41) cases were reach birth while a thirty three (33) were aborted, a blood samples were also collected from 41 a new born babies whom with or with out congenital anomalies. All the samples were divided into two tubes 1st with anticoagulant for serological test 2nd without anticoagulant for molecular test.

Serological test:

Initially IgM and IgG were detected using Rapid test which performed to the pregnant women, this test was done according to the kit instruction (CTK Biotech, Inc. USA) this test was acceptable as a golden standard test.

DNA extraction:

DNA was extracted by DNA extraction kit, the step was done as the fallowing according to the Sacace Biotechnologies instruction(Sac.Bio.Tech. Germany).

Nested polymerase chain reaction technique (nPCR):

Nested polymerase chain reaction technique (nPCR) was performed using two set of primers that related to gene B1 of the *Toxoplasma gondii*, leading to amplification of 193 and 96 bp fragments the primers were choose According to (14 and 15).

Table 1 –B1 Primers Sequence, Product size and Target region which were used in polymerase chain reaction.

Primer	Sequence	Molecular weight	Position
Outer primer (sense strand)	5´GGAACTGCATCCGTTCATGAG-3´	193 bp	694–714
Outer primer (nonsense strand)	5´-TCTTTAAAGCGTTCGTGGTC-3´		887–868
Inner primer (sense strand)	5´-TGCATAGGTTGCAGTCACTG- 3´	96 bp	757–776
Inner primer (nonsense strand)	5-GGCGACCAATCTGCGAATACACC- 3´		853–831

The reaction mixture for the first amplification were done as in the table 2

Table (2) :The mixture for the first amplification

Subject	Amount
Tris–HCl, pH 8.3 (at 25°C),	10 µM
KCL	50 µM
MgCl2	2 µM
Each Outer primer	0.1 µM
dNTP	0. 1 µM
Taq DNA polymerase	1.25 U
purified DNA	11.55 µM
Total	75µM

The mixture was transformed into thermocycler and programmed as the table 3.

Table 3: The program of the first amplification in the thermocycler

Processes	Temperature	Time	No. of cycle
Denaturation	93 ºC	10 sec.	40cycle
Annealing	57 ºC	10 sec.	40 cycle
Extension	72 ºC	30 sec.	40 cycle
final-extension	95 ºC	7 min.	

The reaction mixture for the second amplification were done as in the table 4

Table (4) :The mixture for the nested amplification

Subject	Amount
first-round product	1.15 µl
Tris–HCl, pH 8.3 (at 25°C),	10 µM
KCL	50 µM
MgCl2	3µ M
Each Inner primer	0.5 µM
dNTP	0. 1 µM
Taq DNA polymerase	1.25 U
Total	66µM

The mixture was transformed into thermocycler and programmed as the table 5

Table 5: The program of the Nested amplification in the thermocycler

Processes	Temperature	Time	No. of cycle
Denaturation	93 ºC	10 sec.	40cycle
Annealing	62.5 ºC	10 sec.	40 cycle
Extension	72 ºC	15 sec.	40 cycle
final-extension	95 ºC	7 min.	

All sample of PCR Nested amplified product was electrophoresed on 2% agarose gel containing ethidium bromide (0.5 µg/ml). The gel was examined under UV light (14) .

Test performance Characteristics

The performance characteristics (validity) of a test or criteria, include among others: sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

Fallowed formulas were used in calculation according to (16)

$$\text{Sensitivity} = \frac{\text{Number of true positives}}{\text{Number of diseased people}}$$

$$\text{Specificity} = \frac{\text{Number of true negatives}}{\text{Number of non-diseased people}}$$

$$\text{PPV} = \frac{\text{Number of true positives}}{\text{Number of positive test results}}$$

$$\text{NPV} = \frac{\text{Number of true negatives}}{\text{Number of negative test results}}$$

Result :

From 74 pregnant women with history of abortion; 33 were aborted, 15 cases were early birth (29 - 36 week) and 26 were normal period birth (37- 42week) , the IgM result reveled 29/74 were positive titter, and 47/74 gave positive titter for IgG, while the nPCR result showed 37/74 were positive table 6, Figure 1.

Table 6: The positive result of IgM and IgG titter and PCR for pregnant women

Patient	No. of cases	%	IgM	%	IgG	%	nPCR	%
Aborted	33	45	18	54%	21	63%	20	60%
Early birth	15	20	8	53%	10	13%	9	60%
Nine months birth	26	35	3	11%	16	21%	8	30%
Total	74	100	29	39%	47	63%	36	48%

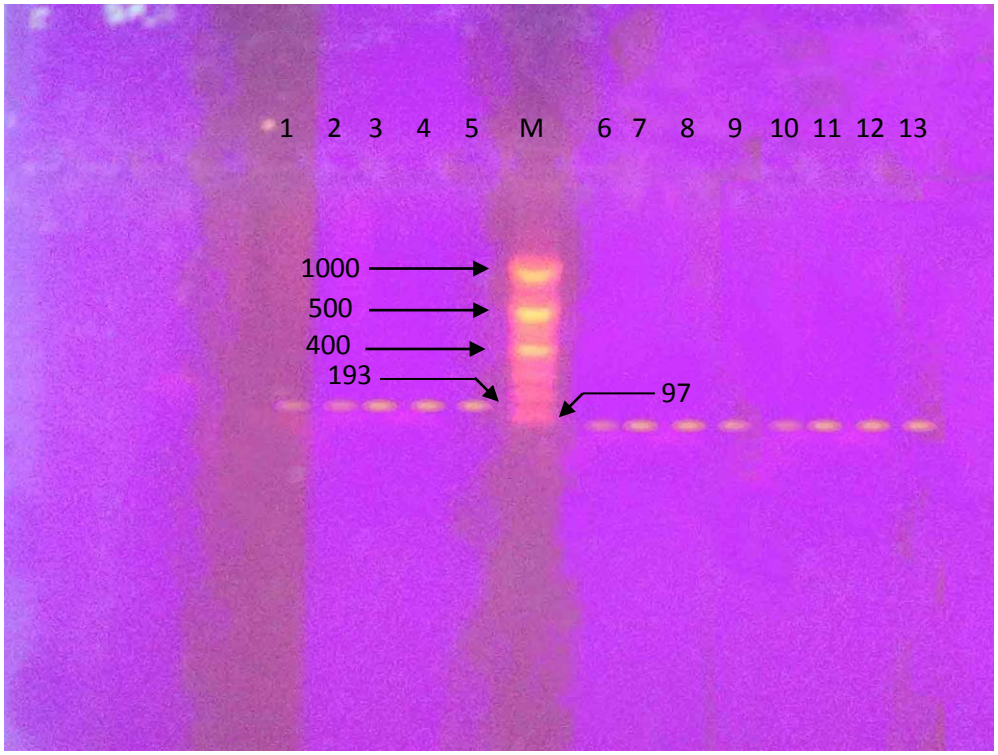

Figure 1: Electrophoresis of nPCR in amplification of B1 gene 1,2,3 and 4: Outer primer amplification in the 1st step of nPCR (193 bp) M: DNA Marker . 6,7,8,9, 10,11,12, 1and 13 Inner primer amplification in the 2nd step of nPCR (97 bp).

From 74 pregnant women with recurrent abortion a 41 reach to the delivery period (early delivery and normal delivery). From the early delivery babies 5/15 with congenital anomalies and 7/15 were positive in PCR while from the normal delivery 11/26 with congenital anomalies and 10/26 were positive in PCR (the congenital anomalies were detected clinically by Gynecologist and Pedestrian or through CT and MRI by Radiologist) Table 7.

Table 7: The result of PCR for new borne babies whom with and without congenital anomalies.

Baby	No. of cases	With congenital anomalies		PCR	
		+	%	+	%
28-29 week pregnancy period	15	5	12	7	17
38-39 week pregnancy period	26	11	27	10	24
Total	41	16	39	17	41

In calculation the validity of B1 gene in prenatally toxoplasmosis showed the sensitivity, specificity, positive productive value and negative productive value were (91%, 81%, 78% and 100%) respectively, while the sensitivity, specificity, positive productive value and negative productive value in postnatal were (88%, 83%, 94% and 96%) respectively table 6.

Table 6: The Validity of B2gene in the postnatal and prenatal toxoplasmosis.

Patient	Sensitivity	Specificity	PPV	NPP
Prenatal (pregnant women)	**91%**	**81%**	**78%**	**100%**
Postnatal	**88%**	**83%**	**94%**	**96%**

PPV= Positive Productive Value
PPN= Negative Productive Value

Discussion:

In present study the abortion rate was revealed 45 %, while other study results showed 38.7% and 29% (11 and 17) on the other hand , it was obtained the abortion rate 7.4% (2).

The current study revealed the IgM positive titter were 39 % and this result was agreed with other study which showed a 37% in pregnant women (11), while other report study revealed 12% (18) , on the other hand the present study showed reactivated toxoplasmosis (positive result for IgG) 63% while other several study reported range from 33 to 66% (18 and 19).

Serology is a sensitive test but is not specific, because serologic studies recommend that asymptomatic toxoplasmosis occurs with huge incidence in the general population (20 and 21), the current study could not conclude the transmission time according to the stage of pregnancy, which can vary widely.

Analysis of local antibody production to confirm a suspected clinical diagnosis of *Toxoplasma* congenitally is a valuable diagnostic tool in the immunocompetent patient; but, false-positive and false-negative results can be a problem (22 and 23).

Besides the detection of specific anti-*Toxoplasma* immunoglobulin, detecting B1 gene of *T. gondii* using nPCR technique was done on the blood samples of both pregnant women and their newborn babies.

The present study, using B1 gene in pregnant women was obtained 91% of sensitivity and 81% of specificity, while the results of other study were obtained the sensitivity 76.2% and the specificity 55% (23), on the other hand the result of recent study was lower than those achieved by (1 and 24).

The false-positive cases could be explained that the PCR can detect *B1* gene of *T. gondii* already broken by drugs used in the management and the false-negatives results could be explained that short time between maternal infection and fetal transmission insufficient, storage of the spacemen and DNA degradation although, PCR was usually performed in 1mL of blood sample , which may not be representative the attendance of parasite, person DNA and particularly blood can interfere in PCR reaction(22).

In the current study revealed the sensitivity and specificity in the newborn babies were 88% and 83% respectively while other reported the sensitivity and specificity of B1 gene in congenital toxoplasmosis were 62.5% and 92.4% respectively (7).

Next birth, kids were clinically followed for 180 days at least, when treatment was intermittent and patients were not seen anymore. Since the patient of sequels appear during grown period , it can be misdiagnosing some cases a slightly may be due to the contamination of the sample by mother blood (24).

It can concluded that a negative prenatal finding based on PCR alone did not always keep out the possibility of congenital infection, as well positive consequences did not forever confirm it. Scientific evaluation of the babies during the first year of life is obviously essential. The final opinion of congenital toxoplasmosis should be based on medical data,
detection of immunoglobulin, nPCR for detecting B1gene of *T.gondii* in blood is a valuable diagnostic method for the prenatal diagnosis of congenital toxoplasmosis because it is safer, dependable and the results are more quickly obtained than the conventional diagnostic technique.

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