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Molecular study of Toxoplasma gondii infection in normal delivery women with previous abortion history at Basrah Province

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ABSTRACT

Toxoplasma gondii is the causative agent of toxoplasmosis, one of the most common parasitic infections of humans and pets. T. gondii was serologically investigated for newborn women who had previous abortion by ELISA and included (70) women with a history of single or recurrent abortion and (30) women with normal delivery without previous abortion as a control group. The study included women attending Basrah Obstetrics and Gynecology Hospital, Al-Moanaa General Hospital, Al-Fayhaa General Hospital, Zubair General Hospital and Dar Al Shifa investment Hospital. The percentage of seroprevalence of T. gondii IgG was (41.4%). No percentage was recorded for IgM antibodies. Significant differences were found between age groups of these antibodies. The examination showed that 7 (23.3%) of (30) in the control group were positive for IgG antibodies, compared to 0 (0%) of IgM. The highest percentage (50%) of IgG antibody was in the age group 20 years or less according to the number of previous miscarriages. The highest percentage of IgG antibody infection was (46.2%) in women who had two previous abortions and no infection rates were recorded for IgM antibody. The highest infection rates (43.8%) were in Zubair district compared to the city center for IgG antibody, and no infection with IgM antibody was recorded. Infection rate (70%) was recorded for the PCR-positive samples using Nested PCR technique. Toxoplasma DNA sequences according to the isolated hosts was carried out. The reading of the genetic tree showed that the sample of women born naturally is identical with the main branch of the kinship tree that carries the sequence MZ717187, MZ717188, MZ717189, MZ717190, MZ717191 in the global gene bank.

1. Introduction

Toxoplasmosis is a zoonotic disease caused by the protozoan parasite Toxoplasma gondii, which is an obligate intracellular capable of infected all warm-blood animals, including humans and birds [1-3]. The disease may result in life-threatening outcomes in risk people such as pregnant women and immunocompromised individuals including cancer patients, people undergoing radiation therapy, transplant recipients, HIV-positive individuals, hemodialysis patients and multi-transfused thalassemia patients [4-6]. Having potential neurotropism, the main effects of the parasite are brain damage, neurological defects, and even encephalitis in immunodeficient people [7-10].

Molecular methods depend on PCR for the specific detection of T. gondii DNA have proved to be simple, sensitive and have been applied to a different clinical samples from animals and humans [11-12].

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When a pregnant woman become acutely infected or has reactivation with *T. gondii* during pregnancy (because of immunosuppression) she can transmit the parasite transplacentally. The risk of occurrence of congenital infection is lowest (10 to 25%) when maternal infection occurs during the 1st trimester and highest (60 to 90%) when maternal infection occurs during the 3rd trimester. The congenital disease is more severe when infection is acquired in the 1st trimester [13].

1. Materials and Methods

2.1. Blood samples collection

The study included (70) women suffering from recurrent abortions had been admitted to Al- Zubair Hospital, Dar Alshifaa Investment Hospital, Basrah General Hospital, Al-Mwanee Hospital, Al- Faiha General Hospital, of Basrah province since February 2020 to 2021. Thirty normal delivery women without abortion history were regarded as a control group. Five ml of venous blood was drawn from each women. Data regarding age, number of pervious abortion and residence had been collected.

Venous blood sample was divided into two volumes, 3ml put into gel tube containing clot activator for serum isolation then transported into centrifuge which was fixed on 3000 rpm for 5 minutes and then centrifuged sera were separated from gel tubes, put in eppendroff tubes and kept at- 20^{o}C to use in subsequent immunological studies. Two ml of whole blood drawn into EDTA vial kept in -20^{o} to use in subsequent molecular study. The Nested polymerase chain reaction Nested PCR were used to detect the *T. gondii* genotype. The work was done in unit of PCR lab in IRAQ – Biotech. Company.

2.2.The Enzyme Linked Immuno Sorbent Assay (ELISA) for the detection of IgM and IgG antibodies:

A total of (100) women blood samples (70 patients, 30 control) were tested for both IgG and IgM according to the protocol of the company.

2.2.1. Antibody Index Interpretation

<0.9 No detectable IgG antibody to Toxoplasma by ELISA
0.9-1.1 Positive result

2.3. Molecular study

The Nested Polymerase Chain Reaction (Nested PCR) were used for blood samples to detect the *T. gondii* genotype. DNA was extracted from 29 blood samples. The extracted DNA was checked by using Nano drop spectrophotometer (U.S.A) which measured DNA concentration (µg/µl) and DNA purity by reading the absorbance at 260/280nm.

2.3.1. Nested PCR reagents preparation

2.3.1.1. Master mix preparation

Nested PCR master mix was done by using PCR premix kit (Bioneer, USA) and according to company instructions.

2.3.1.2. Primary round PCR

The Material	Volume
PCR master mix	25 µl
Template DNA	10 µl
F1: (Forward primer 10 pmol/ µl)	2µl
R1: (Reverse primer 10 pmol/ µl)	2µ
D.D. water	11µl
Total volume	50 µl

Master Mix contents :

Component	Reaction size 50µl reaction
Top DNA polymerase	2.5U
dNTP(dATP,dCTP,dGTP,dTTP)	Each 250µM
Reaction Buffer,with 1.5mM $MgCl_2$	1X

PCR Thermo cycler

First round of amplification was carried out with Nested PCR(10ml template DNA) as follows :

No.	Steps	Time	Temperature	Cycle
1	Initial Denaturation	5 min	94^{o}C	1 cycle
	Denaturation	20 sec.	94^{o}C	
2	Annealing	20 sec.	53^{o}C	30 cycle
	Extension	20 sec.	72^{o}C	
3	Final Extension	5 min	72^{o}C	1 cycle

2.3.1.3. Secondary round PCR

PCR master mix	Volume
PCR master mix	25 µl
Primary PCR product	10µl
F2: (Forward primer 10 pmol/ µl)	2µl
R2: (Reverse primer 10 pmol/ µl)	2µ
D.D. water	11µl
Total volume	50µl

The secondary round of amplification was started with 2ml template from first reaction.

PCR Thermo cycler

Round of amplification was carried out as follows :

No.	Steps	Time	Temperature	Cycle
1	Initial Denaturation	5 min	94^{o}C	1 cycle
	Denaturation	20 sec.	94^{o}C	35 cycle
2	Annealing	20 sec.	45^{o}C	
	Extension	20 sec.	72^{o}C	
3	Final Extension	5 min	72^{o}C	1 cycle

2.4. Gel Electrophoresis

PCR products were analyzed by loading in 1.5% Agarose as following steps:

1. 1.5% Agarose gel was prepared in using 1X TBE and dissolving in water bath at 100°C for 15 minutes , after that, left to cool 50°C.
2. Then 2µl of ethidium bromide stain were added into agarose gel solution.
3. Agarose gel solution was poured in tray after fixed the comb in proper position, after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray and 5µl of PCR product were added into each comb well and 5µl of (100pb Ladder) in one well.
4. The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer.Then electric current was performed at 100 volt and 80 AM for 50 minutes.
5. PCR products were visualized by using ultraviolet transiluminator and the bands were photographed.

2.5. Gel purification

Before PCR products were sent to sequencing, The gel bands were purified by using gel extraction kit as follows:

The DNA bands were cut from the gel using sharp blade. The gel transferred into 1.5 ml eppendrof tube. GSB buffer was added, incubated at 55^0C for few minutes till the gel was dissolved, Then other steps of procedure were followed.

2.6. DNA Sequencing

To identify the genetic variation (Genotype) between *T. gondii* isolate as well as standard NCBI isolates 25 µl volume of PCR product and 17 pica mole for each of the first and second Nested primers were send to Macrogen company, South Korea. The analysis of DNA sequence were done according to genetic analysis V.X. (Mega X) and multiple sequence alignment. Phylogenetic tree was drown according to VPGMA programme.

2.7. Statistical analysis

Chi- square were used to analyzed data obtained in the present study with probability $P \leq 0.05$.

3. Results :

The results of the current study for Enzyme Linked Immuonosorbent Assay (ELISA) revealed that 29 (41.4%) of women with normal delivery whom have recurrent abortion were positive for IgG antibodies, comparing with 0 (0%) of IgM**.**

There were significant differences between IgG values of normal delivery patients according to the age groups, (p. value = 0.001), The higher value is (50%) in IgG at the age 20 years or less, the lower value is (16.7%) in IgG at the age 41-50. The values are (0%) in IgM in all ages, (Fig. 1).

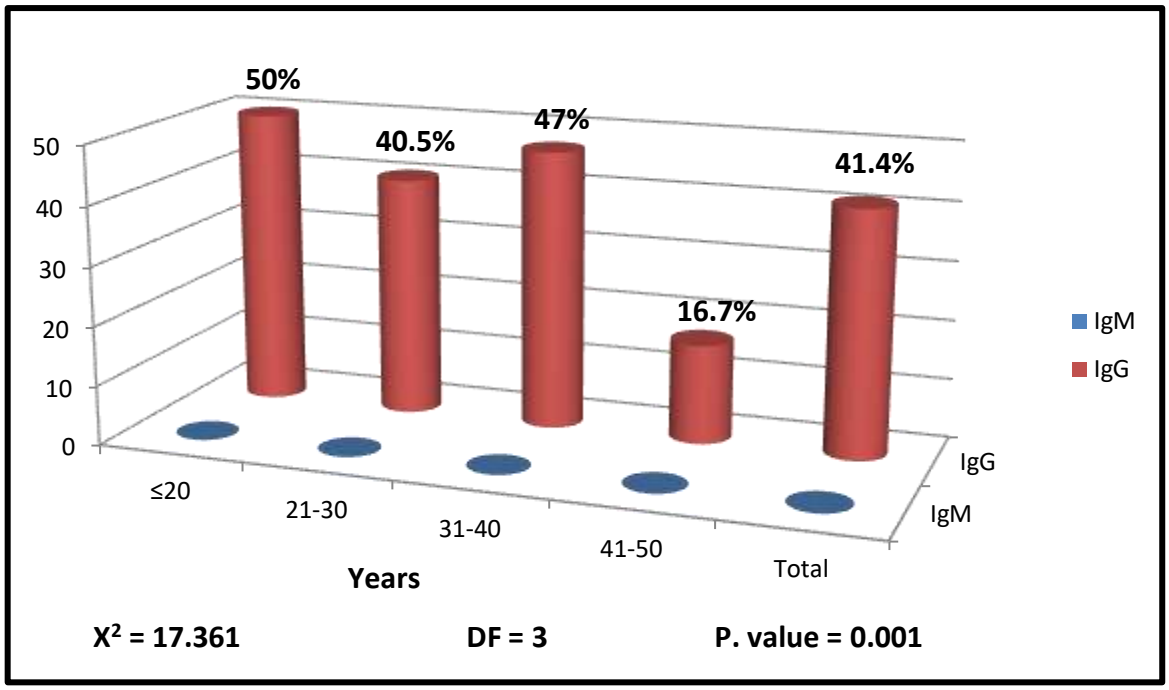

Fig. 1. Seroprevalence of *T. gondii* antibodies of Normal delivery according to the age groups.

The assay revealed that 7 (23.3%) of (30) control were positive for IgG antibodies, comparing with 0 (0%) of IgM**.**

There were no significant differences between IgG values of control according to the age groups, (p. value= 0.257), The higher value is (36.4%) in IgG at the age (31- 40), the lower value is (0%) in IgG at the age (10-20) and the age 41-50. The values are (0%) in IgM in all ages,(Fig. 2).

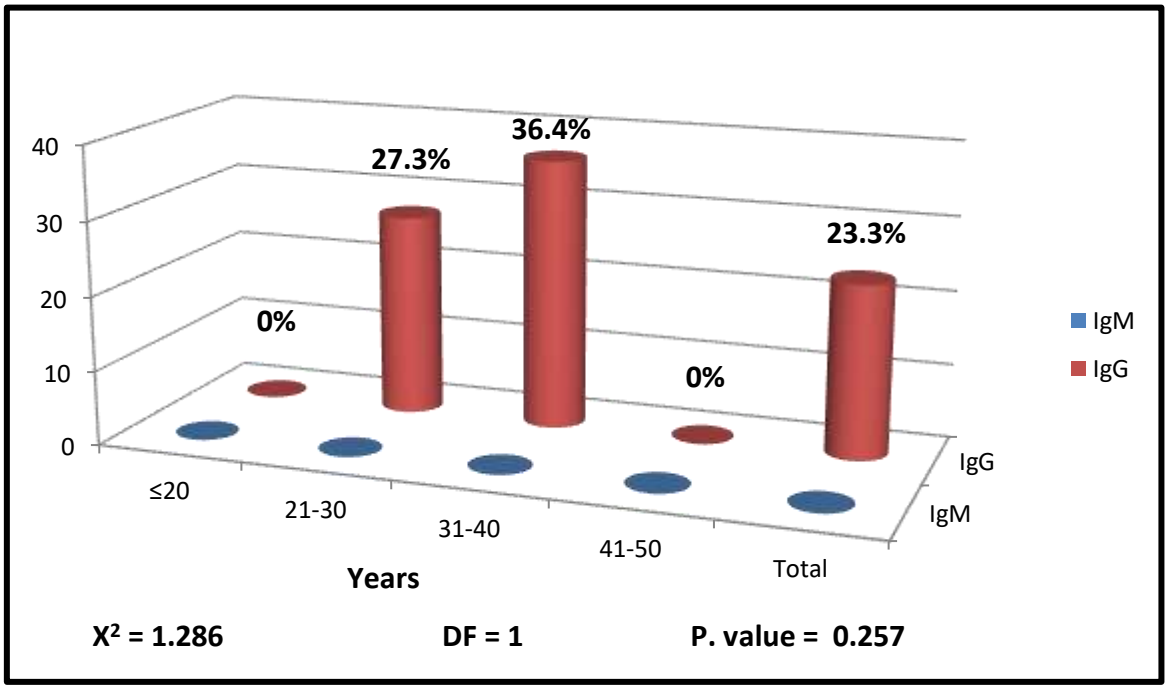

Fig. 2. Seroprevalence of *T. gondii* antibodies of the control according to the age groups.

According to the previous abortion a significant differences between IgG values of normal delivery patients was found, (p.value = 0.001), The higher value is (46.2%) in IgG which had two previous abortion, the lower

value is (18.2%) in IgG which had three previous abortion. The values are (0%) in IgM in all the previous abortion, (Fig. 3).

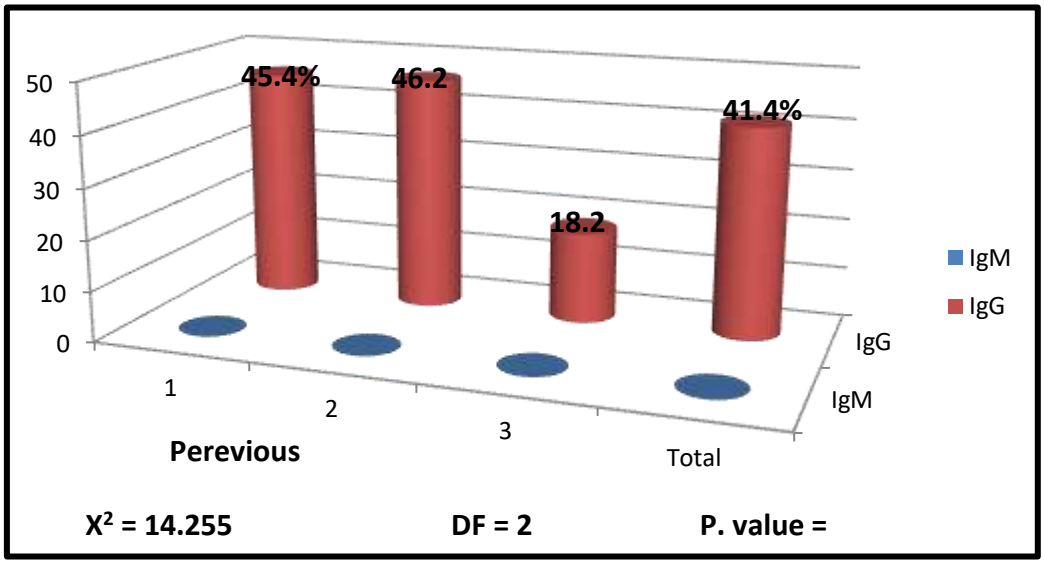

Fig. 3. Seroprevalence of *T. gondii* antibodies of normal delivery patients according to the previous abortion.

According to the locality there were no significant differences between IgG values of normal delivery patients, (p. value = 0.371), The higher value is (43.8%) in IgG on Zubair, the lower value is (36.4%) in IgG on city center. The values are (0%) in IgM in all locations, (Fig. 4).

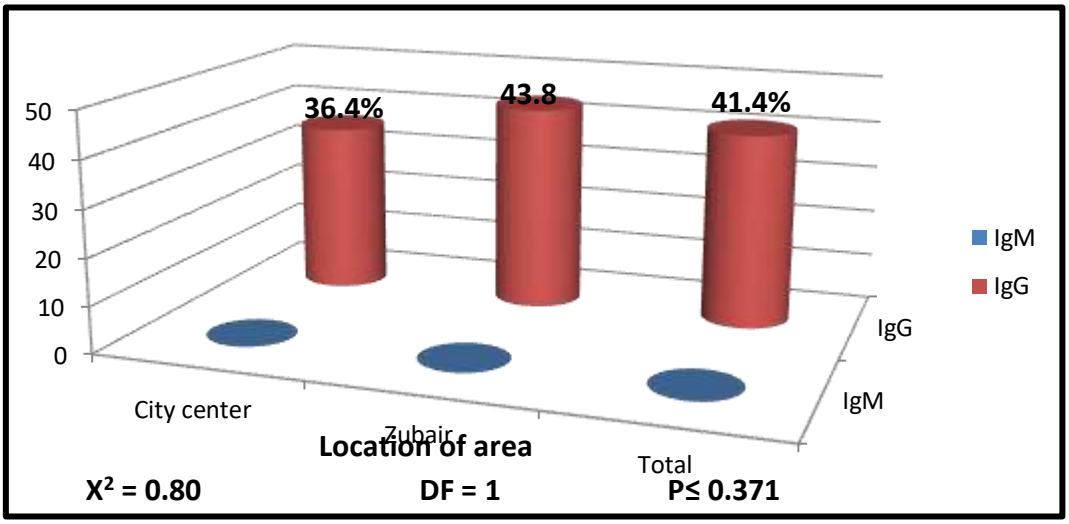

Fig. 4. Seroprevalence of *T. gondii* antibodies of normal delivery patients according to the location of area.

Genomic DNA was isolate from normal delivery women with history of abortion samples subjected to molecular analysis by using PCR for detection of B1 gene. Nested primers were used to identify the genotypes of *T. gondii*. Samples used in present study show a distinct single band of 194bp from the PCR product on agarose gel (Fig. 5).

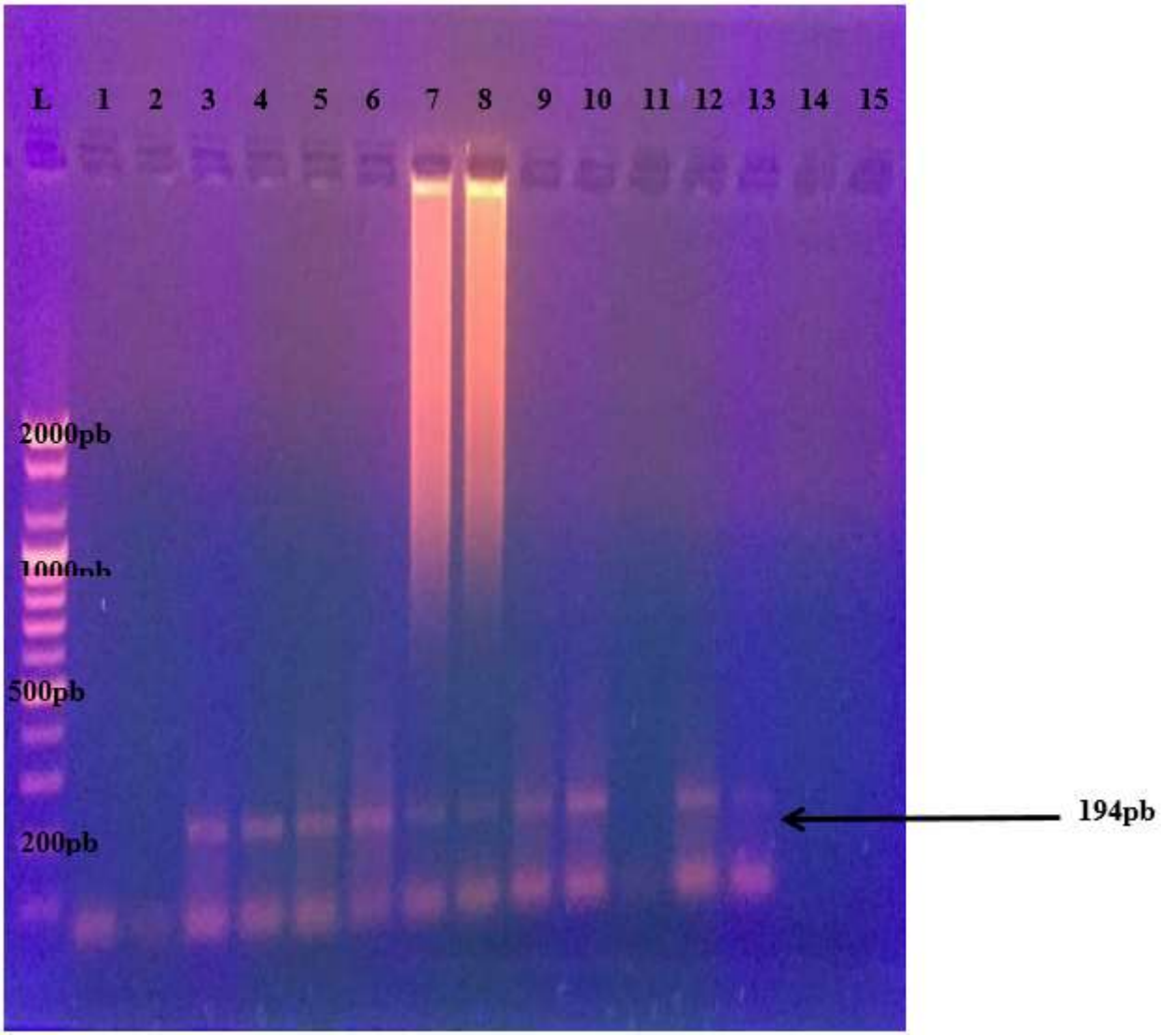

Fig. 5. Nested PCR (Second round) amplification of *T. gondii* from control, normal delivery women with history of abortion samples on 1.5% agarose gel.

L : Ladder (100 - 2000bp)
Lane 1,2 : Negative control
Lane 3,4,5 : Positive control
Lane 6,7,8,9,10,12,13 : Positive normal delivery woman with a history of abortion
Lane 11,14,15 : Negative normal delivery woman with a history of abortion

According to the age groups a significant differences between positive values of PCR (p. value = 0.000) and no significant differences between negative values of PCR was found (p. value = 0.292). The high value (100%) +PCR at age ≤ 20 years and 41-50 years, and lower value (50%) +PCR at age 31-40 years (Table1).

Table 1. The rate of infection by PCR for *T. gondii* of normal delivery patients with history of abortion patients according to the age groups.

Normal delivery Age (years)	+PCR No.	+PCR %		-PCR No.	-PCR %	
≤ 20 n = 2	2	100%		0	0%	
21-30 n = 5	3	60%	X^2 =26.774	2	40%	X^2 =1.11
31-40 n = 2	1	50%	DF =3	1	50%	DF = 1
41-50 n = 1	1	100%	P.value=	0	0%	P. value =
Total n = 10	7	70%	0.000	3	30%	0.292

X^2 = 16 , DF = 1 , p. value = 0.00

There were no significant differences between +PCR (p. value = 0.502) and between -PCR (p. value = 0.294) of normal delivery patients with history abortion. The higher value in +PCR in two previous abortion (75%) , the lower value in three previous abortion (0%), (Table 2).

Table 2. Percentage infection of *T. gondii* of normal delivery patients with history abortion samples according to the previous abortion.

Previous abortion	+PCR No.	+PCR %		-PCR No.	-PCR %	
1 n = 6	4	66.66%		2	33.33%	
2 n = 4	3	75%	X^2 = 0.451	1	25%	X^2 = 1.103
3 n = 0	0	0%	DF = 1	0	0%	DF = 1
Total n = 10	7	70%	p.value =0.502	3	30%	p.value=0.294

X^2 = 16 , DF = 1 , p. value = 0.00

There were a significant differences between +PCR of normal delivery patients with history abortion samples according to the location area, (p.value =0.004), The higher value in +PCR in City center, the lower value in Zubair, (Table 3).

Table 3. Percentage infection of *T. gondii* of normal delivery patients with history abortion samples according to the location area.

Locality	+PCR No.	+PCR %	-PCR No.	-PCR %	
City center n = 2	2	100%	0	0%	X^2 = 8.399
Zubair n = 8	5	62.5%	3	37.5%	DF = 1
Total n = 10	7	70%	3	30%	p.value =0.004

X^2 = 16 , DF = 1 , p. value =0.000

The percentage of sequence identity % between current local normal delivery women with history of abortion samples *T. gondii* isolate and NCBI- Blast homology from different countries was shown in Table (4).

Table 4. Sequence identity % between current local normal delivery women with history of abortion samples *T. gondii* isolate and NCBI- Blast homology from different countries.

No	Accession No.	Country	Host	Organ
1	MK507731.1	Iran	-	Blood
2	KX270373.1	Mexico	Sheep	Skeletal muscle
3	MN542678.1	Iran	Human	Blood from bone marrow
4	MK507732.1	Iran	-	Blood
5	MK521885.1	Iran	-	Blood
6	MK521884.1	Iran	-	Blood
7	MK521883.1	Iran	-	Blood
8	MK521882.1	Iran	-	Blood
9	MK521881.1	Iran	-	Blood
10	MK031701.1	Iran	Human	Blood
11	MK031700.1	Iran	Human	Blood
12	MK031699.1	Iran	Human	Blood
13	MK031698.1	Iran	Human	Blood
14	KX270388.1	Mexico	Sheep	Skeletal muscle
15	KX270387.1	Mexico	Sheep	Skeletal muscle
16	KX270386.1	Mexico	Sheep	Skeletal muscle
17	KX270385.1	Mexico	Sheep	Skeletal muscle
18	KX270384.1	Mexico	Sheep	Skeletal muscle
19	KX270383.1	Mexico	Sheep	Skeletal muscle
20	KX270382.1	Mexico	Sheep	Skeletal muscle
21	KX270381.1	Mexico	Sheep	Skeletal muscle
22	KX270380.1	Mexico	Sheep	Skeletal muscle
23	KX270379.1	Mexico	Sheep	Skeletal muscle
24	KX270378.1	Mexico	Sheep	Skeletal muscle
25	KX270377.1	Mexico	Sheep	Skeletal muscle
26	KX270376	Mexico	Sheep	Skeletal muscle
27	KX270374.1	Mexico	Sheep	Skeletal muscle
28	KX270371.1	Mexico	Sheep	Skeletal muscle
29	KX270370.1	Mexico	Sheep	Skeletal muscle
30	KX270369.1	Mexico	Sheep	Skeletal muscle
31	KX270368.1	Mexico	Sheep	Skeletal muscle
32	KX270367.1	Mexico	Sheep	Skeletal muscle
33	KX270366.1	Mexico	Sheep	Skeletal muscle
34	KX270365.1	Mexico	Sheep	Skeletal muscle
35	KX270364.1	Mexico	Sheep	Skeletal muscle
36	KX270363.1	Mexico	Sheep	Skeletal muscle
37	KC607827.1	India	Mice	-
38	AF179871.1	USA	Human	Culture, Strain = RH
39	KX270372.1	Mexico	Sheep	Skeletal muscle
40	KX270375.1	Mexico	Sheep	Skeletal muscle

The phylogenetic tree was done to show the relationship between NCBI reference sequence of the glycerol 3- phosphate dehydrogenase (GADPH, B1) isolates study.

Analysis of phylogenetic tree was done using UPGMA tree (MEGAG version) (Fig. 6).

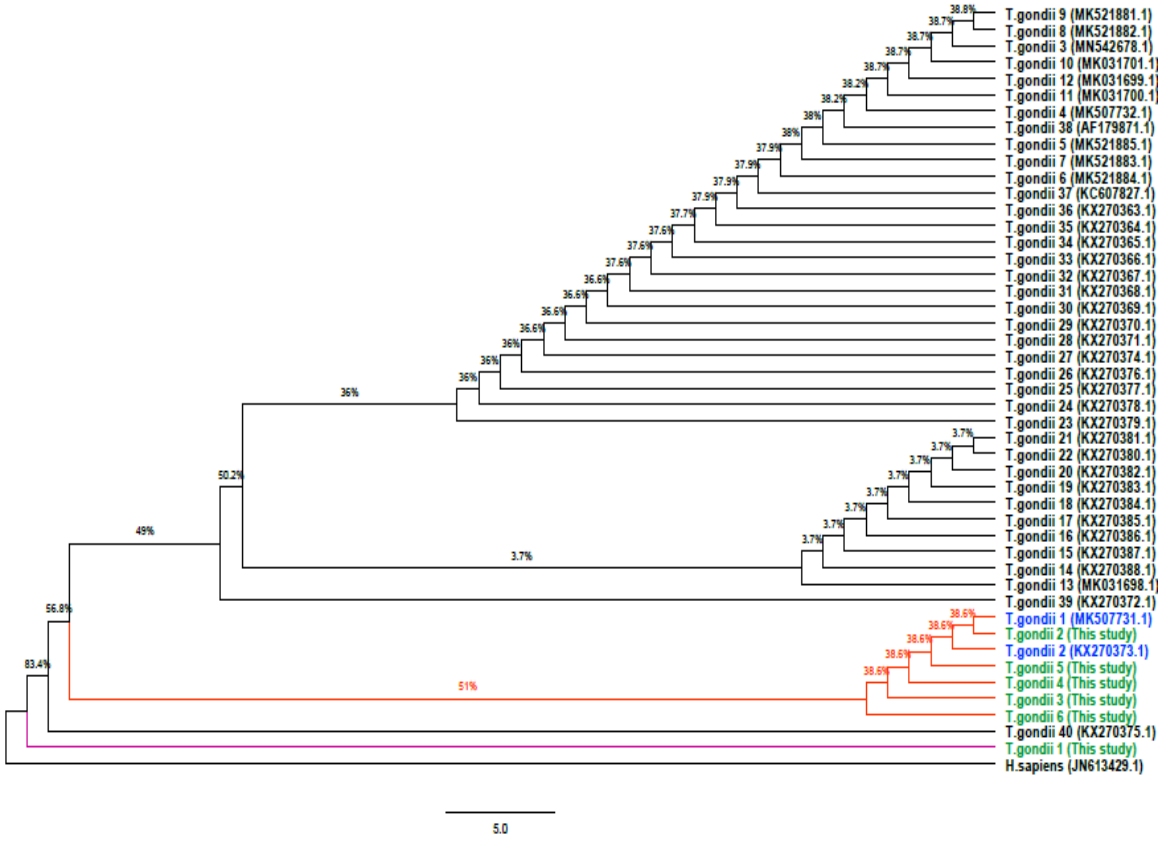

Fig. 6. A phylogenetic tree showing the relationships between references of the glycerol -3- phosphate dehydrogenase (GADPH, B_1) genes from *Toxoplasma gondii* isolates with the GADPH sequence of the present study isolate.

A nucleotide sequence was done to show the alignment GADPH genes from *T. gondii* reference isolates. The GADPH sequence from the present study isolates shown in (Fig. 7).

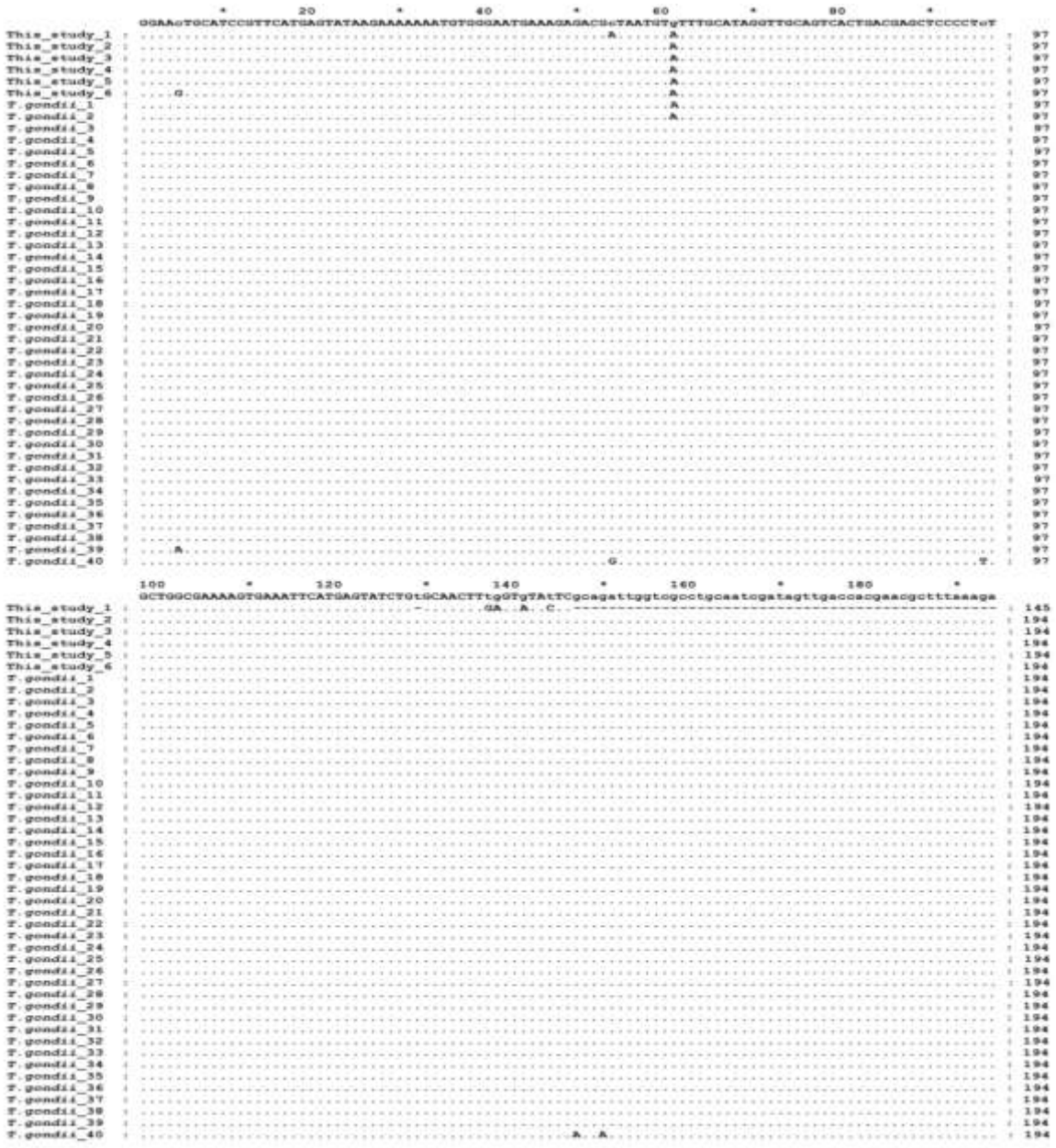

Fig. 7. A nucleotide sequence alignment of GADPH genes from *T. gondii* reference isolates and the GADPH sequence from the present study isolates.

The phylogenetic network which showed the relationship between *T. gondii* haplotypes from 1- 6, (Fig. 8).

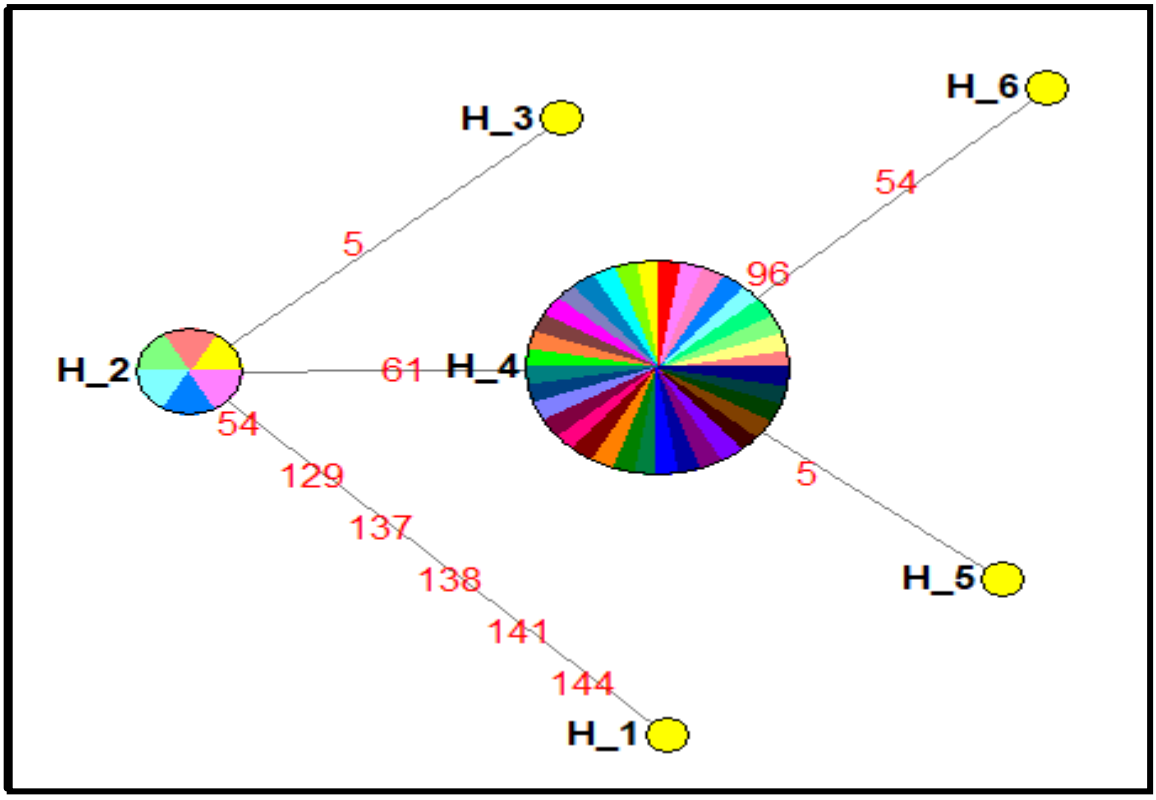

Fig. 8. A phylogenetic network showing the relationships between *T. gondii* haplotypes from 1-6. Each color represents an isolate.

Haplotype_1 (H_1) includes this study isolate(1).
Haplotype_2 (H_2) includes this study isolate(2-5),*T. gondii*_1 and *T.gondii*_2.
Haplotype_3 (H_3) includes this study isolate(6).
Haplotype_4 (H_4) includes *T. gondii*_3 to 38.
Haplotype_5 (H_5) includes *T. gondii*_39.
Haplotype_6 (H_6) includes *T. gondii*_40.

(Fig. 9) and (Fig. 10), (Table 5) showed the nucleotide sequence alignment of *T. gondii* haplotypes and the differences in base pairs 5,54,61,96,129,137,138,141, 144.

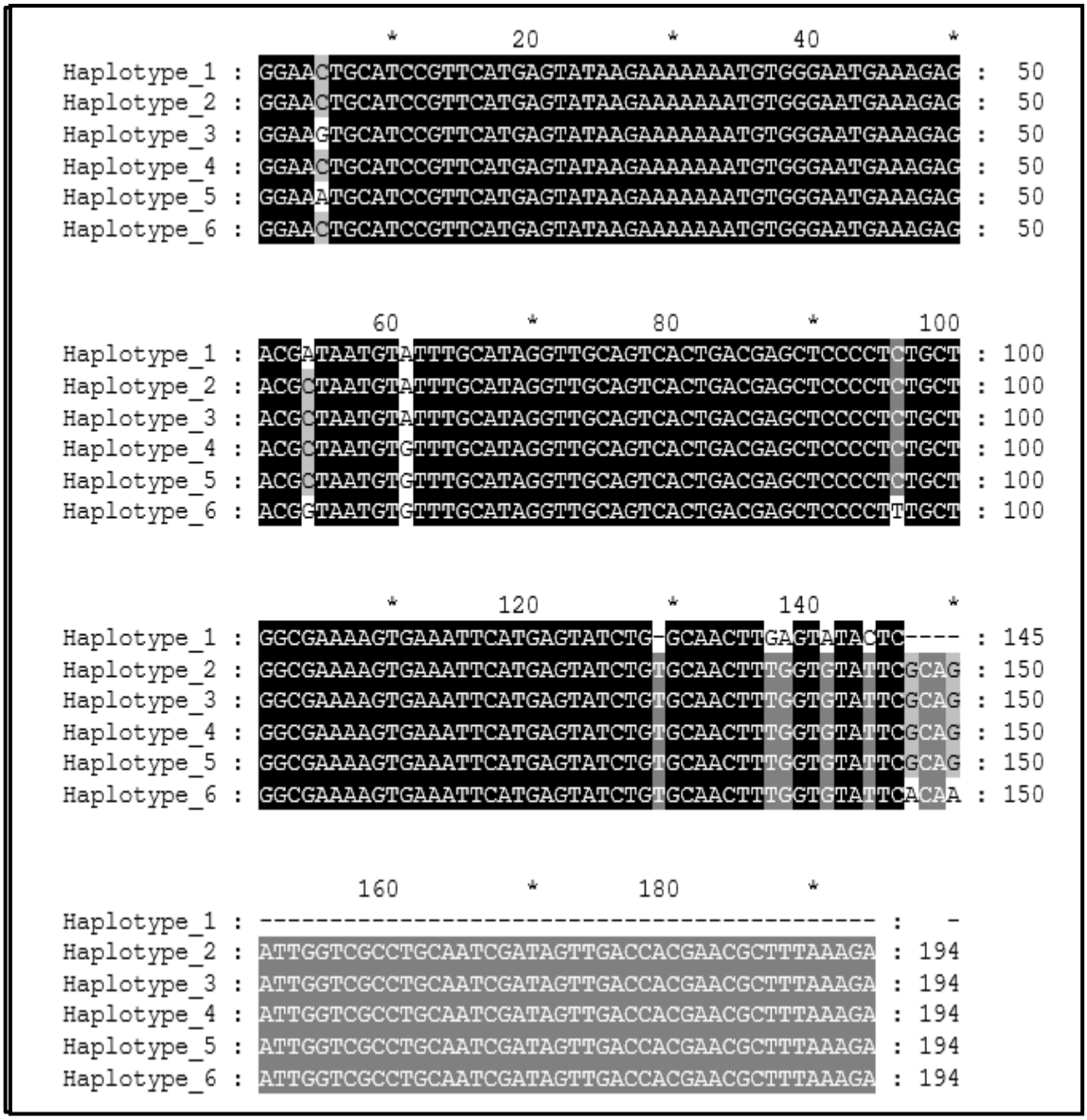

Fig.9. A nucleotide sequence alignment of *T. gondii* haplotypes.

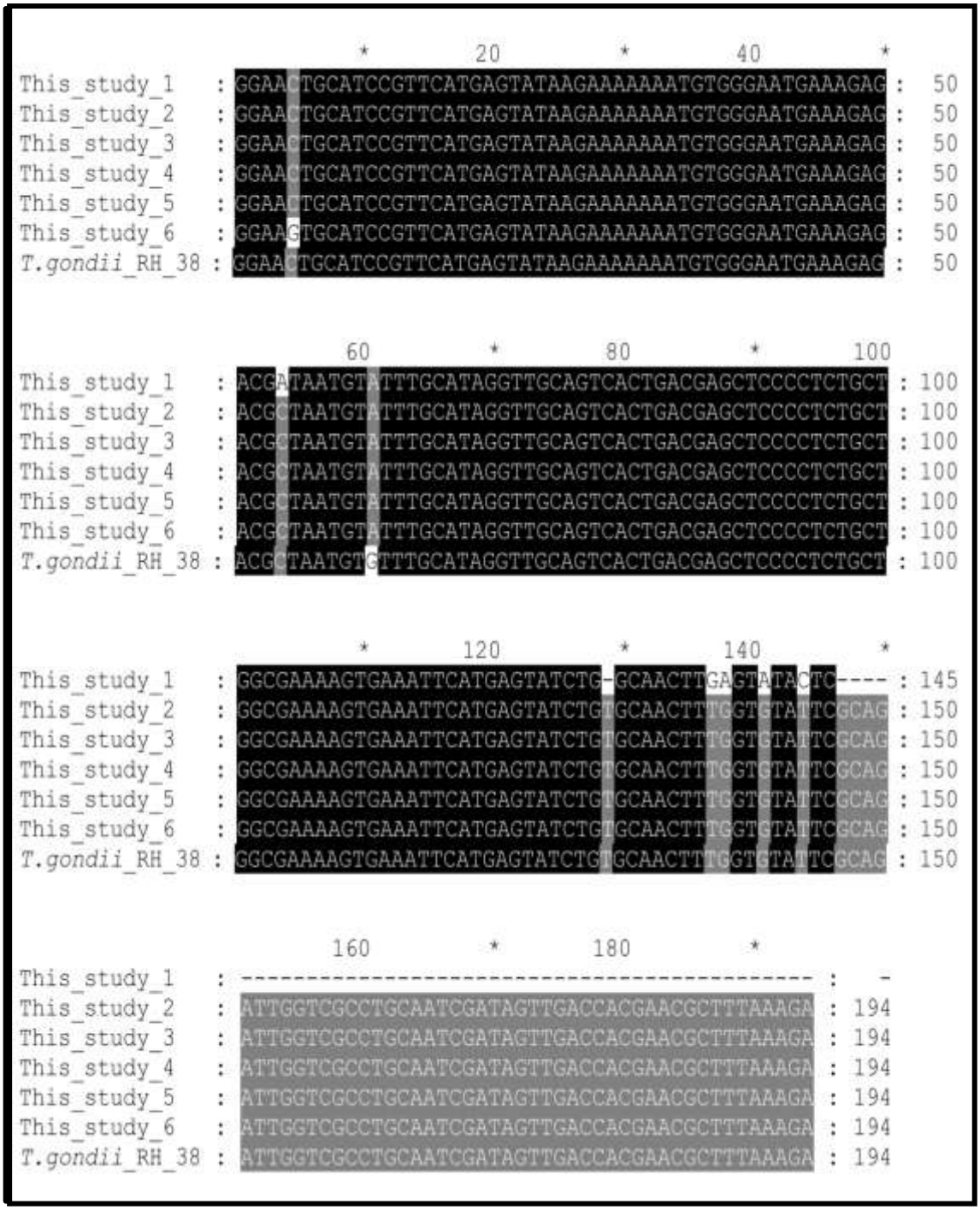

Fig. 10. A nucleotide sequence alignment of *T. gondii* haplotypes.

Table 5. Nucleotide sequence differences of GADPH haplotypes.

	Nucleotide position								
Haplotype	**5**	**54**	**61**	**96**	**129**	**137**	**138**	**141**	**144**
H_1	C	A	A	C	-	G	A	A	C
H_2	C	C	A	C	T	T	G	G	T
H_3	G	C	A	C	T	T	G	G	T
H_4	C	C	G	C	T	T	G	G	T
H_5	A	C	G	C	T	T	G	G	T
H_6	C	G	G	T	T	T	G	G	T

The percentage of DNA identity between haplotype 1 is 100 , between 1 and 2 was 90.34, between 1 and 3 was 89.7, between 2 and 3 was 99.5, between 1 and 4 was 89.7, between 2 and 4 was 99.5, between 3 and 4 was 99, between 1 and 5 was 89, between 2 and 5 was 99, between 3 and 5 was 99, between 4 and 5 was 99.5, between 1 and 6 was 89, between 2 and 6 was 97.42, between 3 and 6 was 97, between 4 and 6 was 98, between 5 and 6 was 97.42 as shown in (Table 6).

Table 6. The percentage of DNA identity among haplotypes

	Haplotpe_1	Haplotype_2	Haplotype_3	Haplotype_4	Haplotype_5	Haplotype_6
Haplotpe_1	100	90.34	89.7	89.7	89	89
Haplotype_2		100	99.5	99.5	99	97.42
Haplotype_3			100	99	99	97
Haplotype_4				100	9.5	98
Haplotype_5					100	97.42
Haplotype_6						100

4. Discussion:

During pregnancy, certain physiological changes, weakness in the body, and immune suppression occur that lead to the activation of the latent parasite during this period [14]. *T. gondii* has been shown to have a direct effect on the fetus leading to spontaneous abortion, fetal death, childbirth, or birth defects [15].

The ELISA assay in the current study revealed that (41.4%) (29/70) of normal delivery women who gave birth naturally and had a history of abortion were positive for IgG antibodies, compared with IgM (0%). This result does not agree with the study [16] in Nineveh, which found 3 positive cases of 25 aborted women with a percentage of (12.0%), and the study of [17] on 125 pregnant women in Al-Diwaniyah city, which indicated that the acute infection rate was (33.6%) and (17.6%), respectively.

The reason for the high levels of antibodies in the sera of women, especially IgG (which is the only antibody transmitted from the mother to her fetus through the placenta) is due to a defect in the transfer of IgG antibodies in the placenta, which leads to the accumulation of its levels in the pregnant mother. The presence of antibodies provides the necessary protection for the fetus until its immune system is completed [18]. *Toxoplasma*-specific IgG usually appears early, peaks after about two months, then decreases gradually but remains measurable for life [19].

In the current study, IgG recorded its highest value (50%) at the age of 20 years or less, and the lowest value is (16.7%) at the age of (41- 50) years. IgM values were (0%) at all ages. The study of [20] in the city of Kirkuk indicated that the highest rate of infection is in the age group between (26 - 30) years, as it reached (30.35%) among pregnant women. The study of[21] found the highest seropositivity (55.9%) at the age of (35- 60) when studied on different groups of the population in Dohuk, followed by a study of [22], in Tikrit, at aged (25-35) (41.2%).

In Basrah, [14] found seropositivity for *T. gondii* increases significantly with age on women with previous miscarriages, reaching (23.7%) in the (35-45) age group. This difference may be attributed to health habits, nutrition, cat populations, human behavior and climate, and possibly that there is wide variation in the prevalence of *T. gondii* in the above mentioned regions.

The results of the current study did not support a relationship between an increase in the number of miscarriages and an increase in *T. gondii* seropositivity. The highest IgG value (46.2%) was found in women who had two previous miscarriages, and the lowest (18.2%) IgG value was for women who had three previous miscarriages. IgM values were (0%) in all previous abortions. The results of the current study agreed with the study of [23] in Mosul, it was found that women who had two miscarriages had a higher percentage of seropositivity (34.14%) using the LAT test and also agreed with a study [24] in Baghdad.

The results of the current study did not agree with [14], they found that women who had five or more miscarriages had a higher prevalence of anti-*T. gondii* antibodies, they also indicated the presence of habitual miscarriage in women with toxoplasmosis. Therefore the immune response must be carefully focused, estimated by advanced immunological methods such as mini-vidas, ELISA and IFAT [25].

The current study included 10 PCR-positive cases with an infection rate of (70%) using Nested PCR technique, which is lower than what was recorded by [26] in Najaf governorate (80.6%) using PCR technique. The results of the current study did not agree with the study of [27] in which qPCR technique was used with infection rate was (38.0%).

T. gondii can live in dormant for the life of the host in the form of cryptic bradyzoites. However, if the host becomes immunocompromised due to the disease. *T. gondii* can shift from its latent stage to the highly virulent tachyzoite stage and cause a recurrence of the infection which can be fetal [28]. Since toxoplasmosis serotype methods is not suitable in immunocompromised patients. Therefore, early and definitive diagnosis is necessary for pregnant women to prevent serious complications that require high and rapid diagnostic tools.

The primer B1 was used in the current study because it has a high sensitivity. The primer B1 may be specific to the *T. gondii* strain found in Iraq [29]. This is consistent with studies of [30] and [31] which found that the B1 gene has high specificity and sensitivity, so it was used in diagnosing *T. gondii* parasites by PCR technique.

Because IgG antibodies are able to cross the placenta, women exposed to toxoplasmosis before pregnancy may not transmit the infection to the fetus due to the ability of this antibody to provide protection for the fetus [32] .It is rare for a woman infected with toxoplasmosis before pregnancy to pass the infection to her fetus, because she will have acquired immunity to the infection, this can happen if the pregnant woman has a previous infection and becomes immunosuppressed and the infection is activated in it [33].

Serological testing is one of the most important diagnostic tools for toxoplasmosis, moreover, the test may fail to detect *T. gondii* infection in some immunocompromised patients due to the fact that anti-*T. gondii* antibody titers may fail to rise in this species. The use of *T. gondii* DNA with nPCR reduces the problems encountered when using serological tests and facilitates diagnosis in difficult cases [34].

Infection with *T. gondii* depends mainly on regular monitoring of pregnant women who are not immunized against the disease. This monitoring begins throughout the months of pregnancy. This is consistent with what was mentioned by both [35] and [36] Where they confirmed that the amount of these antibodies can be relied upon determine the approximate date of infection.

Conclusion

It was concluded from the current study that a high level of parasite infection was observed in delivery women and had previous abortions, compared to control samples and there is a latent chronic infection in them, this infection is active in pregnant women because of their weakened immunity.

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دراسة جزيئية للإصابة بطفيلي Toxoplasma gondii في النساء الولودات طبيعيا واللاتي لديهن اجهاضات سابقة في محافظة البصرة

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الكلمات المفتاحية

Recurrent Toxoplasma gondii, abortion , Molecular study, Nested PCR.

الملخص

يعد طفيلي المقوسة الكوندية *Toxoplasma gondii* العامل المسبب لداء المقوسات، وهو أحد أكثر أنواع العدوى الطفيلية انتشاراً للإنسان والحيوانات الأليفة. تم التحري مصلياً عن طفيلي *T. gondii* للنساء اللاتي ولدن حديثاً و لديهن اجهاضات سابقة باختبار الادمصاص المناعي المرتبط بالأنزيم (ELISA) وشملت الدراسة (70) امرأة لديهن تاريخ من الإجهاض الفردي أو المتكرر و(30) امرأة ذوات الولادة الطبيعية بدون اجهاضات سابقة كمجموعة سيطرة. شملت الدراسة النساء المراجعات لمستشفى البصرة للنسائية والتوليد ومستشفى الموانيء العام ومستشفى الفيحاء العام ومستشفى الزبير العام ومستشفى دار الشفاء الاستشماري. كانت النسب المئوية للانتشار المصلي (41.4%) للأجسام المضادة IgG و لم تسجل نسبة للأجسام المضادة نوع IgM. أظهر الفحص أن 7 (23.3%) من (30) في مجموعة السيطرة كانت موجبة للأجسام المضادة IgG ، مقارنة بـ 0 (0%) من IgM. وجدت فروقات معنوية بين الانتشار المصلي لهذه الاضداد بالنسبة للعمر فكانت اعلى نسبة (50%) للجسم المضاد IgG في الفئة العمرية اقل من 20 سنة بالنسبة لعدد الاجهاضات السابقة كانت اعلى نسبة اصابة للجسم المضاد IgG (46.2%) في النساء اللواتي لديهن اجهاضين سابقين ولم تسجل نسب اصابة للجسم المضاد IgM. كانت اعلى نسب اصابة (43.8%) في قضاء الزبير مقارنة بمركز المدينة بالنسبة للجسم المضاد IgG ولم تسجل اصابة بالاعتماد على الجسم المضاد IgM. اجريت الدراسة الجزيئية على النساء باستعمال الجين B1 من *T. gondii* باستعمال تقنية Nested PCR وسجلت اصابة (70%) للعينات الموجبة لـPCR. حدد تسلسل القواعد النيتروجينية للحامض النووي لطفيلي *T. gondii* بتطبيق تتابعات الحامض النووي DNA وقراءة الشجرة التطورية لهذه العينات واجراء المقارنة بينها تبعا للمضائف المعزولة. اظهرت قراءة الشجرة التطورية وجود تطابق لعينة النساء الولودات طبيعياً مع الفرع الاساسي لشجرة القرابة الذي يحمل التسلسل MZ717187، MZ717188، MZ717189، MZ717190، MZ717191 في بنك الجينات العالمي.

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