Sequence analysis of *Toxoplasma gondii* in Al-Qadisiyah province

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Abstract

The recent study included 111 blood samples from the women and children's health center in Diwaniyah city and from both sexes (male and female) from the beginning of 2017-9-1 until 2018-3-1. Freeze until PCR is checked.

The results of the recent study showed using a nPCR test that the number of infected samples (27) samples and 24.3% out of (111) samples was examined. The results of the present study showed that there were significant differences ($p \leq 0.05$) for the age group, number of births, if the highest level of infection in the age range (35-31) and 43.75%, and the highest level of infection in women with (4) children and 72.72%, and showed no significant differences ($P>0.05$) among the patients and mental problems, number of depression, housing area, mental malformations, marital status , and gender. The results of the sequence analysis showed that all samples were read within one branch and the ratio correspond of local samples was 100% compared with the samples recorded at the NCBI site. The results of the current study on blood standard, which included a (111) sample test, showed a change in the PLT level for people who were infected with nPCR.

Keywords: Parasite , *Toxoplasma gondii* , Sequencing , Nested PCR

Introduction

Toxoplasmosis is an animal disease caused by the protozoa parasite *Toxoplasma gondii*. Humans and warm-blooded animals are highly toxic (1), and its incidence is widespread throughout the *world*. About one-third of the human population has been exposed to this parasite, and the prevalence of infection varies between countries and population groups (2). A person may remain symptom-free unless there is a suppression of immunity (3).

All animals, including humans, and birds are intermediate species, but cats are intermediate and final specimens. They are the only animals that pass through the Oocyst. Sheep and goat meat are an important source of toxoplasmosis (4).

The *T. gondii* parasite is diagnosed in two ways either directly using PCR technique, tissue sections, isolation, or serotonin indirectly. In this way(IgG ,IgM) is detected in the parasite to determine the reactive and chronic infection (5). *T. gondii* has caused severe disease, including spleenomegaly, chorioretinitis, pneumonitis, encephalitis and mental retardation, which have failed in organs of other organs and

may lead to death (6). The current study aimed at the following:
1-Study the spread of parasite among the population of Diwaniyah city through the auditors of hospitals and health centers in the city during the study period.
2-*Toxoplasma gondii* parasite diagnosis by identifying the presence of molecular (PCR)infection methods.
3-Determine the extent of the impact of parasitic infection on some blood components Blood Complete.

- Methods

-Study area

The study was conducted at the woman and children's hospital in the for Diwaniyah province. The study included all the reviewers randomly from both sexes and from different districts and the city center of the province for the period from 2017-9-1 to 2018-3-1. For the purpose of determining the pathological conditions associated with the infection of the parasite after the diagnosis.

-Collection of specimens

A total of 111 random samples were collected from both sexes from the patients to the woman and children's hospital for the period from 2017-9-1 to 2018-3-1. The intravenous blood samples were collected at a volume of 5 ml per sample and placed in test tubes For EDTA coagulation the samples are kept in a freezer until a PCR examine is performed.

Diagnosis of *Toxoplasma gondii* parasite in two ways:

- **Immunological methods** for antibody detection (IgM, IgG) and action steps were taken by manufacturer company for kit (Foresight ,Germany).
- **Molecular methods** by Nested PCR .

- Primers Design

The initiators of this study were designed to diagnose *Toxoplasma gondii* using PCR technology. The design of primers was adopted on the GenBank-NCBI site and by the Primer3plus primer design program. The primers were prepared by the Korean company Bioneer:

Primer	Sequence		Amplicon
Toxo-B1 gene Primary primers	F	**TTTCGCGAAGAGGAGGGAAC**	**628 bp**
	R	**CGCTTGAAGAGACACCAGGT**	
Toxo-B1 gene Secondary primers	F	**ACTGCTCTAGCGTGTTCGTC**	**298 bp**
	R	**ACGGTTGTTGAGGTCCGAAA**	

NCBI-Genbank Toxoplasma gondii B1 gene (AF179871.1)

- Method of diagnosis Toxoplasma gondii parasite using Nested PCR technique

Nested PCR technique was performed to investigate the *Toxoplasma gondii* parasites several steps:

1. DNA extraction of blood

DNA was extracted from blood samples using genomic DNA Extraction Blood Kit processed by Genaid USA.

2. DNA test

By the use of the Nanodrop spectrophotometer (THERMO. USA) , DNA was extracted from the blood samples to detect and measure the concentration of DNA and RNA. DNA is detected by DNA concentration (ng \ µl DNA) And measure the purity of DNA by reading the absorbance wavelength of between (260-280nm).

3. Preparation of the polymerized chain reaction mixture Nested PCR master mix:

The polymerase chain reaction mixture was prepared using the AccuPower® PCR PreMix kit, manufactured by Korea's Bioneer According to the company's instructions as follows:

1 - The polymerase chain reaction mixture was prepared in the PCR tubes with the kit and container on the polymerization chain reaction components and the other components of the reaction mixture were added and according to the instructions of the company as in the following table:

1-Primary Run:

PCR master mix	Volume
DNA template 10-50ng	5µL
Toxo-B1 gene Primary primers Forward primer (10pmol)	1.5µL
Toxo-B1 gene Primary primers Reverse primer (10pmol)	1.5µL
PCR water	12µL
Total	**20µL**

2. After completing the polymerization reaction mixture, the tubes were closed and carefully mixed with vortex for 5 seconds.
3-Transfer tubes for the PCR PC Thermocycler to conduct thermocycler PCR thermocycle conditions.

4. PCR Thermocycler conditions

The polymerization chain reaction was investigated using a PCR thermocycler, the device is programmed as in the following table:

PCR Step	Repeat cycle	Temperature	Time
Initial denaturation	1	95C	5min
Denaturation	30	95C	5sec
Annealing		59.3 C	30sec
Extension		72C	45sec
Final extension	1	72C	7min
Hold	-	4C	**Forever**

2-Secondary Run:

PCR master mix	Volume
PCR product	2.5µL
Toxo-B1 gene Secondary primers Forward primer (10pmol)	1.5µL
Toxo-B1 gene Secondary primers Reverse primer (10pmol)	1.5µL
PCR water	14.5µL
Total	**20µL**

4- After completing the polymerization reaction mixture, the tubes were closed and carefully mixed with the vortex carburetor for 5 seconds.
5-Transfer tubes for the PCR PC Thermocycler to conduct thermocycler PCR thermocycle conditions.

5. Gel electrophoresis

The electrophoresis was carried out using 1% akarose gel to read the result of PCR product analysis.

-Calculation of blood components Calculation of blood components

The blood sample is examined by using the Cell-DYN Ruby / USA device. The blood sample is placed in a tube containing an anticoagulant (EDTA) with the information written for each sample. Each sample is analyzed automatically. After about 5 minutes Or less show the result, which includes the appearance of all components of blood printed paper, and based the current study on the following tests:
1-Total red blood cell count (RBC).
2-Total white blood cell count (WBC).
3- Total platelet count (PLT).

-Statistical Analysis

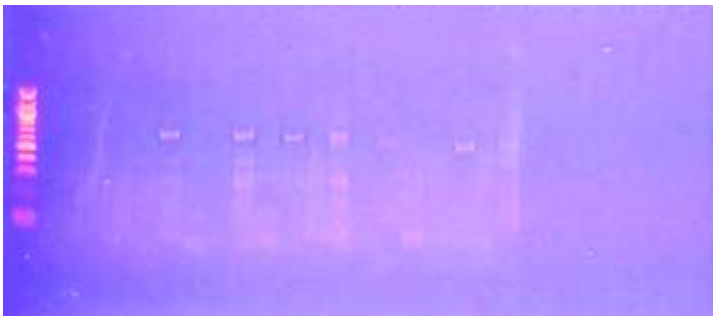
According to the results of the study, the statistical test used the Chi-square test

and the T test to determine the differences between the study groups. Confidence interval is 95% and the probability level is less than 0.05 (P <0.05) (7).

- Resulte & Discussion

The results of the current study showed that the incidence of IgG infection was %28.82, while the infection rate of IgM was %3.6 , and molecular methods were identified to confirm parasite infection.

The samples were first identified using the molecular methods PCR using the Primary primers , which is 628bp long .The results of the present showed the bands as shown in Figure(1).



Figure(1*) T.gondii* test results were performed using PCR assay

Then add the second primer of the first primer product and show the final results of the current study as shown in Figure(2).

Figure(2*) showing the results of samples infected with a parasite using the nPCR test

The results of the present study showed that 24.3% of men with macrophages were diagnosed with nPCR, if there were significant differences between infection and age group. The highest incidence was among the age group (35-31) which was 43.75% and the lowest level of infection in the age group (40-36) as shown in Table(1) The results of the current study were lower than those of (8) in pregnant

women in Hamadan, and (9) in Assam city India, (10) in Slovenia, (11) in Najaf city ,(12) in Egypt for (33.5%, 36.8%, 34%, 80% and 77.3%, respectively, and higher than that of (13) in Jordan, and (14) in Korea and (15) in the United States by 13.5%, 0.88% and 13%, respectively. If the diagnosis of PCR is characterized by high privacy and early diagnosis, where it differs from serological tests that need an immune response to the individual.

Table (1) The number of specimens tested and exposed to Toxoplasmosis is determined by age groups using the nPCR test

Age group	samples examined	infected samples	%
5-10	13	0	0
11-15	10	0	0
16-20	13	2	15.38
21-25	29	9	31.03
26-30	23	8	34.78
31-35	16	7	43.75
36-40	7	1	14.28
Total	111	27	24.32
X2	13.696		
P Value	0.033		

The results of the present study showed significant differences between the infection and the month of the year and the level of probability 0.05 where the highest rate of infection in October and 52.94% In the month of December and by 25% as shown in Table (2).

Table (2) The number of specimens tested and exposed to Toxoplasmosis is analyzed by month of year using the nPCR test:

Months of the year	Number of samples examined	Number of infected samples	%
September	28	8	28.57
October	17	9	52.94
November	35	9	25.71
December	4	1	25
January	18	0	0
February	9	0	0
Total	111	27	24.32%
X2	20.299		
P Value	0.0011		

The results of the current study on the analysis of the genetic sequence of parasites of *Toxoplasma gondii* by the(company macrogen in Korea) showed a clear correlation between the sequence of rules B1 gene in local *Toxoplasma gondii* isolates with Toxoplasma gondii isolates registered at NCBI-GENbank as shown in Fig.(3).

Figure 3: Multiple sequencing alignment analysis using the MEGA6 program of the B1 gene sequencing reaction to Toxoplasma gondii, where the apparent similarity of the sequence of the B1 gene sequence in local toxoplasma gondii isolates is observed Toxoplasma gondii parasites recorded at the NCBI-Genbank gene site shown in the picture.

Showed the results of the present study through the analysis of the tree genetic own Toxoplasmosis the presence of convergence clear between isolates parasite local with isolates recorded in the site NCBI through the comparable with isolates other phenomenon in the tree genetic as shown in Fig.(4).

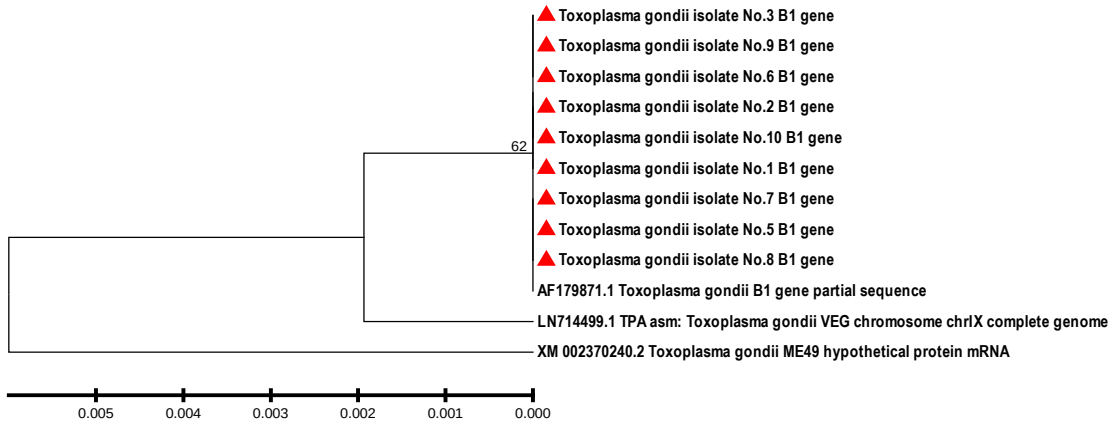


Figure 4): The analysis of the phylogenetic tree analysis using MEGA 6, where the analysis of the UPGMA tree (Unweighed Pair Group Method with Arithmetic Mean) was used. The results of the analysis showed a clear convergence in the isolates of parasite Toxoplasma local gondii with parasitic isolates of Toxoplasma gondii recorded in NCBI-BLAST sequentially (AF179871.1) compared to other isolates shown in the analysis of the genetic tree.

Showed the results of the persent study of the parasite ratios congruence between samples recorded in the site NCBI as shown table (3):

Table (3):The tables represent the homogeneity ratio of local isolates and isolates recorded in NCBI-BLAST Homology sequence identity

Toxoplasma gondii isolates Local	Match ratio with site NCBI-BLAST Homology sequence identity	
	Isolation is identical NCBI-BLAST Toxoplasma gondii	Match ratio
T. gondii isolate No.1	AF179871.1	100%
T. gondii isolate No.2	AF179871.1	100%
T. gondii isolate No.3	AF179871.1	100%
T. gondii isolate No.5	AF179871.1	100%
T. gondii isolate No.6	AF179871.1	100%

T. gondii isolate No.7	**AF179871.1**	**100%**
T. gondii isolate No.8	**AF179871.1**	**100%**
T. gondii isolate No.9	**AF179871.1**	**100%**
T. gondii isolate No.10	**AF179871.1**	**100%**

The results of the study showed that there were significant differences only in one blood component index (PLT) using this nPCR test as shown table (4). The results of the current study showed that there was no effect on the blood components (WBC,RBC) of parasitic infection, which is consistent with the findings of (16).

Table (4) titer for blood components of infected and not infected samples using nPCR examination:

Blood components	RBC	WBC	PLT
Not infected	4.40±0.08	10.09±0.41	271.01±9.49
Infected	4.46±0.09	12.46±1.58	243.88±8.22
T test	0.442	1.439	2.159
P Value	0.660	0.161	0.033

-Conclusions

1-The results of the study showed the highest rates of infection in the age group (35-31) using the nPCR test .

2-There is no effect on the blood parameters except the effect on blood platelets .

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