

Study The Some Aspects of The Immune Response For Pregnant Women Infected with *T.Gondii* and Determine The Genotyping of This Parasite

دراسة بعض جوانب الاستجابة المناعية للنساء الحوامل المصابات بداء القطط وتحديد النمط الجيني للطفيلي
Hashim Ali Abdulameer/ Master's student /Collage of medicine/University of kufa
Hashim Raheem Tarish /PHD, Parasitology/Collage of medicine/University of kufa
Alaa Jawad Hassan/ PHD, Immunology/Collage of Science/University of Babylon.
hashimaa49@yahoo.com

الخلاصة:

الخلفية: تهدف هذه الدراسة الى معرفة الاستجابة المناعية (الاجسام المضادة والحريكات الخلوية) للنساء المصابات بداء القطط ، ومعرفة العلاقات بين تلك المعايير وتأثيراتها على المرضى في فئات عمرية مختلفة.

المنهجية : تم جمع (200) عينة من الدم و (30) عينة من الحبل السري من النساء والحوامل المصابات بداء القطط والمراجعات إلى مستشفيات النجف الاشراف ومختبراتها لأغراض الإجهاض أو العلاج من المرض، أما عدد عينات النساء السويات (السيطرة) فقد بلغت 30 عينة وقسمت النساء الحوامل و السيطرة الى ثلاثة فئات عمرية وهي (15-25)،(26-36)و(37-47) على التوالي ، تضمنت الدراسة جمع العينات و فحصها الاولي بواسطة اختبار لاتكس وبعدها فحصت بواسطة تقنية الامتزاز المناعي المرتبط بالانزيم (الاليزا) للكشف عن الأجسام المضادة IgG و IgM أشارت الدراسة إلى أن تقنية الاليزا أكثر أهمية من اختبار لاتكس وكانت ($P=0.007$) وبإضافة الى السايوتوكينات.

النتائج: أوضحت نتائج هذه الدراسة اختلاف بين الفئات العمرية ونسبة الإجهاض في النساء وكانت الفئات العمرية (15-25)و(26-36) هي الأعلى بنسبة 40% و 51% على التوالي و مقارنتها مع النساء الحوامل والمصابة بداء القطط. وأشارت الدراسة إلى وجود فرق معنوي في نسبة الأجسام المضادة في مختلف الفئات العمرية عند مقارنتها مع مجموعة السيطرة . وتبين وجود فروقات معنوية بين الإصابة الطفيلي وحالات الإجهاض وسجلت أعلى نسبة 40(50%) في الشهر الثاني مقارنتها مع الأشهر الأول والثالث من فترة الحمل وكان تأثيرها لها أهمية. بينت الدراسة ان هناك علاقة بين جينات *Toxo-B1* *ITS-1*, *Tg-8*, *Tg-9*, *SAG-1* و *SAG-2* من خلال فحص DNA المأخوذ من الدم والمشيمة من النساء المصابات بداء القطط . وسجلت أعلى عدد من العينات الموجبة (52) عينة في *ITS-1* من بقية الجينات الأخرى . وكانت العلاقة الإحصائية الأكثر أهمية في التشخيص الطفيلي ($P=0.001$) .

الاستنتاج: هناك العلاقة بين من السيتوكينات وداء المقوسات معها المسببة للإجهاض

التوصيات: الكشف عن الأنماط الجينية التي تؤدي إلى الإجهاض التلقائي

Abstract:

Background: This study aims to find out the immune response (antibodies and cytokines) for women with disease *T.gondii* and correlation between them calibrated and the effects on the patients of the difference age groups.

Martial and method: Were collected (200) samples of blood and (30) samples of umbilical cord from women and pregnant women with disease *T.gondii* and revisions to hospitals in Najaf and laboratories for the purposes of abortion or treatment of disease, while the number of women samples control amounted to 30 samples . Pregnant women and control were divided into three age groups, namely, (15-25), (26-36) and (37-47) respectively. The study involved collecting samples and tested the first test by latex and then examined by the technique of adsorption-linked immunosorbent assay (ELISA) for the detection of antibodies (IgG and IGM). The study reported that technique is more important than the ELISA test for latex which was ($P = 0.007$).

Results: The results of this study include following : the difference between the age groups and abortion rate in women was age groups (15-25) and (26-36) is the highest at 40% and 51%, respectively, and compared with the pregnant women and the disease infected *T.gondii*. The study pointed to the existence of a significant difference in the proportion of antibodies in different age groups when compared with the control group. Adding to the cytokines. The study showed that there is a relationship between genes *Toxo-B1*, *ITS-1*, *Tg-8*, *Tg-9*, *SAG-1* and *SAG-2* by examining the DNA taken from the blood and placenta of women with *T.gondii*. And recorded the highest number of positive samples (52) in the sample *ITS-1* from the rest of the other genes. Statistical relationship was the most important in the diagnosis of the parasite ($P = 0.001$).

Conclusion: The correlation between of the cytokines and toxoplasmaosis with according of abortion .

Recommendations: To detect genotypes that lead to spontaneous abortion.

Keyword: *T.gondii* , PCR , cytokines , *ITS-1*, *SAG-1*, and *SAG-2*

INTRODUCTION:

Toxoplasmosis is disease caused by the protozoan parasite *Toxoplasma gondii*, human and other warm blooded animals are its hosts ⁽¹⁾ .

Toxoplasma gondii affects a wide variety of avian and number of mammals as intermediate hosts with cats' family as definitive hosts ⁽²⁾ . *Toxoplasma gondii* infection stimulates both cell mediated immunity(CMI) in addition to humeral immune response as antibody production, which includes IgM and IgG antibodies ⁽³⁾ . Cell mediated immunity (CMI) is considered one of the most distinctive immunological features of *T.gondii* infection, CMI is elicited by the parasite,

resulting in host protection against rapid tachyzoite growth and consequent pathologic changes⁽⁴⁾.

The *Toxoplasma gondii* causes a very strong response of T-helper1(Th1) focused on Interferon- gamma (IFN γ), Tumor Necrotizing Factor alfa (TNF α) and Interleukin-12 (IL $_{12}$), secreted by dendritic cells and macrophages. This immune response limits the tissue extension of the parasite, and lead the parasite to convert to bradyzoite⁽⁵⁾. The macrophage, T lymphocytes and natural killer (NK) cells on the one hand, and the cytokines, on the other are the major elements involved in immune response against *T. gondii*. Antibodies appear to play a minor role but remain essential means for preventing reinfection by the parasite⁽⁶⁾. The IgG, IgM, IgA and IgE antibodies are increased in patients with acute and chronic toxoplasmosis depending on the strain and the stage of the parasite⁽⁷⁾. It is therefore essential to estimate the time of infection as precisely as possible to properly manage the risk to the patients.

T-helper1(Th1) are proinflammatory cytokines produced by Th-17 cells that induce local epithelial cells to produce chemokine CXCL8 that recruit neutrophils to the site of infection early in the adaptive immune response. Meantime, the chronic infection with *Toxoplasma gondii* is associated with marked pathology caused by over production of IFN- γ by T-cells, also IL-12 produced by dendritic cells in response to parasite antigens. IL-10 is a cytokine secreted by regulatory cells suppressing many inflammatory responses⁽⁸⁾.

The aim of this study:

1. Evaluate anti-*Toxoplasma* IgM and IgG.
2. Evaluate certain pro-inflammatory cytokines (IL-12, IL-17 and IFN- γ) and anti-inflammatory cytokines (IL-10).
3. Evaluate the molecular diagnosis of toxoplasmosis and cytokines through using polymerase chain reaction (PCR).

PATIENTS AND METHODS

Time of collection:

Blood and tissue samples collection was started from October-2013 to May 2014, 200 blood and 30 tissue samples were collected from females with age range (15-47) years.

Biochemical test

A-Latex agglutination test

The kit is provided from Biokit Company – Spain, the principle of the test is based on antigen – antibody reaction directly. The sensitivity of the test is 10-15 IU/ml.

B-Detection of anti-*Toxoplasma gondii* antibodies (IgG & IgM) by MiniVades technique:

The bioMérieux kit was used the Immunity by Minivades[®] kit

C-Detection of cytokines by ELISA technique

The Thermo scientific (China) kit was used the cytokines by ELISA

Polymerase chain reaction (PCR)

A- DNA extraction-salting –out

Turn on a water bath to 55°C. Take a small amount of tissue (~ 0.5 cm²) and 200 μ l blood. Then transfer the sample to a labeled 1.5 ml microcentrifuge tube. Add 600 μ l of TNES buffer and 35 μ l of Proteinase-K (20 mg/ml). Mix the sample by inverting the tube several times. Incubate the samples overnight (or 1-3 hours) at 50°C. If possible occasionally mix the samples by inverting the tubes. [If you are in a hurry you can add more proteinase-K to speed up tissue digestion and

reduce the incubation time to 2-4 hours]. Add 170 µl of 6 M NaCl. Shake the samples vigorously for 20 seconds. [do not shake too roughly or you may damage your DNA, but shake harder than simply inverting the tubes]. Microfuge the samples at full speed (12-14,000 rpm) for 5-10 minutes at room temperature. [Label new tubes whilst centrifuging samples]. Remove supernatant to a new, labeled 1.5 ml microcentrifuge tube. Add an equal volume (800 µl) of cold 100 % ethanol and gently mix by inverting the tube a couple of times. You should see white DNA precipitate out of solution. [Better yields are achieved if the ethanol is kept at -20°C before use and then in an ice bucket when on the bench]. [More DNA can be obtained if the samples are left at 20°C for a few hours or overnight]. Centrifuge the sample at full speed (12-14,000 rpm) for 10-20 minutes at 4°C. [room temperature will suffice if there is plenty of DNA]. Pour (or pipette) off the supernatant, taking care not to dislodge the pellet of DNA. Wash DNA pellet with 70 % ethanol as above. After removing the 70 % ethanol, briefly centrifuge the samples to get the last of the ethanol to the bottom of the tube; pipette off the remaining ethanol. Leave the sample to air dry. Usually 10-15 min depending upon the temperature. Add 100 µl of Elution Buffer or sterile from Tris-EDTA to the DNA and kept in -30 c⁰

B- Preparation of PCR

Polymerase chain reaction master mix reaction was prepared by using (KAPA PCR MasterMix Kit) and this master mix done according to company instructions (10µ Taq ,4µ primer(forward & reverse),5µ DNA template,1µ PCR water) become of final volume 20µl. After the PCR amplification conditions performed with a thermal cycler were specific to each single primer set the depending on their reference procedure, as follows: 40cycle of **Toxo-B1**(94/2min, 94/40sec,57/ 10sec,72/ 30sec,72/5min).35 cycle of **ITS-1**(94/5min,94/30sec,59/30sec,72/30sec,72/7min). 30 cycle of **SAG-1**, (94/2min ,94/1min,65/ 60 sec,72/60 sec,72/5min).40 cycle of **SAG-2**(94/5min,94/1min,60/ 1min,72/ 1min,72/2min).

C- Primers

All the primers and primers with their sequences used in this study and obtained from Bioneer (Korea) are

Primer	Sequence (5' – 3')		(bp)
<i>Toxo-B1</i>	F	TCTTTAAAGCGTTCGTGGTC	193
	R	GGAAGTGCATCCGTTTCATGA	
<i>SAG-1(P30)</i>	F	TTGCCGCGCCCACACTGATG	914
	R	CGCGACACAAGCTGCTGCGA	
<i>SAG-2F3</i>	F	TCTGTTCTCCGAAGTGACTC	241
	R	TCAAAGCGTGCAATTATCGC	
<i>ITS-1</i>	F	AATATTGGAAGCCAGTGCAG	178
	R	CAATCTTCACTCTCTCTCAA	

D- Gel electrophoresis

All PCR products of detection and serotyping genes were analyzed by loading in agarose gel in different percent's as described ⁽⁹⁾.

Statistical analysis:

The Statistical Analysis System-Spss(9 version) was used to effect of difference factors in study parameters (percentage and level). The following statistical tests of significance were used:

- **Chi-square test (X^2)** to test the relationship between two qualitative categories.
- The **independent-sample t-test** procedure to compare means of two quantitative variables.
- Graphs were plotted using **Microsoft Excel program2010**.
- The order of references are using **Endnote program**

Study design was a **case control study**.

Ethical considerations were the student received from the Committee on Research.

RESULTS

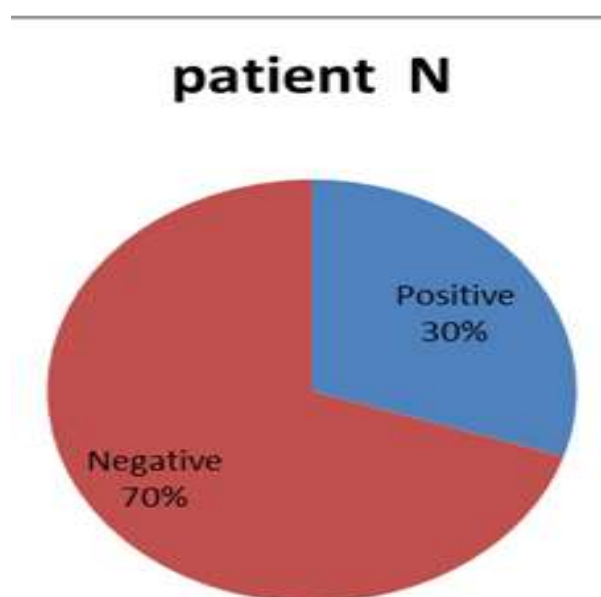


Figure (1): Distribution of tested sera by Toxoplasma Latex agglutination test (LAT):

This figure shows 60 (30%) sera out of 200 were positive for anti- Toxoplasma antibody, while 140 (70%) were negative for anti- Toxoplasma antibody in comparison with control sera.

Table (1): The concentration of anti-Toxoplasma (IgG& IgM) antibodies by minividas

Groups Parameter		Concentration			
		Patient		Control	
		No.	%	No.	%
IgM mIU/ml	Positive	3	75	1	25
	Negative	16	62	10	38
IgG mpIU/ml	Positive	37	95	2	5
	Negative	4	19	17	81

Table 1 shows the serum samples from the study population (patients and controls) were successfully analyzed for IgG & IgM by minividas. Results indicated that 3 (75%) of the patients had elevated IgM levels (>1.0 mIU/ml), while only 1 (25%) of control groups was elevated in IgM levels. The negative results for IgM levels was 16 (62%) patients groups and 10 (38%) were in the control group. The Results indicated IgG that 37 (95%) of patients had elevated levels (>1.0 mIU/ml), while 2 (5%) were control IgG levels. The negative results in IgG levels were 4 (19%) in patients and 17 (81%) were in the control group.

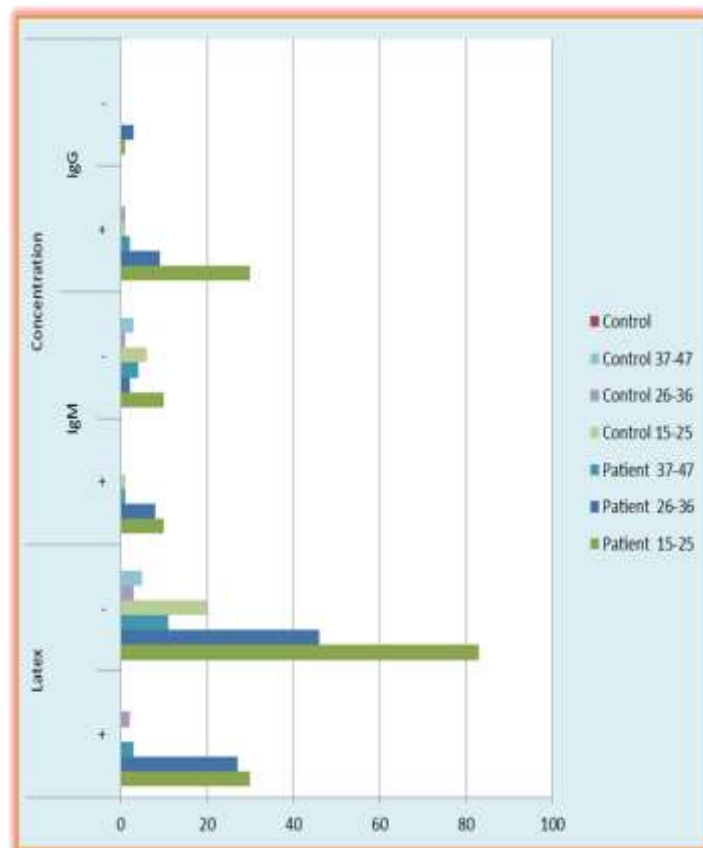


Figure (2): Distribution of anti-Toxoplasma IgG & IgM antibodies among different age groups:

Figure 2 shows that the distribution of anti-Toxoplasma (IgG & IgM) antibodies among ages groups by taking the total number of patients in each age group; which represented by the highest number of patients 30 (27%) in the age category (15-25) years and 27 (37%) were in the age group (26-36) years, while the lowest number 3 (21%) was shown in the age group (37-47) years using latex agglutination test, however in minividas the concentration of IgG & IgM antibodies were different in total number of patients for each age group were 10 (50%) of (IgM) patients shows positive results in the age groups (15-25) years, while 8 (80%) in the age groups (26-36) years. Furthermore, only 1 (20%) was positive in the age group (37-47) years and (the positive results for IgG antibodies were 30 (97%), 9 (75%) and 2 (100%) in first, second and third age groups respectively.

Table (2): The distribution of abortion according to the age groups by Latex agglutination test:

Groups Ages	Abortion No.	%	Non abortion No.	%
15-25	26	40%	39	60%
26-36	20	51%	19	49%
37-47	2	40%	3	60%
Total	48	44%	61	56%

Table 2 shows the age groups of repeated abortion according to the studied samples were illustrated in table and figure (4-6). The 26 (40%) patients were positive by using latex agglutination test in the age group (15-25) years and 20(51%) were in the age group (26-36) years, while only 2 (40%) were in the age group (37-47) years. The non-abortion women reveal positive results with distributions in 39, 19 and 3 in the first, second and third age categories respectively.

Table (3): The detection of parasitic by PCR technique:

Test		PCR			
		<i>Toxo-B1</i>	<i>SAG-1(P30)</i>	<i>SAG-2F3</i>	<i>ITS-1</i>
Patient No.	+ve	30	10	20	52
	-ve	200	220	210	178

Table 3 shows that, there was a significant difference between blood and placental tissue samples in giving positive PCR. Two hundred DNA extracts from blood samples and thirty DNA extract from placental tissue of the same patients revealed with toxoplasmosis. The highest number recorded was in *ITS1* gene of (52) followed by the rest of the gene (30,20 and 10) respectively in the genes *Toxo-B1*, *SAG-1*, *SAG-2*.



Figure(3): Ethidium bromide-stained agarose gel of PCR amplified products from extracted DNA isolates from (blood & tissue) and amplified with *Toxo-B* primers (forward and reverse).

The lanes (1) show positive(blood). The lanes (5-6,8) show positive(tissue). The lanes (2-4,7,9) show negative.(DNA molecular size marker(M) (10000-bp ladder)).

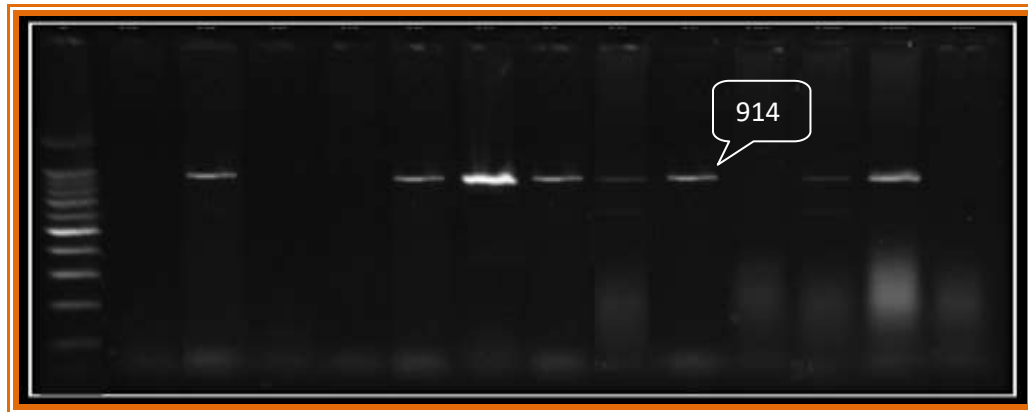


Figure (4): Ethidium bromide-stained agarose gel of PCR amplified products from extracted DNA isolates from (blood & tissue) and amplified with SAG-1 primers (forward and reverse). The lanes (5-6) show positive (blood). The lanes (2,7-9,11-12) show positive(tissue). The lanes (1,3-4,10,13) show negative.



Figure(5) : Ethidium bromide-stained agarose gel of PCR amplified products from extracted DNA isolates from (blood & tissue) and amplified with SAG-2 primers (forward and reverse). The lanes (5-6) show positive(blood). The lanes (2,7-9,11-12) show positive(tissue). The lanes (1,3-4,10,13) show negative.

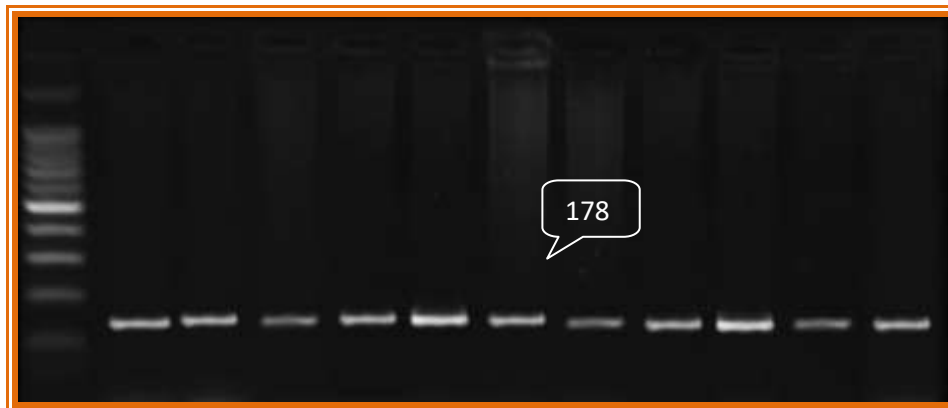


Figure (6) : Ethidium bromide-stained agarose gel of PCR amplified products from extracted DNA isolates from (blood & tissue) and amplified with *ITS-1* primers (forward and reverse). The lanes (1-8) show positive (blood). The lanes (9-11) show positive (tissue).

DISCUSSION

Toxoplasma gondii is a wide spread intracellular pathogen and its infection is usually asymptomatic but can cause severe disease in emerging fetuses or immunocompromised adults. Fetal toxoplasmosis may result in death of a fetus, abortion or congenital infection ⁽¹⁰⁾.

This parasite is a strong stimulator of type1 cytokines like IL-12 and IFN- γ , that prompt early abortion ⁽¹¹⁾. Predisposition of the pregnant women to toxoplasmosis may be enhanced by type 2 cytokines such as IL-4, IL-10 and IL-5, which are kept during pregnancy ⁽¹²⁾. The study involved 30 healthy and (200) of blood and (30) of the placenta from the patient toxoplasmosis, and all these patients were limited to only females at reproductive age (15-47 years).

Distribution of the sera for patients with Toxoplasma by Latex agglutination test (LAT)

The LAT positive patients was 60(30%) among women to have toxoplasmosis ,and this results in assessment to other Iraqi studies carried out in other cities and nearly similar to that reported in Basra,(35%) by ⁽¹³⁾, in Baghdad, (38.8%) by ⁽¹⁴⁾, in Kirkuk, (36.6%) by ⁽¹⁵⁾. This result indicate ahigh scattering of toxoplasmosis among women in the study area, and local results that had been reported by ⁽¹⁶⁾ in Al-Diwanya, ⁽¹⁷⁾ in Thi-Qar and ⁽¹⁸⁾ in Al-Najaf .The similarity in results may be ascribed to ease of use of the same appropriate conditions, including temperature and humidity that allow to the longer infectivity and viability of the oocysts which considered as a main source for infection spread in these cities. Furthermore results were similar to that described in Mosul,(69.2%) by ⁽¹⁷⁾ and (49.85%) by ⁽¹⁷⁾, in Duhok, (46.9%) by ⁽²⁾ and in Baghdad, (60.21%) by ⁽²⁾,but it is lower than that illustrate (80.6%) by ⁽¹⁰⁾. Comparing the current study with those of other Arab countries; it is lower than that stated in Egypt (14.57%) by ⁽¹²⁾ and in Saudi Arabia,(25%) by ⁽¹⁾. The infection rate of this study was higher than the rates of many other revisions that were recorded by LAT in other countries. ⁽⁵⁾ reported that, 30.1% of tested patients in Turkey were seropositivity to the toxoplasmosis. ⁽¹¹⁾ pointed out in study concerning three rural communities in Mexico revealed that the dominance rate was 23.8% and in Korea ⁽¹⁷⁾ . This result indicates a relatively lower frequency of this disease according to this test, on the other hand this result was higher to that reported in AL-Najaf by means of ⁽⁸⁾ which was 52.2%, also higher than that result existing by ⁽¹⁾ which was 59.9%. Which was 33.07% and higher than result recorded by ⁽¹¹⁾ and 24.5% and 18%, in that order, it is also higher than the rest (6.6%) registered in the Korean study by ⁽⁹⁾.

The concentration of anti-Toxoplasma (IgG& IgM) antibodies by minividas

The result has found that the sensitivity and specificity of minividas for IgG and IgM anti-toxoplasma antibody were 95 and 75% in that order ,therefore minividas kit on the basis of the current results can be considered a good screening test outstanding to its high (sensitivity and specificity).⁽¹⁹⁾, who demonstrated that seropositivly rate was (37%) by using ELISA method, and with⁽²⁰⁾, who recorded (37.5%) seropositivly rate among aborted woman by using VIDAS Toxoplasma competition kit. The present result was moreover lower than earlier studies in Iraq ⁽²¹⁾ they revealed higher percentage of seropositive in woman infected with toxoplasmosis 40.6%, 49.7%, and 51.52%, respectively. The description of this variation may be related to variable sample size, location, type of kit used and the different strains of *T.gondii* that plays an important role in differences of infection rates. The present study also showed lower percentage than several studies in some worldwide countries it was ⁽¹⁶⁾ showed whole IgG seroprevalence rate of human toxoplasmosis at 45% in India and 77% in Iran respectively. The results of all these studies showed high prevalence of *T.gondii* in those communities who eat raw or

undercooked meat, unwashed vegetable or fruit ⁽²¹⁾. Also, owing domestic animals like cats, and dogs or other animals and exposure to contaminated soil is another reasons for infection ⁽²²⁾.

The results rate of toxoplasmosis by using ELISA IgG test was lower than those reported by other studies . ⁽¹⁷⁾ and ⁽²²⁾ were found that 48% and 34% of LAT positive patients in Najaf situated positive by IgG-ELISA test, and also ⁽¹⁰⁾ reported that IgG-ELISA test was positive in 46.75% of total. but similar seroprevalent study showed wide difference ranging between 5% to 81% among different areas in Beirut ⁽²⁰⁾ ,when the results were much higher than a result conducted in Gaza by ⁽¹³⁾ , The existing result was nearly similar with the results recorded by ⁽²²⁾ .

Distribution of anti-Toxoplasma (IgG & IgM) antibodies among the different age groups:

The current study revealed there is non-significant difference ($p=0.242$) in relation to LAT technique for diagnosis followed in the age group(15-47) years , while significant increases were showed in IgM and IgG technique by Minividas, among the age group (15-47) when compared with control groups.

Concerning seroprevalence versus age, the results were in covenant with ⁽²³⁾ and ⁽¹⁵⁾ in Iraq , also similar to data obtained by ⁽²¹⁾. A study of pregnant women revealed a seropositivity of 31.7% and 48.3% among age group (15-24) and (25-34) years respectively, while it was in 20% and (35-45) ⁽¹²⁾, in addition , seroprevalence was 23% and 40% in the age groups (40-49) and (60-69) years respectively. The increasing trend of Toxoplasma seropositivity may be related to increasing the chance of exposure to the infections agent.

This critical period of women's life has higher chances for activation of latent infection of *T.gondii* that can be transmitted vertically to the fetus which is considered as one cause of abortion as mentioned by ⁽²³⁾, It has been concluded that Toxoplasma infection is associated with age and this is confirmed statistically (P value <0.01) and this result is consistent with almost all other studies, as the study of ⁽⁴⁾ and ⁽⁵⁾ in Iraq, ⁽⁶⁾ in Saudi Arabia.

The study showed that patients with toxoplasmosis patients were seen among the (15-47) years old with a non-significant difference ($P>0.01$) in spreading of infection among different women age groups according to the LAT. While significant difference ($P<0.01$) in distribution of infection among different women age groups by minividas.

Evaluation of patients by PCR technique according to the type of specimen.

Infection with *T.gondii* usually leads to a big problem among pregnant women, In addition to cause the loss of a pregnancy, it may cause a wide range of congenital malformations, for the reasons above, and early diagnosis of toxoplasmosis in pregnant women allows early intervention and prevention of congenital illnesses that usually lead to the death of the fetus ⁽²⁴⁾.

The study uncovered that out of 30 samples of placental tissue was only 20(67%) of positive patients for the result of the DNA, while that of the 200 blood samples from the same patients gave 60(30%) patients only positive result of DNA, this was the result similar to ⁽²⁵⁾, who reported that 58.6% of the samples PCR assay was positive before, and it was also nearly identical to the results from the ⁽²⁴⁾ who found that 63.49% of the samples tested were from Brazilian women were positive by PCR using the B1 gene. The result was less than that recorded by the company ⁽²⁵⁾ and ⁽²⁶⁾, who reported that 17.65% and 83.3% of the total samples that were tested were positive, in that order.

The conventional diagnosis of toxoplasmosis relies on sociological methods, but cannot be relied upon in vaccines may be individuals immunodeficient,. In addition serological methods may fail to identify at an early stage of infection ⁽²⁷⁾. ⁽²⁸⁾ that the serological diagnostic methods have confines because antitoxoplasma specific antibodies may not be present in the infection

early, and antibodies, especially IgM may not go up over and done with the activation of the cystic form of the parasite.

The current detection technique of parasite DNA by PCR may improve early diagnosis ⁽²⁹⁾.

CONCLUSIONS

It can be concluded that the PCR assay in blood and tissue has a role in detection of recent and active toxoplasmosis. The effect of *Toxoplasma gondii* with the differentiator of ages and the number of abortions .The cytokine has a significant role in the immune response against *Toxoplasma gondii*. The correlation between of the cytokines and toxoplasmaosis with according of abortion .

RECOMMENDATION

- 1- To study of correlation between ITS-1 gene with toxoplasmosis.
- 2- The working on the remove of the gene that cause of spontaneous abortion.
- 3- Further the detection of genotypes that leading to spontaneous abortion.

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