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Research Article

# Immunological and Molecular Study of *Toxoplasma gondii* in Al-Najaf Governorate - Iraq

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**ABSTRACT**

The study was conducted on 350 aborted women and thirty healthy women, whom have visited Central public Health Laboratory in Al-Najaf Governorate during the period from November 2014 to May, 2015. This study was designed to investigate of some tests which determine the infection with *T.gondii* in clinical suspected women in an- Najaf governorate by using two methods, detected specific IgG and IgM by VIDAS technique and PCR technique to indicate *Toxoplasma gondii*. Furthermore, it is designed to determine the effects of *T.gondii* infection on some parameters such as blood cells, cytokine and immunoglobulins besides, the results indicate that PCR were the best methods that can be used in diagnosis and to determine the prevalence of *T.gondii*. Yet the blood parameters gave a clear understanding to physicians to cure patients. Whereas the numbers, percentage, sensitivity and specificity of infected women vary with different tests were 67 (19.14%) (93.05) (98.31), while PCR gave the highest numbers, percentage, sensitivity and specificity were 71 (20.28%) (98.61) (99, 64) respectively for each test. DNA of *T.gondii* parasite was determined by PCR was using three specific primers ($B_1$, 18S and P30) whereas the number, percentage and sensitivity of infected women of these primers were 71 (20.28%) (98, 61%), 66 (18.85%) (91.66%), and 62 (17.71%) (87.32%) respectively. By comparing these primers to evaluate the efficiency in diagnostic of *T.gondii.* The results indicated that $B_1$, 18S are the highest primers and P30 is the lowest primers in diagnostic efficiency. The results showed a significant elevation ($P<0.05$) in serum concentration of pro inflammatory cytokines interleukin-5 (IL-5), interleukin -27 (IL-27) and immunoglobulin M, while no significant ($P>0.05$) in level of immunoglobulin G in *T.gondii* infection patients compared to control group. However, the total Leukocyte count showed significant increase ($P< 0.05$) in infected women compared to the control group due to elevation percentage of neutrophil, monocyte, eosinophil, and lymphocytes while the number of Basophil, remain normal. There were positive correlation between proinflammatory cytokines interleukin-5 (IL-5), interleukin-27 (IL-27) and neutrophil, lymphocytes and IgM levels. The current study has also revealed positive correlation between lymphocytes and immunoglobulin M. The results have also exhibited a negative correlation between Il-5, IL-27 and IgG. Also, revealed a negative correlation between neutrophil, lymphocyte and IgG. The current study has concluded that the infection with *T.gondii* effect on blood cells, immunity of the body and the PCR test are the best methods and B1is the best primer used in the diagnosis of *T.gondii* parasite.

**Keywords**: IL-27, *Toxoplasma gondii*, IL-8, Al-Najaf**.**

## INTRODUCTION

The most common pregnancy complication is fetal loss, occurring in 25-30% of recognized pregnancies. Recurrent pregnancy loss affects at least 1% of all couples[1] and can be defined as two or more failed pregnancies[2]. *T. gondii* is one of the important obligate intracellular protozoan parasites, classified in the phylum Apicomplexa, a significant human and veterinary pathogen. It is enters the host via the digestive system and poses a severe risk for congenitally infected infants[3]. There are three types' strains of *T. gondii*. Type 1, 2 and 3 strains, type 1 is highly virulent[4]. The diagnosis of recently infection of toxoplasmosis has been revealed either by demonstrating a specific immunoglobulin (IgM) antibody, a significant increase in specific IgG antibodies, or both. Study of toxoplasmosis is spurred for essentially three Causes. First, *Toxoplasma* can cause dangerous diseases, e.g. encephalitis, retinitis, myocarditis and pneumonia[5]. Second, *Toxoplasma* is used as model-system of Apicomplexa parasites[6]. Finally, *Toxoplasma* is an important veterinary pathogen with high estimated costs owing to disease, abortion or vaccination in animal farming[7]. There are three stages in complex life cycle of *Toxoplasma*. Tachyzoite develops through the acute phase of infection and replication into the cell. Tissue cysts are produce from Bradyzoite during latent infection[8]. The sporozoite is a third environmentally resistant is third stage found in mature oocyst of the parasite. The life cycle of asexual development represent by the first and second phase which occurs in the intermediate hosts of parasite including man. In the intestinal tissue of cat just the sexual development of life cycle represents the third stage (oocysts)[9]. The individual immune response to infection of *Toxoplasma* is evaluated by status of immune, infection

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timing, and the organism and the host genetic composition[10,11] recorded that the essential resistance of host against the parasite is strong response of immune cells that induced by opportunistic infection of *T. gondii*. Despite of the highly hygiene in human population of developed countries *T.gondii* considered is widely distributed and important public health problem especially in the rural areas, due to the habits in favour of acquiring the disease[12] . It was found in high prevalence in Iraq[13]. *Toxoplasma* infection may lead to severe if not fatal infection of the fetus during pregnancy[14]. Humoral immune response and cell mediated immunity, in addition to the role of cytokines stimulates during toxoplasmosis infection[12].

In mice infected with *T.gondii* IL-5 was investigated and believed that IL-5 have a role in oral infection at early stage with *T.gondii*[15,16] Suggested that role in the production of interlukin-12 and interlukin-5 has a protective role against toxoplasmosis[17] recorded that interlukin-27 and tumor necrosis factor-b act as anti-inflammatory factors were secreted from intestinal epithelial lymphocytes during *T. gondii* infection. Also, Il-27 may be promoting immunopathology by suppressed production of cytokines from T helper17 cells[18]. The present study has been conducted to seek the use of specific IgG and IgM of *T. gondii* by VIDAS technique and PCR technique to investigate the prevalence of *T .gondii* infection in aborted women to determine the most accurate test that can be used in diagnosing this parasite and investigates the effect of *T .gondii* infection on blood cell indices. This aim was achieved by the following objectives:

- Identification of *T. gondii* by specific IgG and IgM VIDAS technique.
- 2- Extraction of DNA to detection and amplification of DNA of *T .gondii* by using PCR technique.
- Determination and compared the sensitivity and specificity of different published primer sets.
- Estimation the levels of IL-5, IL-27 and white blood cell.

## MATERIALS AND METHODS

*Blood Specimens collection*

Five ml was the total blood collected from each clinical suspected woman with *T.gondii* infection and non-suspected women (as control group) by disposable syringe, 2.5 ml of blood kept at room temperature for 30 minutes. The collection of samples was approved by the Institutional Ethics Committee of the Faculty of Science at the University of Kufa and all participants signed informed consent forms. The blood samples have been centrifuged at 3000 rpm for 5 minutes to isolated of serum and have been collected in other sterile tubes, each sample of serum was distributed into four parts; each of them was kept in deep freeze at -20C ºuntil used for serological test and other part of blood 2.5 ml from each of blood samples were drawn in EDTA tubes were divided into two parts the first 0.5 for Haematological Assessments , the second part 2 ml of blood was drawn in tube with anti-coagulated EDTA (Abott /Jordan) which was used for DNA extraction.

*Hematological Methodology*

*Procedure*

Differential count was performed by using CYANHemato analyzer (automatic hematology analyzer. Catlog No.CY006, Diagnostic, Langdorpseteenweg 160, B-3201 Belgium).

*VIDAS TOXO 1gG –ELFA kit*

VIDAS TOXO IgG II is a computerized quantitative exam for usage on the intimate apparatuses for the estimate of quantitation of antitoxoplasma (IgG) of serum, by the Enzyme Link Fluorescent Assay (ELFA).

*Procedure*

- The reagents have been put at RT for 30 minutes
- For each sample one strip of each (TXG and TXG SPR) have been used, control test. The required SPRs were carefully opened.
- By the code "TXG" on the device the test was recognized. The calibrator was recognized by "S1", and screened in twice. The positive and negative controls have been tested by C1 and C2 respectively.
- By using Vortex- type mixer the calibrator controls and samples were mixed.
- 100 microliter from controls and samples was used in test.
- 6-TXG SPRs and TXG strips" introduced in the device. The labels tested by the code of the assay on the reagent and the (SPRs) strips were coordinated.
- All the test steps were done automatically.
- After pipetted the vials were re-stopper and returned then to 2-8˚C.
- The SPRs and strips have been removed from the device after the assay was completed at 40 minutes.
- Strips and SPRs were disposed in to an appropriated recipient.

*VIDAS ® TOXO IgM II (TXG) –ELFA Kit*

The assay max human toxo IgM ELISA kit was conducted according to the manufacturing company (usbio, U.S.A.) using the same procedure described in toxo IgG

*Molecular detection of T gondii by PCR*

*Procedure*

- Blood sample collected in EDTA tubes.
- 300 ul of blood was added in 1.5 ml microcentrifuge tube.
- 900 microliter of red blood cell lysis buffer was added and mixed.
- The tube was incubated for 10 minutes at room temperature.
- Centrifugation for five minutes at 3000 xg to remove the supernatant.
- The cell pellet was resuspended in 100 microliter of cell lysis buffer.
- Before mixed by vortex 200 microliter of GB buffer added to the 1.5 ml microcentrifuge tube.
- The mixture was incubated at room temperature for 10 minutes.
- The mixture inverted every 3 minutes during incubation.
- 200 microliter of sample lysate was added to absolute ethanol and mixed by vortex immediately for 10 min, then transferred to GD column and the GD column was put inside 2ml collection tube.
- Centrifugation at "14000-16000 Xg for five minutes".

- The collection tube has been discarded with the flow-through.
- The GD column putted in a new 2 ml collection tube.
- (400 µl) of W1 buffer added to the GD column.
- The mixture has been centrifuged at 14000-16000 Xg for 30 seconds.
- The flow-through discarded and the same GD Column reused.
- 600 microliters of wash buffer added to the GD Column.
- Centrifugation for 30 seconds at 14000-16000 Xg.
- The flow-through in the collecting tube discarded and placed back of the GD Column.
- Centrifugation for three minutes at 14000-16000 Xg to dry the column matrix.
- Dried GD Column was transferred to clean 1.5 ml microcentrifuge tube.
- 100 microliter of preheated elution buffer (70ºC) to the center of the column matrix.
- The elution buffer was absorbed by the matrix via standing for 3-5 minutes.
- The purified DNA eluted by centrifugation at 16,000 x g for 30 seconds.
- Purified DNA kept in -20 degree up to use.

*DNA Amplification*

The infections of *T gondii* confirmed by PCR amplification of B1 gene, P30 and 18S using specific gene primers as following:

- 8 µl of purified DNA transferred to 0.2 ml PCR tube of master mix kit (contains 5 µl of master mix).
- 2.5 µl (10 uM) of each primer (forward and reverse) has been added.
- 2 µl of deionized water added to completed of reaction volume (20 µl).
- The mixture was mix briefly by centrifugation for 3000 x g for 10 seconds to homogenize the contents.
- PCR tubes the thermo-cycler, and then started the program table 2.

*Analysis of Amplification Products*

The products of PCR was analyzed by 1.5% agarose gel electrophoresis which prepared according to [19](Sambrook, 2001) 1.5 gm of agarose added to beaker contains 100 ml of TBE (PH 8.0). The mixture was melting to homogenizer mixture by heating with microwave at 600 Wt. for 1-2 minutes. Allowed the mixture to cool down at room temperature approximately 50°C, then added of 0.5 µl ethidium bromide was added. The agarose gel was poured in gel tray and the comb put after sealing both edges of tray. The comb was removed and the gel placed in the chamber, and the chamber filled with TBE buffer. Ladder loaded carefully in the first left well then 5µl of DNA sample loaded in the wells. The gel electrophoresis runs at 80 Volts. For 90 minutes. The DNA band was observed by U.V. Transilluminator.

*Detection of cytokines*

These tests were intended for quantification of serum levels of certain pro inflammatory cytokines (IL- 5and IL-27) through the immune-enzymatic technique Enzyme-linked Immunosorbant Assay (ELISA) using bio Elisa reader ELx800 (USBIO, U.S.A.) in zoology laboratory of faculty of science .

*Detection of Interleukin -5*

The Assay Max Human Interleukin -5 kit of ELISA (Cat.NO L14103161) was conducted by (usbio, U.S.A.).

*Assay Procedure*

- Before starting assay procedure prepared Wash Buffer (1X) and IL-5 Standards.
- Each well of micro plate was filled with 50 microliter of Sample and standard and incubated at RT for one hour after sealed with cover.
- 1 drops of l8427-048 Anti-human IL-5 (Biotin) was added to each well without washing the plate and gently mixed and incubated at RT for one hour after sealed with cover.
- Incubation mixture was removed from plate by washing with Wash Buffer (1X). Repeated this method five times and drying the plate by hitting it onto paper towels incubated for 1 hour at RT and covered after adding 100 microleter of I8427-04C (Avidin) to each well.
- Before 15 minutes of second incubation substrate Solution was prepared, repeat washing in previous step.
- Each well filled with 100 microliters of substrate solution and incubated at RT for 15 minutes after sealed with cover.
- 100 microliter of stop solution (8427-04K) was added to each well and mixing.
- A micro plate reader set used to read of 450 nanometer (O.D.) during 30 min.

*Detection of Interleukin -27*

The Assay Max Human Interleukin -27 kit of ELISA (Cat.NO 143914) was conducted by (USBIO, U.S.A.) using the same procedure described in IL- 5.

*Statistical analysis*

Data were analyzed using the software packages Graph pad prism for windows (5.04, Graph pad software Inc. USA), Data are presented as the mean ± standard error (SE). The comparison between the patients and healthy groups were analyzed by one-way ANOVA. In addition the correlations between parameters were performed by Pearson's correlation coefficients (r). A p-value $< 0.05$ was considered significant.

## RESULTS

*Toxoplasma gondii* Detection

The parasite was recognized after examinations of the serum by using IgG and IgM specific VIDAS test, sixty seven out of 350 (19.14 %) women were found infected with *T.gondii* by this test as shown in Table 4.

*Polymerase Chain Reaction PCR*

A total of 350 specimens were tested with different primer set. The number and percentage of *Toxoplasmosis* infection, as defined by positive IgG and IgM specific VIDAS test and PCR technique. The number and percentage of *T.gondii* obtained by IgG and IgM specific VIDAS test 67 (19.14%), and PCR were the number and percentage 71 (20.28%), as seen in table (3). Seventy one specimens were positive by three primer sets, as seen in table (1), number and percentage of blood sample were

Table 1: Oligonucleotide Primers Used in Current Study.

| S. no | Oligonucleotide Primers | company | Country | Primers name |
|---|---|---|---|---|
| 1 | GGAACTGCATCCGTTCATGAG | Bio Synthesis | USA | B1 |
| 2 | TCTTTAAAGCGTTCGTGGTC | Bio Synthesis | USA | |
| 3 | TTGCCGCGCCCACACTGATG | Bio Synthesis | USA | P30 |
| 4 | CGCGACACAAGCTGCGATAG | Bio Synthesis | USA | |
| 5 | CCTTGGCCGATAGGTCTAGG | Bio Synthesis | USA | 18S |
| 6 | TAGGCATTCGGGTTAAAGATTA | Bio Synthesis | USA | |

detected by using primer $B_1$ 71(20.28) ,while P30 were 62 (17.71%) respectively, by using 18S primer were 66 (18.85%) respectively. The result revealed that the two primers sets $B_1$ and 18S gave the highest sensitivity (98.61%) and (91.66%) respectively, but the P30 has a lesser one of sensitivity (87.32%) than the other primers, these results are shown in Table (3). The present study has shown the effected of three primer (B1, P30 and S18) on DNA genomic of *T.gondii* are shown on agarose gel electrophoresis, as seen in Figures 1, 2 and 3 respectively.

*Leukocyte Count*

*Total Leukocyte Count ($X10^3/mm^3$)*

Table 4-3 exhibited significant increase ($P < 0.05$) of TLC in patients infected with *T.gondii* parasite as compared to the control group.

*Differential Leucocyte Percentage %*

The result of differential type of lymphocyte, monocyte, neutrophil and eosinophil shown that the significant increase ($P < 0.05$) in patients suffering from *T.gondii* in compared to control group .but the basophil showing non-significant ($P > 0.05$) change in patients group as compared to control group, as seen in Table (5).

*Interleukin – **5** (IL – 5) and interleukin-27 (IL-27)*

The current study revealed that concentration of (IL-5) and IL-27 in women infection with *T.gondii* were significant increase ($P< 0.05$) (44. 30 ±1.21 pg /ml), (751.7± 32.012) respectively in compared to the control group (13.56 ± 0.721 pg /ml), (1721± 89.153 pg/ml) respectively, as seen in figure (4) and (5).

*The Immunoglobulin Status in Patient and Control Group*

The results of present study as shown in figure (6) and (7) revealed that the concentration of immunoglobulin M was significant increase ($P< 0.05$) (0.2831± 0.082) compared with control group (0.2492± 0.190), while IgG was no significant ($P< 0.05$) (37.02±0.481) compared to control group (36.94± 0.301).

## DISCUSSION

*Toxoplasma gondii* can remain dormant for the lifespan of the host as encysted bradyzoites. However, if the host becomes immunocompromised due to illness, *T. gondii* can reconvert from its dormant stage into the highly virulent tachyzoite stage and cause a recurrence of infection that may prove fatal[20].

The present study has revealed the number and percentage 71 (20.28%) out of 350 women examined and were found to be infected with *T. gondii*. This indicates that is considerable number of females in this society harboring the parasite, transmitter to other people and it is representing a real problem that should not be neglected and must receive attention from health authorities. The aim of the current study was the detection of Toxoplasmosis by using specific primer sets for PCR technique and also evaluation the sensitivity and specificity between the PCR technique and other identification methods which used in current study (detection of specific IgM and IgG) as well as comparison between the sensitivity of primer sets which used.

The results revealed that the elevated positive cases of the number and percentage were 71 (20.28%) respectively from the 350 suspected cases examination by the PCR technique .These results agrees with study of Majeed et al., 2014 [21] Who showed that PCR method more sensitivity than the other method, which used to diagnose the parasites. Detection of *T gondii* by using gene B1 is more sensitivity (98.61%), gene 18s less sensitivity from gene B1 (91.66%) and more sensitivity from P30 (87.32%).Out of 350 suspected blood samples detected 71 positive cases (20.28%) by using gene B1, 66 positive cases (18.85%) by using gene 18s and 62 positive cases (17.71%) by using gene P30.

The highest sensitivity of (B1 and 18S) primers maybe because that the two primers were specific to strain of *T. gondii* found in Iraq, but P30 primer was less specific than the primers recorded above[22]. Results by[23,24] agreed with results of the current study which found B1 gene and 18S have high specificity and sensitivity, therefore, the have been used in diagnosis of *T. gondii* parasites by PCR technique.

B1primer used in PCR technique is highly specific in magnification of *Toxoplasma* DNA and fruitful in the detection of *Toxoplasma* DNA from blood sample of aborted women infected with *T. gondii*.

Also, the results of this study correspond to the study of [25] who reported that P30 gene is single copy genes therefore qualitative PCR appeared less sensitive test and rarely used to diagnose the example of this gene.

DNA does not suspect degradation due to the distinguishing endogenous nuclease activity of *Toxoplasma*, which would have caused in the cleavage of DNA fragments and thus primarily affect the PCR assay with larger target sequences[22].

The product of P30 primer (914 bp) was longer than target of other primers set 18S (88 bp) and B1 primer set (193bp).

The results found that the efficiency of different primers sets were different. The elucidations for these variances belong to numerous strain variability of *T. gondii* and suitability the primer set[26]. In spite of sensitive molecular methods negative PCR may be due to small volume of blood specimens which used as source of *T.gondii* DNA in compared to whole blood in the body of human and small

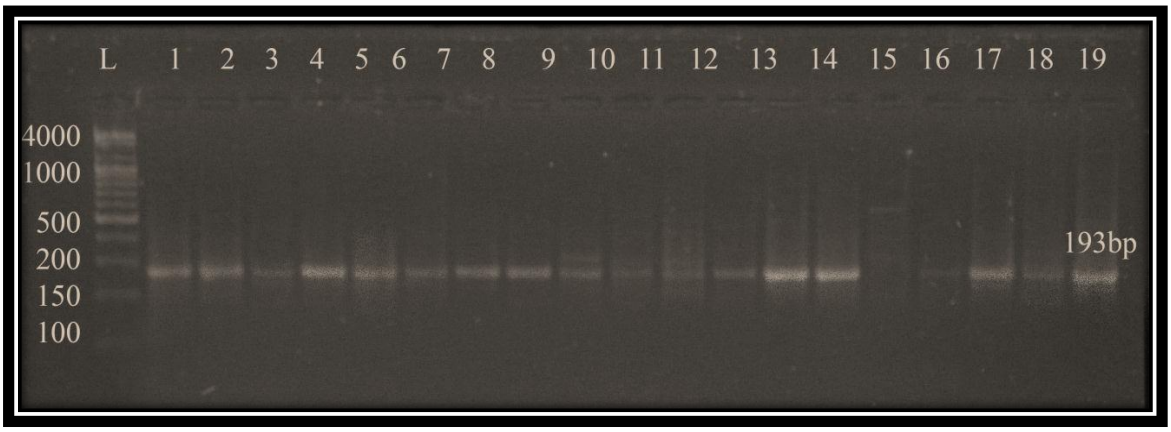


Figure 1: Ethidium bromide-stained agarose gel electrophoresis of monoplex PCR amplified products from extracted total DNA of *Toxoplasma gondii* isolated from blood specimen. Lane: (1 to 19 isolates) amplified with diagnostic B1 *gene*, show positive results at 193 bp . The electrophoresis was performed at 80 volt for 90 Minutes. (L): DNA molecular size marker (100bp ladder, 100 to 4000 bp).

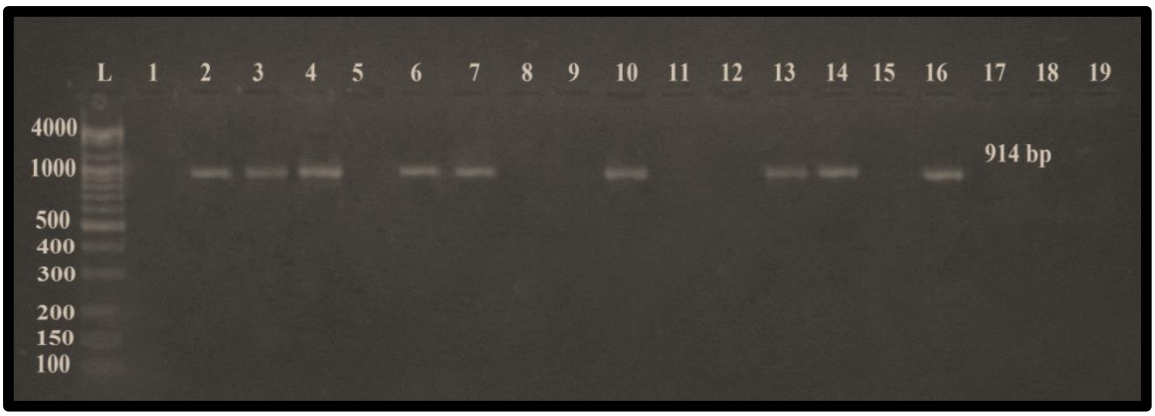


Figure 2: Ethidium bromide-stained agarose gel electrophoresis of monoplex PCR amplified products from extracted total DNA of *Toxoplasma gondii* isolated from blood specimen. Lane: (1 to 19 isolates) amplified with diagnostic P30 gene, show positive results at 914 bp . The electrophoresis was performed at 80 volt for 90 Minutes. (L): DNA molecular size marker (100bp ladder, 100 to 4000 bp).

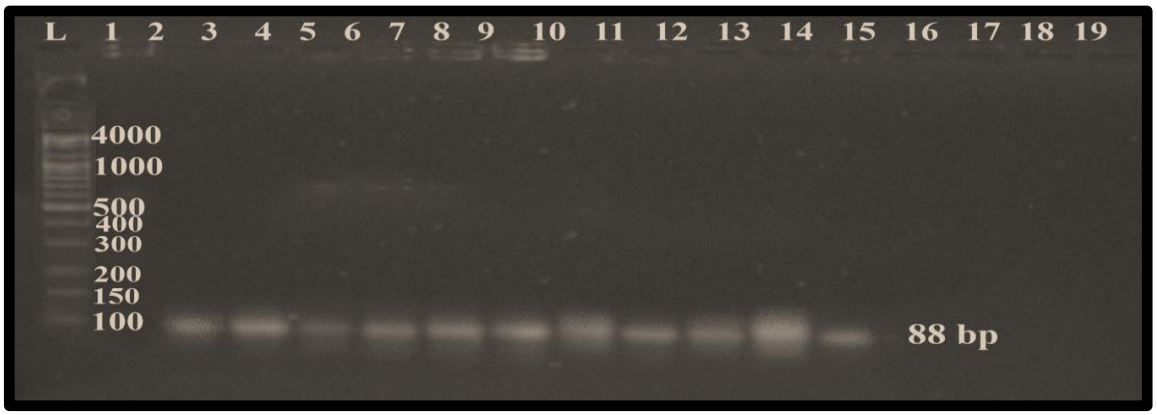


Figure 3: Ethidium bromide-stained agarose gel electrophoresis of monoplex PCR amplified products from extracted total DNA of *Toxoplasma gondii* isolated from blood specimen. Lane: (1 to 19 isolates) amplified with diagnostic 18S gene, show positive results at 88 bp . The electrophoresis was performed at 80 volt for 90 Minutes. (L): DNA molecular size marker (100bp ladder, 100 to 4000 bp).

number of parasite in peripheral blood as well as due to many inhibiter materials in the blood lead to inhibit the PCR reaction, such as hemoglobin, Lactferrin, immunoglobulin G and haeme[27]. Also, may be due to few quantity of *Toxoplasma* DNA may be extracted from clinical samples[28,29].

The current study corresponds to the study of[30], Who conducted for gene primer is less specific than those of the

Table 2: Cycling Parameters of Genes Amplification (19).

| Primers | steps | Initial denaturation | Denaturation | Annealing | Extension | Final extension |
|---|---|---|---|---|---|---|
| B1 | Temp.(C°) | 95 | 93 | 62.5 | 72 | 72 |
| | Time | 5min. | 10sec. | 10sec. | 15sec. | 1min. |
| | Cycle | 1 | 40 | | | 1 |
| 18S | Temp.(C°) | 95 | 95 | 60 | 74 | 74 |
| | Time | 5min. | 10sec. | 10sec. | 1min. | 1min. |
| | Cycle | 1 | 35 | | | 1 |
| B30 | Temp.(C°) | 95 | 95 | 65 | 74 | 72 |
| | Time | 5min. | 1min. | 1min. | 3min. | 7min. |
| | Cycle | 1 | 35 | | | 1 |

Table 3: Compared between the Sensitivity of Primer Sets Used in Detection Methods of 71 Samples of *T.gondii*.

| Primer | Positive case | | Sensitivity % |
|---|---|---|---|
| | No. | % | |
| B1 | 71 | 20.28 | 98.61%* |
| 18S | 66 | 18.85 | 91.66% |
| P30 | 62 | 17.71 | 87.32% |

*The highest sensitivity primers used in detection of *Toxoplasma gondii*.

Table 4: Comparison the Sensitivity and Specificity of Different Detection Methods of 350 Samples of *Toxoplasma gondii.*

| Detection methods | Positive case | | Sensitivity % | Specificity % |
|---|---|---|---|---|
| | No. | % | | |
| Immunological test | 67 | 19.14 | 93.05 | 98.31 |
| PCR | 71 | 20.28 | 98.61* | 99.64* |

*The highest sensitivity and specificity of different detection methods of *Toxoplasma gondii*.

B1 gene were used in PCR amplification of DNA of diagnosis *T.gondii* parasite but does not agrees with his study of that P30 primer greater sensitivity than amplification of 18S primer, these differences may be due to a new strain in Iraq or due to genetic mutation in this parasite.

The results showed a significant increase in the serum level of IgM in aborted women infection with *Toxoplasma* in comparison to healthy group, while they showed no significantly differences in the serum level of IgG in aborted women infection with *Toxoplasma* in comparison to healthy group. These results mean all infected women were in acute stage, the results of study agree with study of [31,32,33,34]. All these studies explain the high serum levels lead to acute phase of Toxoplasmosis and the high avidity of IgG lead to chronic phase of Toxoplasmosis.

In women infected with *T.gondii* the first Abs appears in serum is IgM**,** through the begin of infection. In the human the finest activators of the complement system is IgM because their high level of cytotoxicity and his structure they facilitate good agglutination; this sensation is used especially in serological diagnostic techniques. The major outer Ages of these IgM are the shallow proteins of the *T.gondii*. The concentration of IgM individual difference and may be remain for year in maximum cases. The second Abs appears in toxoplasmosis is IgG, many type of IgG are found through *T.gondii* infection such as IgG1, G2 and G3.due to the ability of IgG of passage the placenta They play a role in defense of the fetus. The chief goal antigens of IgG are the shallow antigens of the *T.gondii*[35,36] .

This increase in the concentration of IgM cooperates with the increase in the B-lymphocyte, which generates IgM responses[37]. Another study done by[38] proved that the concentration of IgM significantly increase in serum of women infection with *Toxoplasma* in comparison with healthy group. This study concluded that there is a significant increase in the concentration of IgM due to the increase in the percentage of B-lymphocyte in peripheral blood in women infection with *Toxoplasma* in comparison with healthy group. This indicates a stimulation of the humeral immune response during the infection with *T. gondii.*

The data of this study indicated a significant increase in WBCs; these due to an increase in the number of monocyte, lymphocyte, neutrophils, and eosinophil because the infection with this parasite causes stimulation in the immune system of host humoral and cellular. The results revealed eosinophilia associated with patients who suffering from *T. gondii* infection. The present study indicated a significant increase in lymphocyte in *T. gondii* infection patients compared to control group. This result corresponds to the result of[39,40] who recorded that the B-lymphocyte is significantly higher in patient with *T. gondii* infection. The results of this study revealed neutrophilia associated with patients suffering from *T.gondii* infections; only a few studies have been carried out on the response of neutrophils to *T.gondii* infection and these results agrees with[41,42,43]who provided an increase in the neutrophil cell in the women infected with *T.gondii* patients compared to control group. The increase of neutrophil count maybe due to immune response of neutrophil against *T.gondii* infection to produce IL-12 which acts against this parasite protozoan[44]. The current study indicated significant increase in monocyte in women infected with *T.gondii* compared to control group, and this result corresponds to that of[44] who recorded that the monocyte count increase in women infection with *T. gondii* to produce IL-12.

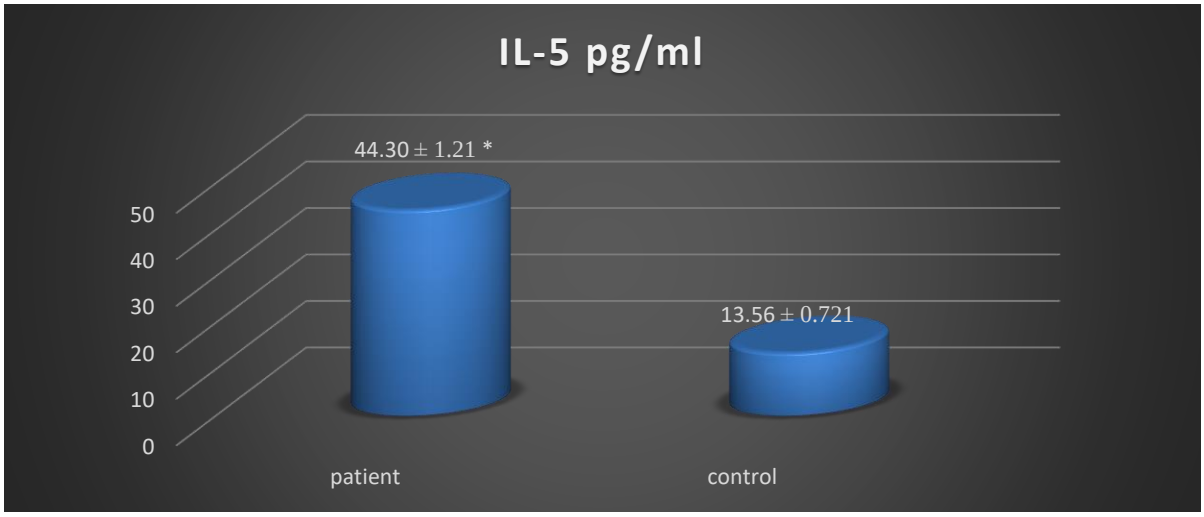


Figure 4: Concentration of IL-5 (pg/ml) Comparison between Patients Suffering from *Toxoplasma gondii* Infection and Control Group.
* Significant difference P<0.05 between control group and patients

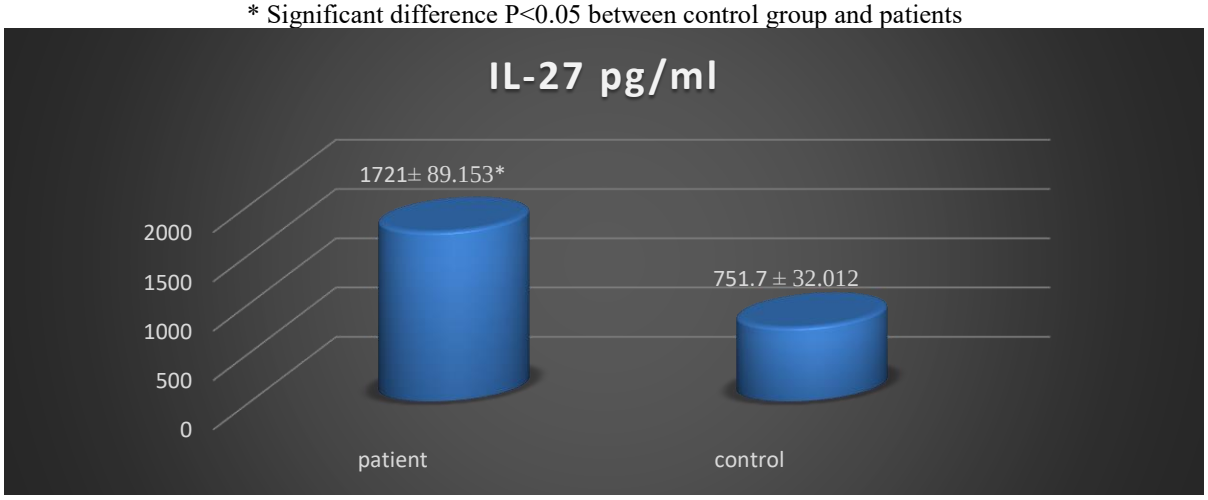


Figure 5: Concentration of IL-27 (pg/ml) Comparison between Patients Suffering from *Toxoplasma gondii* Infection and Control Group
* Significant difference P<0.05 between control group and patients

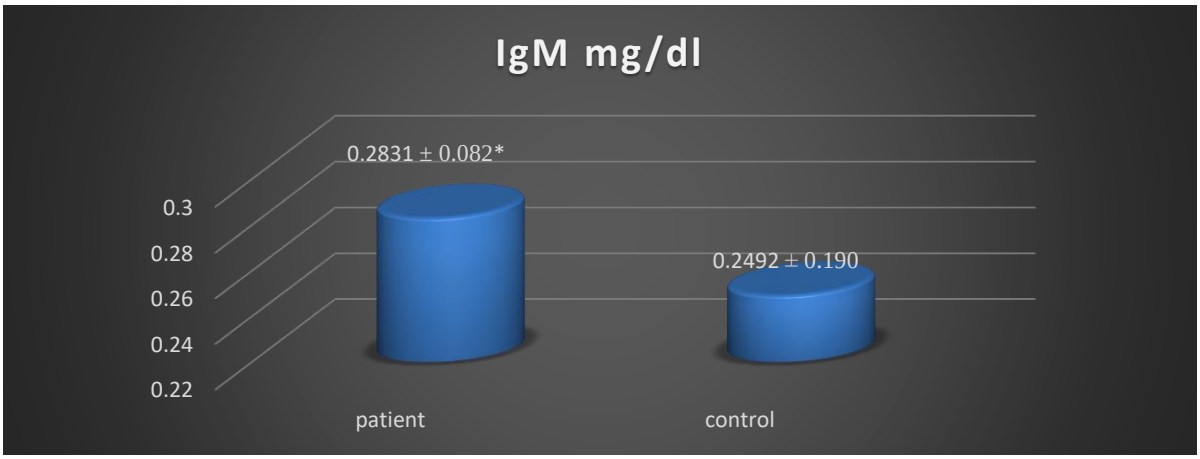


Figure 6: Concentration of IgM (ml/dl) Comparison between Patients Suffering from *Toxoplasma gondii* Infection and Control Group.
* Significant difference P<0.05 between control group and patients

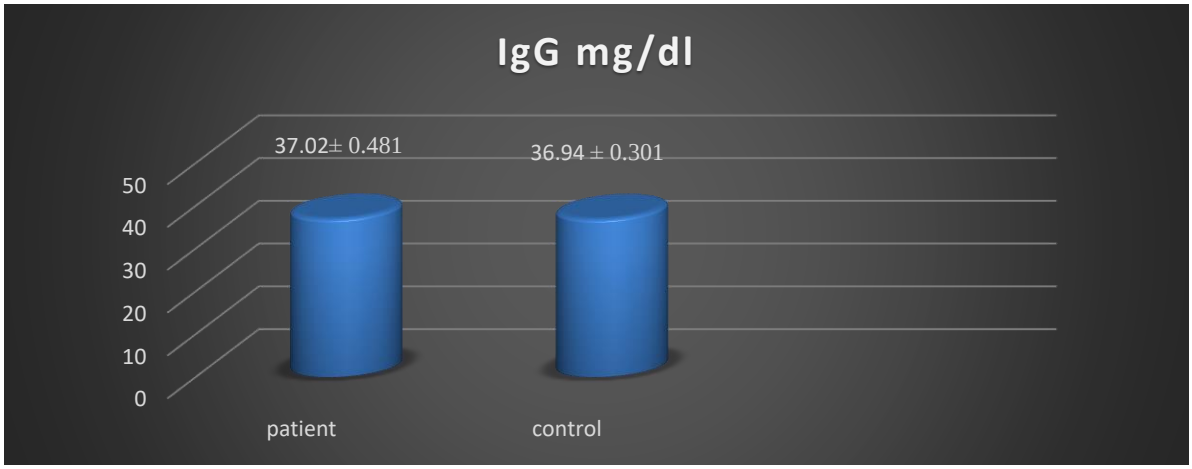


Figure 7: Concentration of IgG (ml/dl) Comparison between Patients Suffering from *Toxoplasma gondii* Infection and Control Group.

Identification of anti-Toxoplasma-specific IgM antibodies is a sensitive indicator for new infection and anti Toxoplasma-specific IgG indicator chronic infection[45].

The current study showed a significantly increase in the concentration of IgM in serum of infected with *T. gondii* patients compared to control group but no significant differences in levels of IgG in serum of infected with *T. gondii* patients compared to control group. This means most of study samples were from women with acute infection but in some cases the existence of IgM is not continuously referred to of an early infection because the IgM continue for months or year after the acute phase of the disease[33].

Results conducted that molecular assay more Sensitivity and Specificity than immunological assay where the percentage reached 98.61 & 99.64 respectively compared with immunological assay where the percentage reached 93.05 & 98.31 respectively, this may be due to delay or failure the body to produce antibodies or for presence some inhibitory substances such as calmodulin, myosin, actin and tubulin intra cytoplasmic of *T.gondii* may be elucidate false positive results through serological diagnosis of parasite infections[46].

This result corresponded with the study by[47,21]. The two researchers diagnosed *T.gondii* by serological and molecular test and they found PCR method was more sensitive and specific than IgG and IgM specific VIDAS test.

The results of the current study corresponded with the study of [47] which that reported that molecular diagnosis from blood specimens giving from women suspected of acute infection with *T. gondii* more specific and sensitive the serological assay (*T. gondii* specific IgM and IgG VIDAS test), found about (29%) of the suspected specimens with DNA of *Toxoplasma* was identified in compared to (20%) positive bioassay. Similar results have been conducted by (3) that reported the negative serological test for women with low-avidity to Abs and negative IgM were sure negative for *T.gondii* DNA by PCR technique.

Higher significance molecular test of DNA detection in peripheral blood of aborted women infected with *T. gondii* may be due to the only technique for both the diagnose and noting of the Genotyping and molecular identification clinical specimens for administered anti parasitic drugs[48,49,50,51].

Negative PCR of blood samples may be due to few number of *T.gondii* In the peripheral blood short remain time of parasitaemia or small size of blood sample which used to DNA is extracted compared to the total volume of blood in the human body and presence some inhibitory substance in human blood that may impede the reaction of PCR assay such as hemoglobin, haeme, immunoglobulin G and Lactferrin[47].

A successful PCR technique in detection of parasite DNA in acute infection may belong to PCR assay not dependent on the viability of parasite which detected all the *T.gondii* dead and viable. In peripheral blood of human *T. gondii* rapidly killed by the immune system but the DNA remains for some time in peripheral blood of human[52].

Toxoplasmosis infection stimulates humoral immune response and cell mediated immunity, in addition to the role of cytokines[13]. *T.gondii* is strongly stimulates type1 cytokines during infection, that induced early during infection, that will induce abortion early in pregnancy[13,53,54].

The results showed a significantly increase in the concentration of (IL-5 and IL-27) cytokines in serum of patient infected with *T. gondii* compared to control group. Increasing the (IL-5 and IL-27) level maybe due to increasing the monocyte or macrophages which, is stimulated by *T. gondii* infection caused abortion leading to stimulate host immune response cellular and humoral[55,65] increased serum level of IL-5 in infected women may be due to a defensive role of interleuken-5 against *Toxoplasmosis* and recommend that interleuken-5 may play a role in the formation of interleukin-12[57]. conducted that IL-5 has important role in induce the eosinophilia and this lead to induction of T-helper2 response to interleukin-4 release by eosinophils in begin of Toxoplasmosis infection.

Table 5: Total Leukocyte Count and Differential Leukocyte Percentage in Control Group and Patients Suffering from *Toxoplasma gondii* Infection.

| Parameters | Control (n = 30) | *T.gondii* patients (n = 60) |
|---|---|---|
| TLC ($X10^3$/ $mm^3$) | 6.211 ± 0.176 | *8.110 ± 0.282 |
| Neutrophil % | 51.368 ± 0.121 | *57.814 ± 0.247 |
| Lymphocyte % | 34.026 ± 0.074 | *42.821 ± 0.124 |
| Monocyte % | 8.438 ± 0.033 | *14.616 ± 0.035 |
| Eosinophil % | 5.268 ± 0.189 | *8.801 ± 0.072 |
| Basophil % | 1.061 ± 0. 058 | 1.023 ± 0.021 |

* Significant difference P<0.05 between control group and patients.

(15) reported that there is a significant increase of serum IL-5 level during infection with *T gondii*.[38] was reported that *Toxoplasma* infection leads to increase the interleuken-5 but decrease in the tumor necrosis factor-α and IL-12.

Serum level of IL-27 increased in women infected with this parasite in compared with healthy women may be due to important role of Interleukin-27 as immune-regulatory properties during infection as well as autoimmunity[58,59]. Also IL-27 is initially considered a proinflammatory Th1-polarizing cytokine because of its capacity to raise sensitivity of CD4+ T lymphocyte to stimulate T-bet Expression and interleuken-12[18,60] demonstrate increasing of serum IL-27 concentration to promoting expression of T-bet and CXCR3 in Treg cells. This result does not correspond with the study of [18,61] where they found some eukaryotic pathogens such as *T. gondii* and *Trichuris muris* induce interleukin-27 released but do not account for the elevated expression of this interleukin during infection.

## CONCLUSION

*Toxoplasma gondii* has an important role in change of cytokines levels and immunoglobulins titer in aborted women that infected with this parasite. Also B1gene is the best gene used for diagnosis of *T.gondii* isolated from aborted women and it has a crucial effect on white blood corpuscles in aborted women.

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