# Detection of *B1 Gene* in *Toxoplasma Gondii* and the Role of Interleukin 2 in Abortive Women Infected by this Parasite

**Hanan Mohammed Saied Jassam[1], Thaer Abdulqader Salih[2]**

*[1]Post graduate/Department of Biology/ College of Science/ University of Anbar/Iraq,*
*[2]Assist prof. Department of Biology/ College of Science/ University of Anbar/Iraq*

## Abstract

The study included 80 blood and serum samples of aborted women with *Toxoplasma gondii* infection and healthy, uninfected and non-aborted women in the control group. For the period from August-2018 to June-2019, in collaboration with the Falluja teaching hospital for women and children and the Ramadi teaching hospital for women and children, the Enzyme-Linked Immunosorbent Assay (ELISA), also to the *Toxoplasma gondii (B1) gene*, the Nested polymerase chain reaction technique are used. The results of the study showed by that Elisa *Toxoplasma gondii*, IgG infection (50%), IgM infection (12.5%), and IgG & IgM Infection (12.5%), were mean (108.16±47.02) (99.02±31.47) (96.45±20.68) respectively compared with the control group (25%) (0.94±0.93) and Interleukin-2 shown in IgG group (48.66±12.01) and control group (14.28±6.84) decreased compared to IgM (81.19±9.66), and IgG & IgM (80.71±9.18) group. Nonetheless, using nested PCR, the *B1 gene* showed just (12.5%) of all study samples in IgM and IgG&IgM alone and all IgG negativly.

***Keywords:*** *Toxoplasma gondii, ELISA, Interleukin 2, nPCR.*

## Introduction

*Toxoplasmosis* is the main common parasitic disease, in which the coccidian protozoan caused by *Toxoplasma gondii,* (from 10% to 80%) in different parts of the world. The human infection pathway is the consumption of tissue cysts found into uncooked or raw eating as well as the ingestion of oocysts through polluted vegetables or water, another path of infection is the transmitting of tachyzoites (the fast-dividing) from mother which pregnant to the growing fetus which can be resulting in abortion, chorioretinitis, including severe developmental defects **(1).**

In *T. gondii*, it was found that three different phases of the life cycle depend on the phase of the parasite into the host. Tachyzoites and bradyzoites found mainly in the intermediate host (asexuality, within the tissue cyst) appear to be a morphological form. The most recent phase of sporozoites can only be seen in the most recent host (sexuality reproduction, oocysts) **(2).**

The most significant sequel to *toxoplasmosis* is a congenital infection in women pregnant (3). The congenital transmission of *T. gondii* happens predominantly for the first time during pregnancy (4). The incidence of congenital toxoplasmosis is greatest for the first and second trimesters of pregnancy, usually leading to abortion or stillbirth **(5).**

Many serological methods have been used to detect *T. gondii* as an enzyme-linked immunosorbent assay. Recent research has confirmed that PCR is critical to assessing the prevalence of *T. gondii*, as the only method of DNA detection, PCR has been used professionally in women pregnant to identify *toxoplasmosis* **(6)**. The *B1 gene* appears highly specialized and has 35 replicates in its genome, resulting in the amplification of the target for polymerase chain reaction (PCR) to identify blood and tissue parasites **(7).**

**Corresponding author:**
**Hanan Mohammed Saied Jassam**
Hananmohammed395@gmail.com

Interleukin-2 (IL-2) is a very considerable inflammatory cytokine, with IL-2 production abnormalities identified in patients receiving transplant rejection. A number of studies have shown that the higher IL-2 outflow levels have already been an association with recurrent spontaneous abortion [8].

## Material and Method

### Collection of samples

Between August-2018 and June-2019, in collaboration with Falluja teaching hospital for women and children, Ramadi teaching hospital for women and children, 80 blood samples were collected over the age range (18-45) years. In this study, four different women's groups, control group included (20 samples) for healthy women non-abortion before, but infected women with *T. gondii* IgG group (40 samples), IgM group (10 samples) and IgG&IgM group (10 samples) which had an abortion before on the basis of the first diagnosis.

5 ml of venous blood samples were collected from the patient and the control subjects, 3ml was placed in a gel tube (without anticoagulant) and kept at room temperature until blood was clotted; the serum was separated by centrifugation at 3500 r.p.m. for 10 min and then stored at -20 ˚C. Also 2 ml placed in the EDTA tube and stored at-20 ° C until DNA was extracted. The DNA extraction by Kit extraction Geneaid from the blood as well as stored at-20 ° C until used for PCR.

## The Diagnostic Methods:

1-Serological tests

### A. Estimation antibodies of *Toxoplasma gondii*

The serological test was used for the diagnosis of *Toxoplasma gondii* by the ELISA test IgG according to the NO.TOXG01 catalog kit and IgM according to the NO.TOXG02 catalog kit.

### B. Estimation of IL-2 concentration

Estimation of the level of IL-2 in the serum of women with T. Gondii was done using the ELISA method, according to the use of the Cloud-Clone Crop Catalog kit NO. SEA073Rb.

### 2- Molecular detection test

Nested Polymerase chain reaction (nPCR)

Blood DNA extraction was carried out according to the DNA extraction kit protocol with NO catalog. Nested PCR performed a two-stage amplification of the fragment of the B1 gene with separate primer pairs shown in table (1) for all DNA samples.

**Table (1): *Gene B1* Specific Primers**

| ***B1 gene Primers*** | ***B1*gene fragment** | **Fragment Length(bp)** | **Annealing temperature Tm** |
|---|---|---|---|
| *B1 (F1)*<br>5′-GGAACTGCATCCGTTCATGAG-3′ | 694-714 | 193 bp | 56.6 °c |
| *B1 (R1)*<br>5′-TCTTTAAAGCGTTCGTGGTC-3′ | 887-868 | | 51.9 °c |
| *B1 (R2)*<br>5′-TGCATAGGTTGCAGTCACTG-3′ | 757-776 | 96 bp | 51.7 °c |
| *B1 (F2)*<br>5′-GGCGACCAATCTGCGAATACA CC-3′ | 853-831 | | 63.2 °c |

* F: Forward sequences, R: Reverse sequences.

Primers were added in the first stage, as instructed by the manufacturer, and are then placed into the PCR, then the first stage product is used as a template in the second stage and is also put into the PCR thermocycler.

## Result and Discussion

Serological tests are beneficial for the diagnosis of T. gondii by detecting antibodies in serum and determining the phase of infection in the acute and chronic phase [9].

The result of this study shown in Table (2), *T.gondii* antibody level (UI / mL) in control group was (0.94±0.93) a significant difference with group IgG infection, IgM infection, and IgG & IgM Infection (108.16±47.02) (99.02±31.47) (96.45±20.68) respectively. But in group IgG had shown a non-significant difference with both groups IgM and IgG & IgM. Also group IgM had shown a non-significant difference in rates of *T.gondii* antibodies opposed to group IgG & IgM.

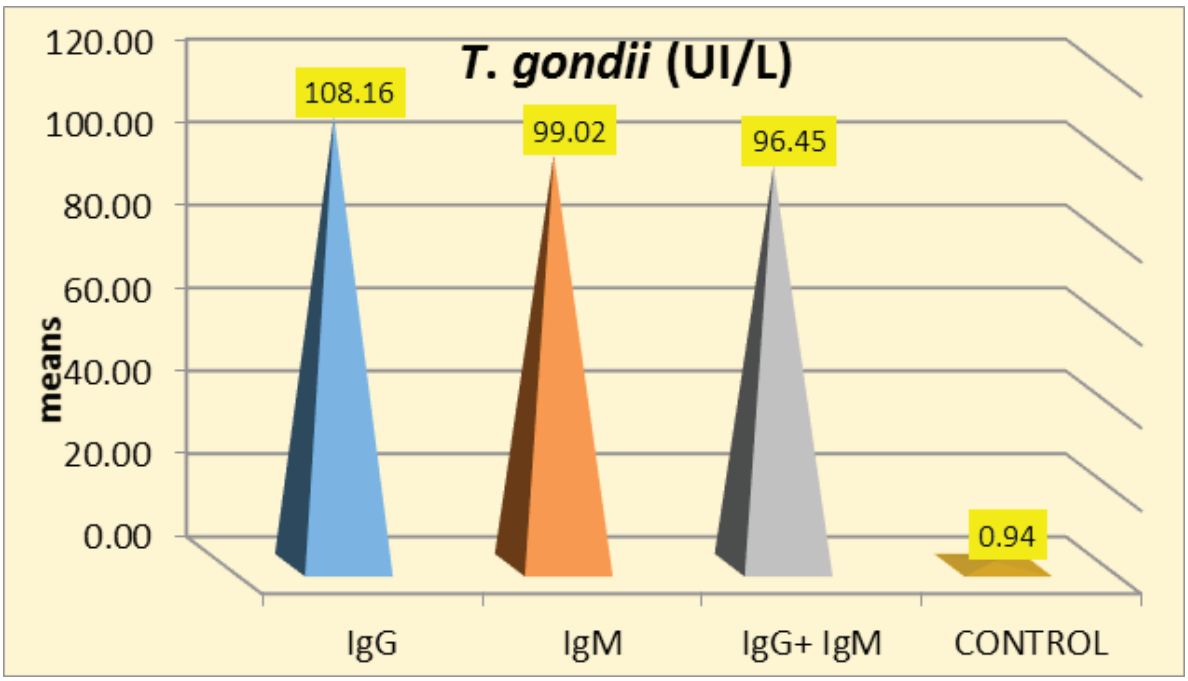


**Figure (1): Levels of *mean T.gondii* infection in Patients and Control**

The result of this study was shown to be a percentage of *T. gondii* seroprevalence IgG group (50 %) which is significantly greater than IgM and IgG&IgM which is shown (12.5 %) and (12.5 %) respectively, but control group (25 %), as most IgG-positive *toxoplasmosis* incidents were sufficient to determine the presence of chronic infection; IgM-positive titer, in the other hand, leads to the presence of acute infections.

The results of this study coincided with the results of the study [6] which showed *T. gondii* seroprevalence for IgM (4.16 %) and IgG (25.83 %) while IgM and IgG shown (3.33%) of aborted women with toxoplasmosis; and [10] were identified as IgG (31.5 %), IgM (7.6 %) and IgM & IgG (2.5 %).

On the other hand, the results were not in agreement with the result of [11] as shown IgG of *T. gondii* was (18.09 %) whereas IgM (9.79 %) for pregnant having recurrent spontaneous abortions also[12] observed 60 (38.7%) of IgG & IgM *T. gondii* were positive (4 % and 44 % respectively to IgG and IgM for aborted women).

*Toxoplasmosis*, which can lead abortion at any time of pregnancy, is indeed controversial, but even this study was been proposed to seek a possible association for both *T. gondii* infection and abortion.

The prevalence of parasite *T. gondii* varies among women in different countries in the world, based on different climate conditions, health, and food habits, economic standing, level of education and age [13].

The reason for high prevalence among aborted women may be reversion to the kind of acute infection or reactivation of chronic infection due to reduced immunity of pregnant mothers since the time of infection during pregnancy have an important role to play in determining the demise of the fetus [14].

Cytokines are divided into two components: Th2 (such as IL-3 and IL-15) to correct the immune system response, while TH1 (such as IL-2, IFR) seems to be harmful to end a pregnancy by influencing either the placenta or stimulation to avoid fetal implantation and to cause abortion or fetal killing.TH1 allows to invade trophozoites and apoptosis and hence generates harmful factors to the fetus [15].

In these study Interleukin-2 (IL-2) in women with *T. gondii* infection had a high level especially in comparison to the control group, which showed a lower level of infection. Furthermore, the increase in women infected with *T. gondii* IgM and IgG&IgM (81.19±9.66) (80.71±9.18) was higher than in the IgG (48.66±12.01) and control (14.28±6.84) respectively.

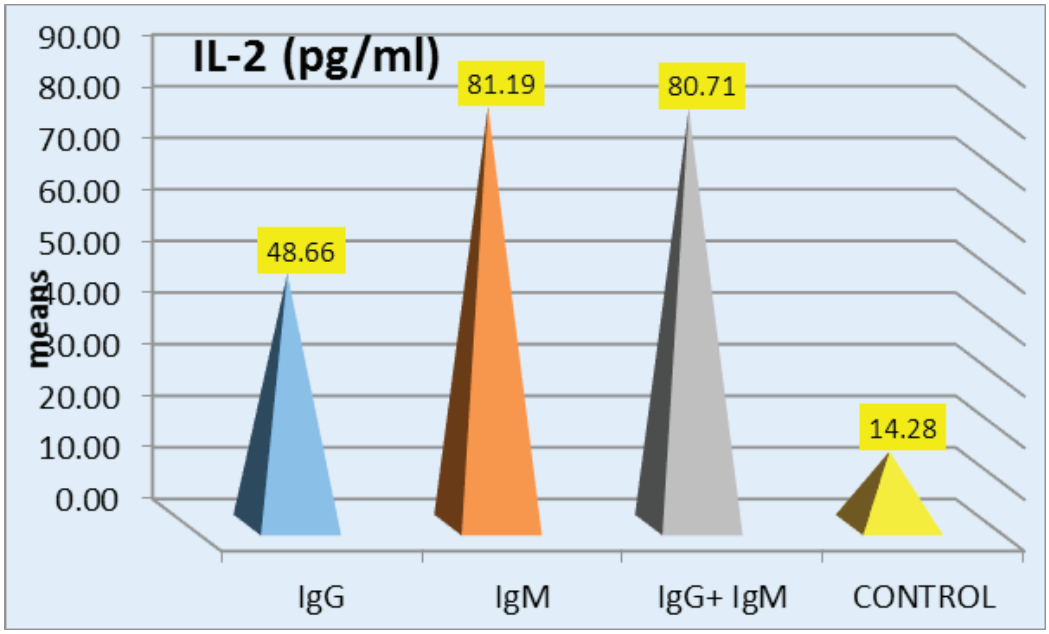


**Figure (2): Levels of *mean* IL-2 in Patients and Control**

These results have been similar to previous studies done by [16], [8] which found in a group of women diagnosed with *T. gondii*, the rate of IL-2 level was increased compared to healthy women as a control group.

IL-2 manages the activities essential for the immunity of white blood cells (particularly lymphocytes). It is part of the human body's normal response to microbial infection, besides being detectable in both nonself (foreign) and self. IL-2 effects have been mediated by CD4 and CD8 T-cell lymphocyte bind to IL-2 receptors of the immune response [17].

Also many unknown abortions are related to immunological reasons when cytokines mainly work on the loss or completion of pregnancy whereas Cytokines of kind Th1, such as (IL-2) are widespread in cases of pregnancy failure [18].

Following the initial ELISA serological examination, the nPCR technique is used to detect the existence of *T. gondii* DNA in circulating blood is an opportunity for an actual diagnosis in aborted women with *T. gondii* infection.

Results of n PCR in samples of patients (12.5%) of the DNA fragment band indicated an identification of the *B1 gene* and this explained the existence of the infection and had shown (92 %) that there is no amplified DNA fragment stating that there is no *B1 gene* and that there is may be, no infection.

The first step, of n PCR test has been used to amplification of *B1 gene* fragment (DNA) using the first primers to amplify the 193 bp fragment such as figure (3).

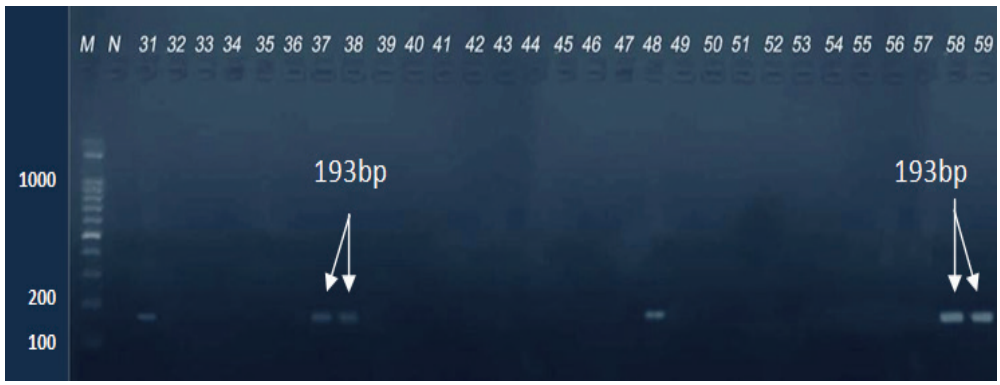


**Figure (3): Amplification of the 193bp fragment of the *B1 gene* of *T. gondii* DNA**

The second step, of the n PCR experiment has been used to amplification a 96bp fragment of the isolated DNA *B1 gene* from first product, that only in 10 samples (12.5%) for IgM and IgG&IgM showing positive and all other IgG negative being excluded such as figure (4).

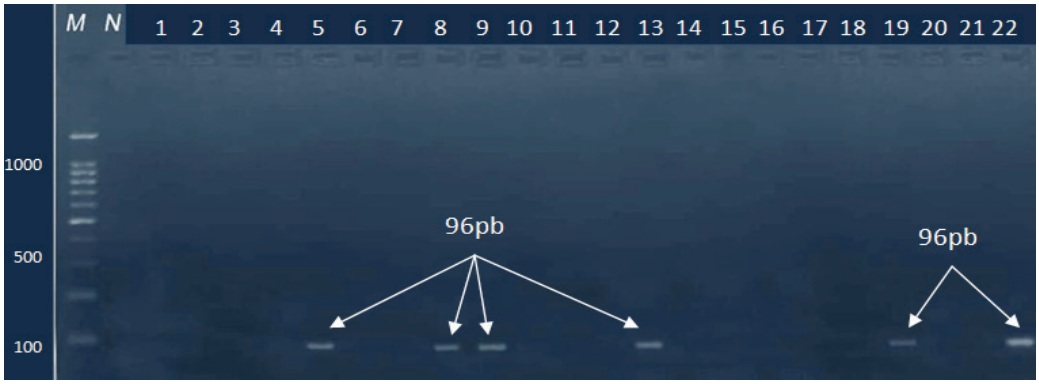


**Figure (4): Amplification of the 96bp fragment of the *B1 gene* of *T. gondii* DNA from first product**

These result may be due to IgM Ab can also be recognized in both acute and chronic *toxoplasmosis,* at that same time as *T. gondii* IgG Ab continued for even a long time, until for years. Therefore, the serology method may well be ineffective because it is focused on the level of antibodies generated which either fail or are delayed significantly whereas n PCR is based on the existence of the parasitic genetic material [19].

Also perhaps result due to the *B1* primers have the highest sensitivity due the two primers have been very specific to *T. gondii* strain was detected in Iraq. It is also very specific to the magnification DNA of *T. gondii* used in the PCR technique [20].

The result of the present study using n PCR were accepted with [6] as the result of the *B1 gene* was detected in (15.83 %) infected women with *T. gondii* and results of [21] also were consistent with the resulting study of investigation report *T. gondii* rate (31.57%) in donor blood. But, the results of [22], [19] were far from the result of these study *B1gene* was identified in (77 %) and (87.5 %) respectively of both the study samples.

**Ethical Clearance**: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest**: The authors declare that they have no conflict of interest.

**Funding**: Self-funding

## References

**1.** Shojaee S, Teimouri A, Keshavarz H, Azami

SJ, Nouri S. The relation of secondary sex ratio and miscarriage history with Toxoplasma gondii infection. BMC infectious diseases. 2018 Dec;18(1):307.

2. Dubey JP. History of the discovery of the life cycle of Toxoplasma gondii. International journal for parasitology. 2009 Jul 1;39(8):877-82.

3. Mahmood OI. Effect of Toxoplasmosis on hematological, biochemical and immunological parameters in pregnant women in Tikrit city, Iraq. Tikrit Journal of Pure Science. 2018 Sep 26;21(3):24-7.

4. Paquet C, Yudin MH, Allen VM, Bouchard C, Boucher M, Caddy S, Castillo E, Money DM, Murphy KE, Ogilvie G, van Schalkwyk J. Toxoplasmosis in pregnancy: prevention, screening, and treatment. Journal of obstetrics and gynaecology Canada. 2013 Jan 1;35(1):78-9.

5. Ghasemi FS, Rasti S, Piroozmand A, Bandehpour M, Kazemi B, Mousavi SG, Abdoli A. Toxoplasmosis-associated abortion and stillbirth in Tehran, Iran. The Journal of Maternal-Fetal & Neonatal Medicine. 2016 Jan 17;29(2):248-51.

6. Zghair KH, Hassani HH, Mahmood SH. Detection of B1 gene of Toxoplasma gondii in blood of pregnant and abortive women infected with this parasite. Iraqi Journal of Medical Sciences. 2010;8(3):42-8.

7. Sardarian K, Maghsood AH, Farimani M, Hajiloii M, Saidijam M, Rezaeepoor M, Mahaki H, Zamani A. Evaluation of Toxoplasma gondii B1 gene in Placental Tissues of Pregnant Women with Acute Toxoplasmosis. Advanced biomedical research. 2018;7.

8. Saleh DS, Kharibet KE. Detection of Cytomegalovirus, Rubella virus, and IL-2 Levels in a Sample of Recurrently Aborted Iraqi Women. Iraqi Journal of Science. 2015;56(3A):1890-4.

9. ATAEIAN A, Tadayon P. Prevalence of Toxoplasma gondii Antibodies in Women of Zanjan Hakim-Hidajy Hospital, 1999.

10. Sultan BA, AL-Fatlawi SN. Relationship between Toxoplasma gondii and abortion in aborted women in Najaf province. journal of kerbala university. 2016;14(1):177-85.

11. Kadhim, R. A. The relationship between some physiological and biochemical aspects in persons infected with Toxoplasmosis. PhD. Thesis. College of Science. University of Babylon. 2013; p193.

12. Hadi HS, Kadhim RA, Al-Mammori RT. Seroepidemiological aspects for Toxoplasma gondii infection in women of Qadisiyah province, Iraq. International Journal of PharmTech Research. 2016;9(11):252-9

13. Ayi I, Edu S, Apea-Kubi KA, Boamah D, Bosompem KM, Edoh D. Sero-epidemiology of toxoplasmosis amongst pregnant women in the greater Accra region of Ghana. Ghana medical journal. 2009;43(3).

14. AL-Khshab EM. Some antioxidants level in seropositive toxoplasmosis woman in Mosul. Tikrit Journal of Pure Science. 2010;15(2):17-22.

15. Feliciano MA, Silva AS, Crivelaro RM, Oliveira ME, Coutinho LN, Vicente WR. Profile of IL-2, IL-4, IL-10, IFN-γ, TNF-α and KC-like cytokines in pregnant bitches. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2014 Aug;66(4):1067-72.

16. Mohaimen NA, Majid A. Evaluation of IL-2, IL-8, IL-10 expression in trophoplastic tissue of women with spontaneous miscarriage infected by Toxoplasma gondii. Al-Anbar Medical Journal. 2011;9(1):50-8.

17. Miller CM, Boulter NR, Ikin RJ, Smith NC. The immunobiology of the innate response to Toxoplasma gondii. International journal for parasitology. 2009 Jan 1;39(1):23-39.

18. Daher S, Mattar R, Gueuvoghlanian-Silva BY, Torloni MR. Genetic polymorphisms and recurrent spontaneous abortions: an overview of current knowledge. American Journal of Reproductive Immunology. 2012 Apr;67(4):341-7.

19. Ghareeb AM, Kawan H. Direct Amplification of B1 gene of Toxoplasma gondii DNA using Nested Polymerase Chain Reaction Following Microwave Treatment for Whole Blood Samples. The Iraqi Journal of Veterinary Medicine. 2015;39(1):23-7.

20. Al-Hadraawy SK, Hadi FA. Immunological and Molecular Study of Toxoplasma gondii in Al-Najaf Governorate-Iraq. Int. J. Pharmacognosy and Phytochemical Res. 2017;9(4):482-92.

**21.** Al-Jubory, R.H.; Kadir, M.A.; Al-hadedy, E.H . Seroprevalence of toxoplasmosis among blood donors in Kirkuk main blood bank. Kirkuk University J. for Scientific Studies.2015; 2(10), 107-128.

**22.** Mohammad M, Ahmed S, Hussain A. Seroprevalence of Toxoplasma gondii between couples in Ramadi city using enzyme linked immunosorbent assay (ELISA). International Journal of Medicine and Medical Sciences. 2013 Jun 12;5(6):295-9.