

**INTERLEUKIN- 5 AS A BIOMARKER IN ABORTED WOMEN
INFECTED WITH *TOXOPLASMA GONDII* IN AL-NAJAF
GOVERNORATE – IRAQ**

Saleem K. A. Al-Hadraawy* and Phadel Abbas Hadi

Department of Biology, Faculty of Science, University of Kufa, Iraq.

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***Correspondence for
Author**

**Dr. Saleem K. A. Al-
Hadraawy**

Department of Biology,
Faculty of Science,
University of Kufa, Iraq.

ABSTRACT

The aim of this research was to diagnosis of *Toxoplasma gondi* by use PCR technique and evaluate interleukin-5 (IL-5) in aborted women infected with *T. gondi*; blood specimens were collected from 350 suspected women whom have visited the department of infertility at Al-Sadder medical city, Al-Zahra Hospital in Najaf governorate during the period from November 2014 through May, 2015. Thirty healthy looking age matched women taken to serum tubes and serum was separated. Serum was used for evaluation of the IL-5 by using VIDAS technique. *T. gondi* was isolated from seventy one with a prevalence rate 20.28% by using PCR technique. The results

revealed a significant increase in IL-5 in women infected with *T. gondi* in compared to control group and the PCR is accurate method used in diagnosis of *T. gondi* parasite.

KEYWORD: *T. gondi*, *Toxoplasma gondi*.

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 (Ford and Schust, 2009) and can be defined as two or more failed pregnancies (Asrm, 2012). *T. gondii* is one of the important obligate intracellular protozoan parasites, classified in the phylum Apicomplexa, a significant human and veterinary pathogen. It enters the host via the digestive system and poses a severe risk for congenitally infected infants (Montoya and Liesenfeld, 2004). There are three types' strains of *T. gondii*. Type 1, 2 and 3 strains, type 1 is highly virulent (Black and Boothroyd, 2000).

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 (McAllister, 2005). Second, *Toxoplasma* is used as model-system of Apicomplexa parasites (Kim & Weiss, 2004). Finally, *Toxoplasma* is an important veterinary pathogen with high estimated costs owing to disease, abortion or vaccination in animal farming (Scott *et al.*, 2007).

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* (Suha, 2011). Zhang (1999) suggested that role in the production of interleukin-12 and interleukin-5 has a protective role against toxoplasmosis. Mennechet *et al.* (2004) recorded that interleukin-27 and tumor necrosis factor- β 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 (Villarino *et al.*, 2006).

MATERIALS AND METHODS

The study was conducted on 350 suspected women with Toxoplasmosis disease and 30 of healthy women as control groups, when all these cases were examined and defined as suspected with *T.gondii* by obstetrician when attended to AL-Zahra, maternity and pediatric, AL-Sadder teaching hospital in AL-Najaf province from November 2014 through May, 2015.

Sample collection

From suspected women was carefully collected three ml of blood was collected from each clinical suspected women with *T.gondii* infection and non-suspected women (as control group) by disposable syringe ,1ml of used to DNA extract and the remain blood samples was drawn in sterile plain tubes and remains for 30 minutes at room temperature. After that the samples were centrifugation at 3000 rpm for 5 minutes (Back man/counter, Germany) to separate the serum and collected in another sterile tube.

DNA Extraction

Toxoplasma gondii DNA extraction by used Genomic kit (geneaid/Taiwan) according following procedure.

Procedure

1. Blood sample collected in EDTA tubes.
2. 300 μ l of blood was added in 1.5 ml microcentrifuge tube.
3. 900 microliter of red blood cell lysis buffer was added and mixed.
4. The tube was incubated for 10 minutes at room temperature.
5. Centrifugation for five minutes at 3000 xg to remove the supernatant.
6. The cell pellet was resuspended in 100 microliter of cell lysis buffer.
7. Before mixed by vortex 200 microliter of GB buffer added to the 1.5 ml microcentrifuge tube.
8. The mixture was incubated at room temperature for 10 minutes.
9. The mixture inverted every 3 minutes during incubation.
10. 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.
11. Centrifugation at "14000-16000 Xg for five minutes".
12. The collection tube has been discarded with the flow-through.
13. The GD column putted in a new 2 ml collection tube.
14. (400 μ l) of W1 buffer added to the GD column.
15. The mixture has been centrifuged at 14000-16000 Xg for 30 seconds.
16. The flow-through discarded and the same GD Column reused.
17. 600 microliters of wash buffer added to the GD Column.
18. Centrifugation for 30 seconds at 14000-16000 Xg.
19. The flow-through in the collecting tube discarded and placed back of the GD Column.
20. Centrifugation for three minutes at 14000-16000 Xg to dry the column matrix.
21. Dried GD Column was transferred to clean 1.5 ml microcentrifuge tube.
22. 100 microliter of preheated elution buffer (70⁰C) to the center of the column matrix.
23. The elution buffer was absorbed by the matrix via standing for 3-5 minutes.
24. The purified DNA eluted by centrifugation at 16,000 x g for 30 seconds.
25. Purified DNA kept in -20 degree up to use.

DNA Amplification

The infections of *T gondii* confirmed by PCR amplification of B1 gene using specific gene primers as following:

- 1- 8 μ l of purified DNA transferred to 0.2 ml PCR tube of master mix kit (contains

- 5 μ l of master mix).
- 2- 2.5 μ l (10 μ M) of each primer (forward and reverse) has been added. 3- 2 μ l of deionized water added to completed of reaction volume (20 μ l).
- 4- The mixture was mix briefly by centrifugation for 3000 x g for 10 seconds to homogenize the contents.
- 5- PCR tubes the thermo-cycler, then started the program (table 1):

Table (1): Cycling Parameters of Genes Amplification (Sambrook, 2001)

| Primers | steps | Initial denaturation | Denaturation | Annealing | Extension | Final extension |
|---------|-----------|----------------------|--------------|-----------|-----------|-----------------|
| B1 | Temp.(C°) | 99 | 39 | 62.5 | 27 | 27 |
| | Time | 5min. | 10sec. | 10sec. | 15sec. | 1min. |
| | Cycle | | | | | |

Analysis of Amplification Products

The products of PCR was analyzed by 1.5% agarose gel electrophoresis which prepared according to (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 μ l 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 5 μ l 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 Interleukin -5 (IL-5)

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

Statistical analysis

Data were analyzed using the software packages Graphpad prism for Windows (5.04, Graphpad software Inc. USA), Data are presented as the mean \pm standard error (SE). The comparison between the patients and healthy groups were analyzed by T- test. A p-value < 0.05 was considered significant.

RESULTS

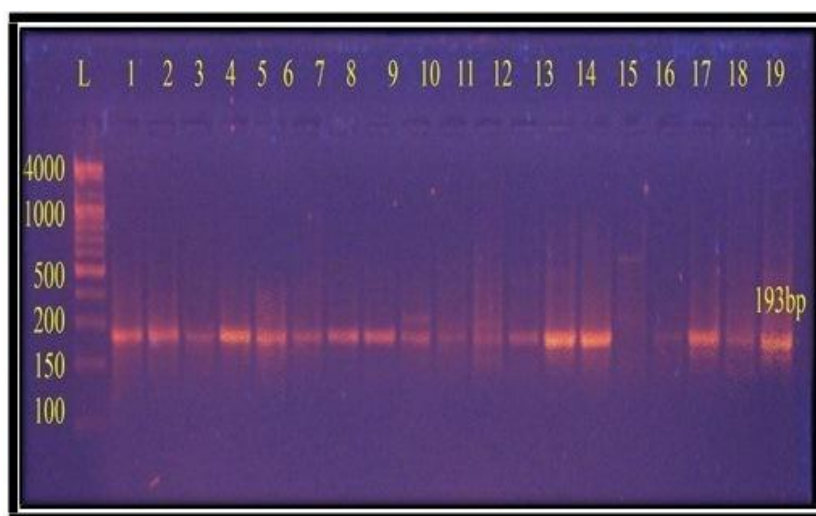


Figure 1: Agarose gel electrophoreses for PCR products using specific primer to amplify specific target sequence (B1 gene) from genomic DNA of *T.gondii* .L-DNA ladder 100bp.15 and 16 negative samples. Other lanes represent the PCR product (193 bp).

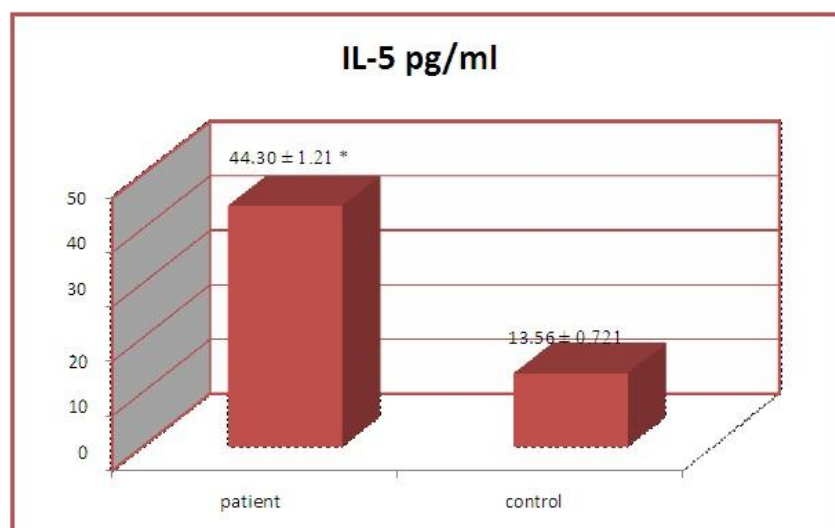


Figure 2: IL-5 Concentration (pg/ml) Comparison between Patients Suffering from *T. gondii* Infection and Control Group.

Significant difference $P < 0.05$ between control group and patients

The present study has shown the effected of three primer (B1) on DNA genomic of *T.gondii* are shown on agarose gel electrophoresis, as seen in Figures (1). Also the results revealed that the number and the percentage of women infection with *T. gondii* were 71 (20.28%) present study on Toxoplasmosis in women revealed a significant increase ($p < 0.05$) in IL-5 concentration of patients serum (44. 30 \pm 1.21 pg /ml) in compared to

the control group (13.56 ± 0.721 pg /ml) .(figure 2)

DISCUSSION

The results of study revealed that the positive cases of the number and percentage were 71 (20.28%) respectively from the 350 suspected cases examination by the PCR technique.

The highest sensitivity of (B1) primer maybe because that the two primers were specific to strain of *T. gondii* found in Iraq. Results by Hurtado *et al.*(2001) , Calderaro *et al.*(2006) and (Chabbert, 2004) were agrees with results of current study who found B1 gene has high specificity and sensitivity therefore used in diagnosis of *T. gondii* parasites by PCR technique. B1primer used in PCR technique is highly specific in the magnification of *T. gondii* DNA and successful in the detection of *T.gondii* DNA from blood sample of aborted women infected with *T. gondii*.

Toxoplasmosis infection stimulates humoral immune response and cell mediated immunity, in addition to the role of cytokines (AL-Fertosi, 2006). *T.gondii* is strongly stimulates type1 cytokines during infection, that are induced early during infection, that will induce abortion early in pregnancy (AL-Fertosi, 2006; Marshal, 1998; Robert, 2001).

The results showed a significantly increase in the concentration of (IL-5) cytokines in serum of patient infected with *T. gondii* compared to control group. Increasing the (IL-5) level maybe due to increasing the monocyte or macrophages which stimulated by *T. gondii* infection caused aborted lead to stimulated host immune response cellular and humoral (Evering & Weiss, 2006; Darcy, 1994) increased serum level of IL-5 in infected women may be due to a protective role of IL-5 against *T. gondii* infection and suggest that IL-5 may play a role in the production of IL-12.

At *T. gondii* infection IL-5 has been the ability to induce eosinophilia, and as a consequence this cytokine has been shown to have an important role in the induction of a Th2 response through the ability of eosinophil to release IL-4 early in infection (Sabin *et al.*, 1996) .Suha and Sami (2011) reported that there is significant increase of serum IL-5 level during infection with *T gondii*. Lang *et al.* (2007) was reported that *T.gondii* infection lead to increase in level of IL-5 but decrease in level of TNF- α and IL-12.

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