

8-15-2021

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Recommended Citation

Hadi, Hadeel S.; Kadhim, Raad A.; and Al-Mammori, Raheem T.O. (2021) "Study The Levels Of Cytokines (IL-12,IFN- γ) And Human Proflin-1 (PFN-1) In The Sera Of Aborted Women With Toxoplasmosis And Healthy Women (non-pregnant and pregnant women)," *Al-Qadisiyah Journal of Pure Science*: Vol. 26: No. 4, Article 17.

DOI: 10.29350/qjps.2021.26.4.1352

Available at: <https://qjps.researchcommons.org/home/vol26/iss4/17>

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Study the levels of cytokines (IL-12, IFN- γ) and human Proflin-1 (PFN-1) in the sera of aborted women with Toxoplasmosis and healthy women (non-pregnant and pregnant women)

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Article History

Received on: 13/6/2021

Revised on: 29/6/2021

Accepted on: 1/7/2021

Keywords:

Toxoplasma gondii, cytokines (IL-12, IFN- γ) and human proflin-1 (PFN-1)

DOI: <https://doi.org/10.29350/jops.2021.26.4.1352>

ABSTRACT

Toxoplasma gondii is an intracellular parasite infected large proportion of the world population, but mostly without visible clinical signs in immunocompetent patients. The present study was performed to determine the levels of cytokines (IL-12, IFN- γ) and human proflin-1 (PFN-1) in the sera of 60 aborted women with seropositive anti-*Toxoplasma* antibodies (55 seropositive IgG and 5 seropositive IgM) as case group and 30 healthy women (15 non pregnant and 15 pregnant women) as control groups in Women's and children Hospital in Al-Qadisiyah province from December / 2015 till March / 2016, Five ml of venous blood sample was obtained from each woman under sterile condition. Serum samples of aborted women were all positive for *Toxoplasma* antibodies by ELISA method as a result of a previous study back to us. Cytokines and proflin-1 were estimated in all samples, a significant increase in the concentration of interleukin-12 was observed in the aborted women with toxoplasmosis and a significant decrease in the concentrations of both interferon gamma (IFN- γ) and proflin-1 (PFN-1) compared to the control group at probability (0.05). These results confirm the overlapping role between *Toxoplasma* infection and abortion on immune factors.

1. Introduction

Toxoplasma gondii is highly prevalent pathogenic protozoa which infect a broad range of warm-blooded animals, including humans [21,31]. The infection has a worldwide distribution, about one-third of humanity has been exposed to this parasite, but seroprevalence differs greatly between countries (less than 10% to more than 90%) and population group [4]. Infection of pregnant

women may result either in abortion or congenital infection of the fetus. The congenital infection of fetuses results in hydrocephalus, intracranial calcification and retinochoroiditis [25].

Women can transmit the infection transplacentally to their unborn fetus, this often can occur during an acute infection acquired during pregnancy. The risk of congenital diseases is lower (10-25%) in case of injury to the mothers during the first trimester but is more likely to produce serious damage, and highest (60-90%) in case of injury to the mothers during the third trimester. The overall risk of congenital infection of the acute *T. gondii* infection during pregnancy ranging about 20-50% [17]. *T. gondii* has the ability to evade the abandonment of the host immune system in killing machine early macrophages, so they infect macrophages and is also considered as a target for T cells because of the intense expression of MHC class II, also pushes the counter regulatory molecules by infections such as cytokines or Interleukins. The host defense through the inflammatory response regulation may be, it will hinder the cytokines produced by Th1 and antimicrobial activity of macrophages [16].

Cytokines play an important role in the pathogenesis of pregnancy loss toxoplasmosis, but equally important, proved to be differences in the genes encoding cytokines to interfere with the expression of these molecules may have a vital part in gene organizing in inflammatory response, and impedance or exposure to infection, including toxoplasmosis [22].

Macrophages, T lymphocytes and natural killer (NK) cells, on one hand, and cytokines, moreover, are the main elements involved in the immune response against *T. gondii*. The antibodies show to play a secondary role, but remain an important means to prevent re-infection by a parasite [3]. Prior studies have appear that IL-12 produced by dendritic cells (DCs), macrophages and neutrophils in infected animals which is responsible for the induction of T helper1 cell (Th1) responses and removal *T. gondii* [13,10,32].

IL-12 supports production of interferon- γ (IFN- γ) during *T. gondii* infection [14], Resistance to *T. gondii* is highly based on IFN γ and therefore on IL-12 [11], and IFN γ is necessary to stimulate T cells responses and resistance to *T.gondii* [29,30]. Together, the IFN γ and IL-12 pathways activate effector mechanisms in a variety of cell types, leading to control of *T. gondii* infection [15]. The current study was planned to discover the relationship between some immune factors and toxoplasmosis and the effect of abortion on those factors, to complete this goal, the levels of some important cytokines (IL-12 and IFN- γ) and PFN-1 in women with or without abortions should be determined.

2. Materials and methods

Blood Samples were collected from 60 aborted women aged 15-44 years and 30 pregnant (15) and non-pregnant (15) women aged 20-37 years. The samples were obtained during their visit to the Women and Children's Hospital in Al-Qadisiyah province. The levels of cytokines interleukin-12, interferon-gamma and profilin-1 by using enzyme-linked immunosorbent assay (ELISA) were estimated. All serum samples of aborted women were positive for anti-toxoplasma antibodies by ELISA as a result of a previous study back to us (55 seropositive IgG and 5 seropositive IgM.), The use of the kits Manufacturer of the company Elabscience/ China to measure the concentration of IL-12, IFN- γ and PFN-1, It was measurement according to the instructions of the manufacturer of the kits.

Statistical analysis was performed with SPSS version 19 for comparison among different groups of study, one way anova test and Post Hoc test as Fisher's Least Significant Difference (LSD) were used.

3. Results

The results in table (1) show that the patients (aborted women) with positive IgG antibody for *Toxoplasma gondii* have significant increase in IL-12 concentration in comparison with pregnant control, while no significant increase in IgM in comparison with control groups at LSD value (30.28).

Table (1) IL-12 concentration among patients and control groups.

The Study groups	N	Mean Pg./ml	Std. Deviation	LSD Value
Aborted women with <i>Toxoplasma</i> IgG Positive	55	91.26	34.74	30.28
Aborted women with <i>Toxoplasma</i> IgM Positive	5	62.34	29.28	
Healthy Pregnant Control	15	55.28	23.43	
Healthy non-Pregnant Control	15	61.38	20.91	
Total	90	78.68	39.33	

The results in table (2) show that the concentration of IFN- γ were lower in positive IgM aborted women than IgG, both groups of patients have significant decreased in comparison with pregnant control. While IgM positive patients have significant decreased in comparison with healthy pregnant and non-pregnant control groups at LSD value (19.33).

Table (2) INF- γ concentration among patients and control groups.

The Study groups	N	Mean Pg./ml	Std. Deviation	LSD Value
Aborted women with <i>Toxoplasma</i> IgG Positive	55	62.26	28.53	19.33
Aborted women with <i>Toxoplasma</i> IgM Positive	5	43.02	10.86	
Healthy Pregnant Control	15	83.98	67.77	
Healthy non- Pregnant Control	15	66.03	33.70	
Total	90	65.44	38.64	

The results in table (3) show that the human profilin-1 level were low in infected patients with IgG and IgM antibodies in comparison with healthy pregnant and non-pregnant control groups at LSD value (230.14).

Table (3) PFN-1 concentration among patients and control groups.

The Study groups	N	Mean Pg./ml	Std. Deviation	LSD Value
Aborted women with <i>Toxoplasma</i> IgG Positive	55	867.74	325.75	230.14
Aborted women with <i>Toxoplasma</i> IgM Positive	5	728.40	262.37	
Healthy Pregnant Control	15	1191.46	386.07	
Healthy non-Pregnant Control	15	1096.40	327.26	
Total	90	952.06	255.12	

4. Discussion

IL-12 required to resist acute and chronic toxoplasmosis, and this goes back to a fundamental role in stimulating the production of IFN- γ and facilitates the formation of TH1 type, response [11,28]. The results of the current study showed that patients with toxoplasmosis have a higher concentration of IL12 compared to the control group. This is due to the fact that the infected with *Toxoplasma gondii* stimulates strong CMI characterization by Th1-cell high response [28,20]. These findings are compatible with Kareem [18] where the results showed a significant difference between aborted women with *T. gondii* and healthy women in the level of IL-12.

IFN- γ has a huge role in the immune response against *T. gondii*, it is working to resist the parasite and its inhibition through several of mechanisms and materials that works to stimulate to kill the parasite, or transferred from the phase rapid multiplication to the slow phase by a range of influences, including nitric oxide [12], as well as increase the effectiveness Indoleamine2,3-dioxygenase, which works to break down tryptophan, which is important for the growth of the parasite [23], also IFN- γ works to stimulate IgG positive antibody, Dendritic cells , Natural killer cells and Macrophages to swallow and kill the parasite and is produced from natural killer cells and white blood cells neutrophil [26].

The present study show that lower level of INF- γ was seen in patients with acute infection in association with anti-toxoplasma IgM infection in comparison with chronic infection or patients with high IgG level , this result might be show that the immunocompromised condition due pregnancy state followed by abortion of female infected with *Toxoplasma*, caused reactivation of the previous exposure or latent infection , so the interferon level not sufficient to activate the immune system, IFN- γ in certain condition such as *Toxoplasma* infection produced by neutrophil rather than other immunological cells, so that in such condition there is no functional neutrophil well produced and the interferon reduced or remain in normal concentration as well as there is no relation to IL-12 induction of immunological cells to produce IFN- γ .

This mechanism was explained by Andrade *et al.* [7] ,who mentioned that , Exposure to gamma interferon (IFN- γ) and members of the tumor necrosis factor (TNF) cytokine family is thought to activate cell autonomous mechanisms that restrict parasite replication and/or promote pathogen clearance . Clearly indicate that elimination of *T. gondii* by primed macrophages is IFN- γ dependent and functions. These toxoplasmacidal and toxoplasmastatic activities explain the pivotal role of IFN- γ signaling in resistance of *T. gondii* in vivo. In other study done by Ohshima *et al.* [24] mentioned that, Neutrophils provide an important innate source for IFN- γ ; the mechanisms that regulate neutrophil-derived IFN- γ are not well understood because this IFN- γ is not regulated by TLRs or by interleukin-12 (IL-12). Interferon- γ (IFN- γ) is crucial for survival during *Toxoplasma gondii* infection. Production of this cytokine by natural killer (NK) cells is dependent on the Toll-like receptor 11 (TLR11)-mediated recognition of *T. gondii* profilin by dendritic cells (DCs). Both CD4⁺ T cell-derived and CD8⁺ T cell-derived IFN- γ is essential for resistance to *T. gondii* during the chronic stage of infection.

IFN- γ is produced at high levels in acutely infection at 7 days post infection and remains even at 3 wks. post infection. Clearly, IFN- γ is a potent immune effector and, yet, *Toxoplasma* is not cleared but persists in immunocompetent hosts. For optimal control of *Toxoplasma* replication in vitro, host cells should be activated with IFN- γ before infection , if the cells are infected first, subsequent

exposure to IFN- γ is unable to control infection and the parasite grows normally. *T. gondii* is an obligate intracellular parasite that induces a strong IFN- γ -driven cell-mediated immune response in its mammalian hosts. This response is critical for the resolution of acute infection and control of a chronic, latent infection in the CNS. The various cell types are activated by IFN- γ to acquire potent toxoplasmodicidal mechanisms [19].

Perhaps the reason for the decline in the level of IFN- γ in aborted women are also other reasons, for example infected with other illnesses women may have caused the decline in IFN- γ . These diseases were diabetes and this was confirmed in a study by Al-Moussawi and Al-Quraishi [5], They have shown that the decrease in the concentration of IFN- γ in aborted women infected with *T. gondii*, and they suffer from diabetes time at the same time (decrease its concentration in patients with diabetes as a result of an imbalance in the functions of the lymphocytes cells that produced by) this does not mean that all aborted women infected with toxoplasmosis who dropped their IFN- γ concentration are Suffer from diabetes, but diabetes is an example of these diseases that has caused this decline. The current study did not coincide with other studies such as the study of both Al-Sorchee [6] and the study of Abdullah *et al.* [2] in Iraq, the study of Abdul-Lateef *et al.* [3] who proved high concentration of IFN- γ compared with the control and Abdul-Mohymen and Hussain [1] Confirmed increase the concentration IFN- γ among aborted women.

Few studies were done on profilin level on human samples, most studies and experimental are done on animal laboratory. In the present study the human profilin-1 level were lowered in infected patients with IgG and IgM antibodies in comparison with pregnant and healthy control, this result might be due to protein consumption by immunological activity against infection (Toxoplasmosis), or the latent *Toxoplasma* infection caused low profilin synthesis because such parasite have intracellular activity, and the profilin is essential in cellular growth, change of cellular shape and repairing of damaged tissues. This result relatively constituent with the other studies, such as study of Di Nardo *et al.*[9] who stated that Profilin is imperative for spatially and transiently controlled development of actin microfilaments, which is a crucial procedure in cell velocity and cell shape changes. This is crucial for procedures, for example, organ advancement, wound recuperating, and the chasing down of infectious intruders by cells of immune system, while most profilin in the cell is bound to actin, profilins have more than 50 diverse restricting accomplices. A large number of those are identified with actin control, yet profilin additionally is by all accounts required in activities in the nucleus, for example, mRNA joining.

Most aborted women suffering from antiphospholipid syndrome have antiphospholipid and anti Cariolipin antibodies, some researches such as study of Bae *et al.* [8] who mentioned that in

mammalian cells four profilin isoforms have been found; profilin-I is expressed in many tissues while profilin-II is overwhelming in cerebrum and kidney. The profilin is bind in some variant of membrane phospholipid, this cooperation is the sequestration of profilin in an "inactive" frame, from where it can be discharged by activity of the enzyme phospholipase C .

The human PFN-1 concentration , sometimes , more acting at higher level than low level because of, at high focuses, profilin block the polymerization of actin, though it improves it at low concentrations. Restrains androgen receptor (AR) and accumulation and binding of G-actin which was fundamental for its restraint of AR [27].This fact might be explain the increased PFN-1 level among control result than patients with toxoplasmosis.

5. Conclusion

Toxoplasma infection in aborted women stimulate strong cell mediated immunity and induced Th1 cell to produce high level of IL-12, that is necessary to regulate the reduction level of INF- γ and PFN-1 caused by *T. gondii* infection to pervent neuronal damage and slow rate of bad prognosis of toxoplasmosis. PFN-1 is involovement in varios neurological syndroms. The intracellular activity of *T. gondii* lead to increase consumption of profilin protein, that it is essential for cellular growth, changing of cellular shape and repear of tissue damage, especialy of CNS tissues, macrophage and denderitic cells. Thus profilin maintain a protein essential for the effectiveness of *Toxoplasma* infection.

5. References

1. Abdul -Mohymen, N. A., and Hussain, A. Detection of IL-10, IFN- γ and IL-8 in sera of patients with recurrent spontaneous abortion. Iraqi J of Medical Sciences, 2008, 6(2):56-58.
2. Abdullah D. A.; Mahmood, M. A. and Mohammed, D.B. The levels of cytokines IL-4, IL-10, IL12P40, IFN- γ during acute Toxoplasmosis. J. Fac. Med. Baghdad, 2011, 53:4.
3. Abdul-Lateef, H. I.; Al-najar, S.A. & Abul Majeed, N.G. The Levels of IFN-gamma, IL-12 and testosterone hormone in persons with asymptomatic Toxoplasmosis. J. Fac. Med. Baghdad, 2012, 54(1).
4. AL-Azawi, B.M. and AL-Waeli, F.M.S. Studies on the effect of infected cases of parasite *Toxoplasma gondii* on some sex hormones. J Pharmaceutical Research, 2015, 4(10): 2413-2423.
5. Al-Moussawi, A. M. and Al-Qureshi, M. A. The study of cellular dynamics levels: interleukin β 1-IL and interleukin IL-2 and interleukin IL-4 and interleukin IL-6 and interferon gamma

- IFN- γ in sero-positive women toxoplasmosis. *J Babylon University Pure and Applied Sciences*, 2015, 3:23.(In Arabic).
6. Al-Sorchee, S. M. A. Immunological study on women infected with Toxoplasmosis with a History of Abortion/Baghdad. M.Sc. Thesis. College of Education (Ibn Al- Haitham), University of Baghdad, 2005.
 7. Andrade, R. M.; Portillo, J.A.C.; Wessendarp, M. and Subauste, C.S. CD40 signaling in macrophages induces activity against an intracellular pathogen independently of gamma interferon and reactive nitrogen intermediates. *Infection and immunity*, 2005, 73(5): 3115-3123.
 8. Bae, Y. H., Ding, Z., Das, T., Wells, A., Gerteler, F., and Roy, P. Profilin1 regulates PI (3, 4) P2 and lamellipodin accumulation at the leading edge thus influencing motility of MDA-MB-231 cells. *Proceedings of the National Academy of Sciences*, 2010, 107.50: 21547-21552.
 9. Di Nardo, A., Gareus, R., Kwiatkowski, D. and Witke, W. Alternative splicing of the mouse profilin II gene generates functionally different profilin isoforms. *J of cell science*, 2000, 113.21: 3795-3803.
 10. e Sousa, C. R.; Hieny, S.; Scharon-Kersten, T.; Jankovic, D.; Charest, H.; Germain, R. N. and Sher, A. In vivo microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas. *The Journal of experimental medicine*, 1997, 186(11): 1819-1829.
 11. Evering, T. and Weiss, L. M. The immunology of parasite infections in immunocompromised hosts. *Parasite immunology*, 2006, 28(11): 549-565.
 12. Fox, B. A.; Gigley, J. P. and Bzik, D. J. *Toxoplasma gondii* lacks the enzymes required for de novo arginine biosynthesis and arginine starvation triggers cyst formation. *International j for parasitology*, 2004, 34(3): 323-331.
 13. Gazzinelli, R. T.; Hieny, S.; Wynn, T.A.; Wolf, S. and Sher, A. Interleukin 12 is required for the T-lymphocyte-independent induction of interferon gamma by an intracellular parasite and induces resistance in T-cell-deficient hosts. *Proceedings of the National Academy of Sciences*, 1993, 90(13): 6115-6119.
 14. Gazzinelli, R. T.; Wysocka, M.; Hayashi, S; Denkers, E.Y.; Hieny, S.; Caspar, P. and Sher, A. Parasite-induced IL-12 stimulates early IFN-gamma synthesis and resistance during acute infection with *Toxoplasma gondii*. *Journal of Immunology*, 1994, 153(6): 2533-2543.
 15. Han, S-J. Visualization of immune cells during *Toxoplasma gondii* infection. 2012. PhD Thesis. Freie Universität Berlin.

16. Kadhim, R. A.; Al-marzoqi, A. H. and Al-tae, Z. M. Genetic susceptibility associated with toxoplasmosis; genetic polymorphism, molecular and immunological study. *Al-Kufa University journal for Biology*, 2016, 8(2): 21–38.
17. Kadir, M. A.; Ghalib, A. K.; Othman, N.F. and Ahmed, I. S. Seroprevalence of *Toxoplasma gondii* among pregnant women in Kirkuk, Iraq. *J Kir Univ-Sci Studies*, 2011, (6): 1-11.
18. Kareem, S. S. Prevalence, serodiagnosis and some immuno-logical aspects of toxoplasmosis among women in Baghdad province. 2008. PhD Thesis. M. Sc. Thesis. College of health and Medical Technology. Technical Foundation. 104.
19. Kim, S. K.; Fouts, A. E. and Boothroyd, J. C. *Toxoplasma gondii* dysregulates IFN- γ -inducible gene expression in human fibroblasts: insights from a genome-wide transcriptional profiling. *The Journal of Immunology*, 2007, 178(8): 5154-5165.
20. Makhseed, M.; Raghupathy, R.; Azizieh, F.; Omu, A.; Al-Shamali, E. and Ashkanani, L. Th1 and Th2 cytokine profiles in recurrent aborters with successful pregnancy and with subsequent abortions. *Human Reproduction*, 2001, 16(10): 2219-2226.
21. Mckee, A. S.; Dzierszynski, F; Bose, D. S. and Pearce, E.J. Functional inactivation of immature dendritic cells by the intracellular parasite *Toxoplasma gondii*. *The Journal of Immunology*, 2004, 173 (4): 2632-2640.
22. Mohammed, L. O.; Ad'hiah, A. H. and Al-Nasiri, F. S. Cytokine gene polymorphisms of interleukin-1 receptor antagonist gene (IL1RNmspa11100) and its role in susceptibility or resistance to toxoplasmosis infection in samples of women from Salahaddin Governorate. *International Journal of Current Microbiology and Applied Sciences*, 2015, 4(9): 625–634.
23. Nascimento, F. R.F.; Calich, V.L.G.; Rodriguez, D. and Russo, F.M. Dual role for nitric oxide in paracoccidioidomycosis: essential for resistance, but overproduction associated with susceptibility. *Infect. Immun.*, 2011, 79(6):2470-2480.
24. Ohshima, J.; Sasai, M.; Liu, J.; Yamashita, K.; Ma, J.S.; Lee, Y. and Takeda, K. RabGDI α is a negative regulator of interferon- γ -inducible GTPase-dependent cell-autonomous immunity to *Toxoplasma gondii*. *Proceedings of the National Academy of Sciences*, 2015, 112(33): 4581-4590.
25. Radostits, O.M.; Gay, C.C.; Hinchcliff, K.W. and Constable, P.D. *Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats*. Saunders, London, 2006, (10):1518-1522.
26. Rich, R. R.; Fleisher, T. A. ; Shearer, W. T.; Schroeder Jr., H. W.; Frew, A. J. and Weyand, C. M. *Clinical immunology principles and practice* , 2008, 3: 1578.

27. Shao, J.; Welch, W. J.; Diprospero, N. A. and Diamond M. I. Phosphorylation of profilin by ROCK1 regulates polyglutamine aggregation. *Molecular and cellular biology*, 2008, 28(17): 5196-5208.
28. Sher, A.; Denkers, E. Y. and Gazzinelli, R. T. Induction and regulation of host cell-mediated immunity by *Toxoplasma gondii*. In: Ciba Found Symp, 1995, 95-104.
29. Suzuki, Y.; Orellana, M. A.; Scheiber, R. D. and Remington, J. S. Interferon-gamma: the major mediator of resistance against *Toxoplasma gondii*. *Science*, 1988, 240(4851): 516-518.
30. Trinchieri, G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nature Reviews Immunology*, 2003, 3(2): 133-146.
31. Waree, P. Toxoplasmosis: Pathogenesis and immune response. *Thammasat Medical Journal*, 2010, 8(4): 487-496.
32. Yap, G.; Pesin, M. and Sher, A. Cutting edge: IL-12 is required for the maintenance of IFN- γ production in T cells mediating chronic resistance to the intracellular pathogen, *Toxoplasma gondii*. *J of Immunology*, 2000, 165(2): 628-631.