www.jmscr.igmpublication.org Impact Factor 3.79
ISSN (e)-2347-176x

IGM Publication Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

IFN-γ A/T Alleles Frequency Among Anti Toxoplasma IgG Seropositive and Seronegative Pregnant Women

Authors

Abdelhaleem A A[1*], Hershan A A[1,2], Aqeely H M[2], Abdelmoula A M[3], Aysha Alvi[3], and Elhassan I M[2].

[1]Tropical& Endemic Diseases Research Unit Medical Research Center, Jazan University, Saudi Arabia.

[2]Collage of Medicine, Jazan University, Saudi Arabia.

[3]Collage of Applied Medical Sciences, Jazan University, Saudi Arabia.

*Corresponding Author

Dr: Aymen A Abdelhaleem

Medical Research Center, Jazan University, Jazan Saudi Arabia,

Email*: aabdelhaleem@hotmail.com.*

Abstract

Background: *Toxoplasma gondii is an obligate intracellular opportunistic protozoan parasite that can infect different vertebrate hosts including humans. Cell mediated immunity has a crucial role in the evolution of toxoplasmosis disease. TH1 cytokines and particularly, IFN-γ plays an important role in resistance to toxoplasmosis. Cytokines genes polymorphisms have been shown an association with susceptibility to parasitic diseases.*

Objective: *the aim of this study was to detect the frequency in the gene encoding IFN-γ (+874T/A) among Toxoplasma gondii seropositive and seronegative pregnant women.*

Methods: *This study was included 94 pregnant women divided into two groups: anti T. gondii IgG sero-positive group 44(46%) and anti T. gondii IgG sero-negative group 50(54%). Presence and absence of IFN-γ A, and T alleles were detected by PCR using specific primers.*

Results: *The differences between A and T alleles distributions were not statistically significant between the seropositive and seronegative pregnant women p value (>0.05), whereas T allele was slightly increased among seronegative group.*

Conclusion: *a seropositive pregnant women that carrying a single A genotype could be associated with a high risk of infection reactivation against T. gondii.*

Keywords: *IFN-γ , T. gondii infection in pregnant women*

.

Introduction

Toxoplasma gondii is an obligate intracellular opportunistic protozoan parasite that can infect different vertebrate hosts including humans [1].

Toxoplasma infection during pregnancy may cause a wide range of clinical manifestations. In immune suppressed patients, toxoplasmosis can cause severe encephalitis by acute infection or reactivation of latent infection [2].

Acute and latent infections during pregnancy are commonly diagnosed by the detection of anti-*T. gondii*-specific IgG and IgM antibodies and by the avidity of *T. gondii*-specific antibodies [3].

In the immunity towards Toxoplasmosis *IFN-γ* was an important cytokines in resistant to *Toxoplasma* infection[4-5], The effectors T cells exert their function both by cytotoxicity and by secretion of cytokines, especially *IFN-γ*. The cell-mediated immunity with resultant production of IL-12 and *IFN-γ* is essential to control infection by *T. gondii* by restricting the multiplication of the parasite during the acute phase and accelerating the progression to the chronic phase[6].

Furthermore, CD8, and CD4 T cells mediate long term protection against reactivated diseases [7]. *IFN-γ* produced by these cells are important to activate infected cells to kill the parasites [8- 9]. There for immune competent individuals are likely ignorant to reactivation of Toxoplasmosis.

IFN-γ is a cytokine that is highly conserved, with few allelic variations in its gene. A single nucleotide polymorphism (SNP) located in the first intron of the human gene for *IFN-γ* at the extremity 5' adjacent to the CA repeated region (*IFNγ*+874 T/A polymorphism) influences the secretion of this cytokine. Individuals carrying the A allele are low producers of *IFN-γ* [10].

A previous study has shown a correlation of *IFNγ+874T/A* polymorphism with ocular toxoplasmosis. The AA genotype showed an increased frequency in individuals with ocular findings[11].

This study was aim to evaluate the association between the presence of polymorphism in the gene coding *IFN-γ* at position +874T/A among anti *T. gondii* IgG sero-positive and sero-negative pregnant woman.

Materials and methods

Ethical consideration

The ethical approval for this study was obtained from the Ethical Committee of the Faculty of Medicine, Jazan University. Informed written consent was obtained from all participants prior to involvement in the study.

Study type

The study type was cross sectional study.

Subject groups

A total number of ninety four pregnant women attended maternity section in Jazan General Hospital were included in this study, divided into two groups: (anti *T. gondii* IgG sero-positive group 44(46%) pregnant women, and anti *T. gondii* IgG sero-negative group 50(54%) pregnant women) according to the anti-IgG screening results for toxoplasmosis by ELISA technique as mentioned recently by Ageely et al, (2014)[12].

Sample collection, and processing

Five mL of venous blood was collected aseptically from 94 pregnant women, transported to the

laboratory of the Medical Research Center Jazan University. Then, serum was separated from the whole blood by centrifugation at 3000 rpm for ten minutes at room temperature, tested for anti-*T. gondii* IgG antibodies using ELISA test kit (Human Gesllschaft for biochemical and diagnostic, Max Plank, Germany) following the manufacturer's instruction. Serum was considered positive if it showed 1.4-fold higher than the ELISA cut-off. The rest of the whole blood sample was used for DNA extraction for *IFN-γ* A/T alleles testing.

Molecular Biology Test

DNA extraction

Procedure

DNA was isolated by the Promega Blood Kit Cat No (Catalog NO 0000089642) according to the manufacturer's instructions.

Polymerase Chain Reaction (PCR) Assays

Allele Specific PCR

PCR was preformed according to Parvaneh, et al (2009) [13] with minor modification. The assay targeted the A/T alleles at two different regions, 260bp bands correspond to IFN-γ A or T allele and the 100 bp bands correspond to universal gene of gamma interferon (Internal control).

Primers sequences used in this study

- Antisense primer (260 bp corresponded to IFN-γ): TCA ACA AAG CTG ATA CTC CA.
- Sense for T allele sequence: 5 - TTC TTA CAA CAC AAA ATC AAA TCT- 3´
- Sense for A allele sequence: 5´ - TTC TTA CAA CAC AAA ATC AAA TC- 3´.
- Internal control amplifies a human β-globin sequence (100 corresponded to β-globin):

BG-forward: ACA CAA CTG TGT TCA CTA GC.

BG-reverse: CAA CTT CAT CCA CGT TCA CC.

PCR reaction

Procedure

The reaction mix with a total volume of 20 µl included:

One µl of dNTPs, 3 µl of Mgcl2, 0.5µl of Taq DNA polymerase and 1 µl from each specific (antisense primers, and sense for A or T allele), 0.5 µ1 of each internal control primers BGF and BGR, 3 µl of buffer, 7.5 µl of distilled water, followed by the addition of 2 µl of the template DNA, the mixture was gently mixed, and then transferred to the PCR machine.

Amplification procedure

Amplification was done in PCR machine (MULTIGENE) included initial denaturation at 95 ºC for 2 min followed by two loops; loop 1 which consisted of 15 cycles with the following program: 95 ºC for 1min, 62 ºC for 1min, and 72 ºC for 1min, and loop 2 included 25 cycles with the following program: 95 ºC for 1min, 56 ºC for 1min, and 72 ºC for 1min, and a final extension step at 72 ºC for 7 min.

Visualization of product

Absence or presence of PCR products was visualized by electrophoresis. The PCR products were loaded into 1.5% Agarose gel stained with Flouro-safe stain instead of Ethidium bromide . The gel was then examined in Gel documentation system(BIORAD-Gel Doc^{tm} XR+).

Statistical analysis:

The collected data was analyzed using the Statistical Package for Social Science (SPSS). The *P* Value was calculated by Z test for two population proportions.

Results:

- The results revealed a 100-bp product for human *IFN-γ* universal gene, and a 260-bp product for allele A or allele T, as shown in figures [1&2].

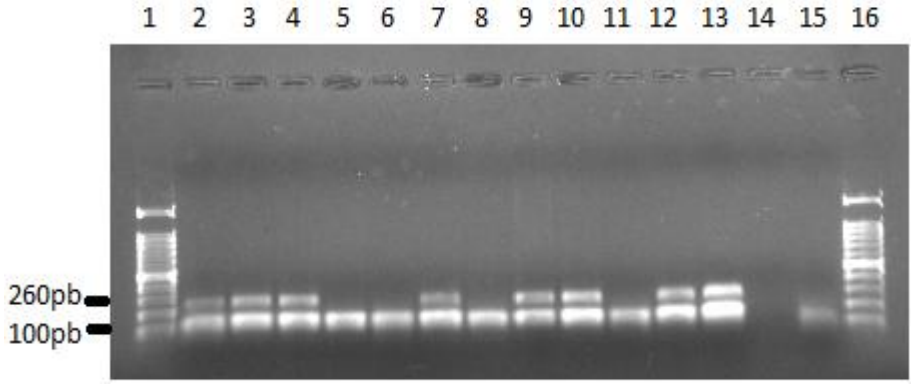

Figure [1]: PCR showed a100-bp product for human *IFN-γ* universal gene, and a 260 bp product for (allele A). Lanes(1&16) a 100-bp PCR MWt marker. Lanes 5,6,8,11, and 15 were negative for allele A. Lane 14 was negative for universal gene.

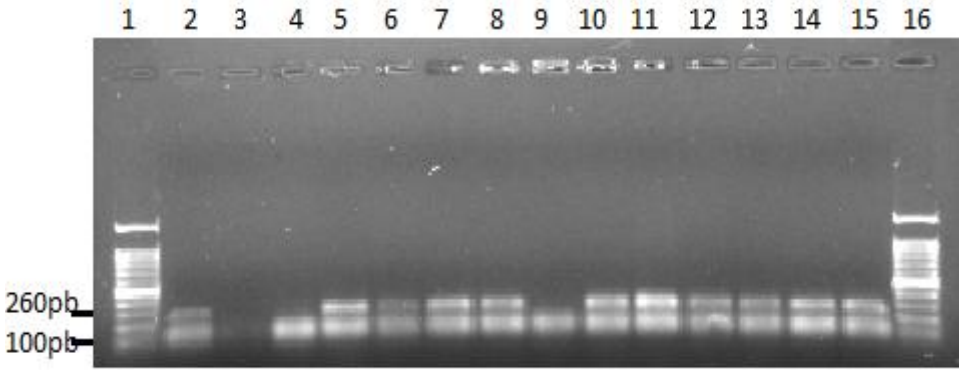

Figure [2]: PCR showed a100-bp product for human *IFN-γ* universal gene, and a 260 bp product for (allele T). Lanes(1&16) a 100-bp PCR MWt marker. Lanes 4, and 9 were negative for allele T. Lane 3 was negative for universal gene.

- *IFN-γ* genotypes was determined in 44 anti *T. gondii* IgG seropositive and 50 seronegative pregnant women. Both groups were categorized for polymorphism into three genotypes by discriminating between the presence and absence of the two alleles tables (1, 2) respectively.

Table (1) *IFN-γ* alleles among anti *T. gondii* IgG sero-positive pregnant women.

Sample	A allele	T allele	Sample	A allele	T allele
1	NO	NO	26	yes	yes
2	NO	NO	27	yes	NO
3	yes	No	28	No	NO
4	yes	yes	29	No	yes
5	No	No	30	yes	yes
6	yes	yes	31	NO	NO
7	yes	yes	32	yes	No
8	yes	No	33	yes	No
9	yes	No	34	No	yes
10	yes	yes	35	yes	yes
11	yes	No	36	yes	yes
12	NO	No	37	No	yes
13	NO	No	38	No	yes
14	yes	No	39	No	yes
15	yes	No	40	yes	yes
16	yes	yes	41	NO	NO
17	yes	yes	42	NO	NO
18	No	yes	43	NO	NO
19	yes	yes	44	NO	NO
20	yes	yes			
21	yes	No			
22	yes	yes			
23	yes	yes			
24	yes	yes			
25	yes	yes			

Table (2) *IFN-γ* alleles among anti *T. gondii* IgG sero-negative pregnant women.

Sample	A allele	T allele	Sample	A allele	T allele
1	NO	NO	26	No	No
2	NO	NO	27	yes	no
3	NO	yes	28	No	No
4	NO	yes	29	No	No
5	yes	yes	30	No	No
6	yes	yes	31	No	No
7	yes	yes	32	yes	no
8	No	No	33	yes	no
9	No	No	34	yes	yes
10	No	yes	35	yes	yes
11	yes	yes	36	yes	yes
12	No	No	37	yes	yes
13	No	yes	38	No	No
14	No	No	39	yes	yes

15	yes	yes	40	No	No
16	No	No	41	yes	No
17	No	No	42	yes	No
18	yes	yes	43	yes	No
19	yes	yes	44	No	No
20	yes	yes	45	No	yes
21	yes	no	46	yes	No
22	yes	no	47	yes	No
23	no	yes	48	No	yes
24	no	yes	49	No	yes
25	yes	yes	50	No	No

Results of allele A

A Allele was detected in 10 (22.7%) in seropositive group, comparing with10(20%) in seronegative group with *p* value (>0.05), the result was no significance difference table [3]

Results of allele T

T Allele was detected in 6 (13.6%) in seropositive group, comparing with 9(20%) in seronegative group with *p* value (>0.05), although there was no significance difference in this results T allele was more detected in seronegative group table [3].

Results of both (A/T) alleles

A/T alleles were positive in 17 (38.6%), and 14 (28%)of seropositive and seronegative groups respectively , with *p* value (>0.05).Indication of no significance difference between the study groups Table [3].

Table [3]: *INF-γ* genotypes frequency in anti- *T. gondii* IgG sero-positive and sero-negative pregnant ladies.

Alleles	**Anti- *T. gondii* IgG positive No(%)**	**Anti- *T. gondii* IgG positive No(%)**
A/A	10(22.7)	10 (20)
T/T	6 (13.6)	9 (18)
A/T	17 (38.6)	14 (28)
Universal gene only	11 (25)	17 (34)
Total	44	50

Discussion

Cellular immune response is critical for the control of the intracellular pathogen *Toxoplasma gondii*. It occurs by activation of a complex immune response, which utilizes cells of the innate and adaptive immune systems.

Recently there have been major advances in understanding of the role of cytokines in the initiation and maintenance of protective immunity to *T. gondii*, and *IFN-γ* cytokine has been identified as one of major resistance to this pathogen[14]. *T. gondii* infection induces a response by TH1 cells, which produce *IFN-γ*, a

cytokine that is involved in resistance to toxoplasmosis [15, 16].

Infection during pregnancy may cause a wide range of clinical manifestations to the mother and the fetus. In immunocompetent patients, toxoplasmosis can cause severe encephalitis by acute infection or reactivation of latent infection[2].

This study was aimed to detect the A , and T alleles frequency of *IFN-γ* in anti-*T. gondii* IgG seropositive and seronegative pregnant women, and the risk of seropositive group of being reactivated with toxoplasmosis.

In this study 94 pregnant women were studied 44 (46%) with anti-*T. gondii* IgG seropositive, and 50 (54%) were seronegative. The results of single A or T, and both A/ T *IFN-γ* alleles frequency were of no significance difference between the two tested groups *p* value (>0.05) as shown in table (3). With minor differences in T allele that it was more detected in IgG seronegative group.

High levels of IFN-γ have been described in both asymptomatic and symptomatic *T. gondii* seropositive individuals [17]. In this study the both A/T genotypes were most frequent in seropositive group (38.6% of individuals) comparing with single genotype , this result was in agreement with previous study done by Maria et al, 2009 [18] in retinochoroiditis toxoplasmosis.

A seropositive pregnant women that carrying both A/T alleles (38.5%) , and a single T allele (6%) were associated with high level of *IFN-γ*, and there for they were highly protected of being reactivated of toxoplasmosis. On the other hands a seropositive pregnant women that had a single A genotype (10%), were at high risk of reactivation, because individuals carrying a single A genotype produce less *IFN-γ* than individuals with other genotypes[10].

For seronegative pregnant women, although some individuals of this group were carrying a single A genotype (10%), they have no risk of being reactivated with toxoplasmosis in the future, because they were seronegative of anti- *T. gondii* IgG, and had no history of past infection.

As a conclusion a seropositive pregnant women that carrying a single A genotype could be associated with a high risk of infection reactivation against *T. gondii*. Further studies involving polymorphisms in other cytokines including (IL-10, and IL-12) should be performed to understand the role of the immune system in the course of *T. gondii* infection.

Acknowledgement

We would like to acknowledge Jazan University and the Directorate of Health Affairs at Jazan Saudi Arabia, with special thanks to the laboratory staff of Jazan General Hospital for their great help and assistance.

References

1. M. W. Black and J. C. Boothroyd, "Lytic cycle of *Toxoplasma gondii*,"*Microbiology andMolecular Biology Reviews* 2000;vol. 64,no. 3, pp. 607–623.
2. J. G. Montoya and O. Liesenfeld, "Toxoplasmosis," *The Lancet* 2004; vol. 363, no. 9425, pp. 1965–1976.
3. L. C. Bertozzi, L. A. Suzuki, and C. L. Rossi, "Serological diagnosis of

toxoplasmosis: Usefulness of IgA detection and IgG avidity determination in a patient with a persistent IgM antibody response to Toxoplasma gondii," *Revista do Instituto de Medicina Tropical de Sao Paulo* 1999; vol. 41, no. 3, pp. 175–177.

4. Freshman, M.M., et al., Invitro and invivo antiviral action of an interferon like substance induced by Toxoplasma gondii. *Pro Soc Exp Biol Med*., 1966; 123(3): p.862-6.
5. Rytel, M. W. and T. C. Jones, Induction of interferon in mice infected with Toxoplasma gondii. *Pro Soc Exp Biol Med*., 1966; 123(3): p.859-62.
6. Suzuki Y, Orellana MA, Schreiber RD, Remington JS. Interferon-gamma: the major mediator of resistance against *Toxoplasma gondii*. *Science* 1988; 240:516-518.
7. Gazzinelli, R., et al., Simultaneous depletion of CD4+ and CD8+ T lymphocytes is required to reactivate chronic infection with Toxoplasma gondii. *J immunol*, 1992; 149(1): p. 175-80.
8. Yap, G.S. and A. Sher, Effector cells of both nonhemopoietic and hemopoietic origen are requiered for IFN-gamma and tumor necrosis factor alpha dependent host resistance to intracelullar pathogen, Toxoplasma gondii. *J Exp Med*., 1999; 189(7): p. 1083-92.
9. Wilson, E. H. and C.A. Hunter , The role of astrocytes in the immunopathogenesis of toxoplasmic encephalitis. *Int J parasitol*, 2004; 34(5): p. 543-8.
10. Pravica V, Perrey C, Stevens A, Lee JH, Hutchinson IV. A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-gamma production. *Hum Immunol* 2000; 61:863-86.
11. Albuquerque MC, Aleixo AL, Benchimol EI, Leandro AC, Neves LB, Vicente RT, et al. The IFN-gamma +874T/A gene polymorphism is associated with retinochoroiditis toxoplasmosis susceptibility. *Mem Inst Oswaldo Cruz* 2009; 104:451-455.
12. Hussein Aqeely, Eman K. El-Gayar, Darakhshan Perveen Khan, Abdullah Najmi, Ayesha Alvi, Ibrahim Bani, Mohamed Salih Mahfouz, Saif Elden Abdalla, and Ibrahim M. Elhassan. Seroepidemiology of *Toxoplasma gondii* amongst Pregnant Women in Jazan Province, Saudi Arabia. Journal of Tropical MedicineVolume 2014, Article ID 913950, 6 pages.
13. N Parvaneh , B Pourakbari, KH Daneshjoo, H Ashraf, A Salavati, S Mamishi. Polymorphism in the First Intron of Interferon-Gamma Gene *(+874T/A*) in Patients with BCG Adenitis. *Iranian J Publ Health* 2009; Vol. 38, No.3, pp.12-16.

14. Lieberman LA, Hunter CA. The role of cytokines and their signaling pathways in the regulation of immunity to *Toxoplasma gondii*. *Int Rev Immunol* 2002; 21:373-403.
15. Suzuki Y, Conley FK, Remington JS. Importance of endogenous IFN-γ for prevention of toxoplasmic encephalitis in mice. *J Immunol* 1989;*143*:2045-2050.
16. Scharton-Kersten TM, Wynn TA, Denkers EY, Bala S, Grunvald E, Hieny S, Gazzinelli RT, Sher A. In the absence of endogenous IFN-gamma, mice develop unimpaired IL-12 responses to *Toxoplasma gondii* while failing to control acute infection. *J Immunol* 1996;*157*: 4045-4054.
17. Fatoochi F, Cozon GJN, Wallon M, Kodjikian L, Peyron F. Systemic T cell response to *Toxoplasma gondii* antigen in patients with ocular toxoplasmosis. *Jpn J Ophthalmol* 2006;*50*: 103-110.
18. Maíra Cavalcanti, et al 2009. The IFN-g +874T/A gene polymorphism is associated with retinochoroiditis toxoplasmosis susceptibility. *Mem Inst Oswaldo Cruz* 2009; Vol. *104*(3): 451-455.