

TNF- α , IL-1 β and IL-10 Gene Polymorphisms and their Association to Toxoplasmosis of Aborted Women in Sulaimania City

Latif Omer Mohammed*

Microbiology Department, College of Medicine, University of Sulaimania, Sulaimania, Iraq

*Corresponding author: Latif Omer Mohammed, PhD, Microbiology Department, College of Medicine, University of Sulaimania, Sulaimania, Iraq, E-mail: latif.mohammed@univsul.edu.iq

Received date: October 03, 2018; Accepted date: October 11, 2019; Published date: October 18, 2019

Copyright: ©2019 Mohammad LO, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Toxoplasmosis is the disease that is caused by an obligatory intracellular parasite *Toxoplasma gondii*. Cytokines have a function in its pathogenesis. The single nucleotide polymorphisms (SNPs) of every cytokine have been reported to have functioned in etiologic. So the target of the introduced study, is to examine the relationship between the SNPs of mentioned interleukins and toxoplasmosis of 50 toxoplasmosis – aborted women who attend to maternity hospital in Sulaimania city through the period Jan 2017 – March 2017 to ending their pregnancy and 40 healthful women (University staff and wellbeing staff) as control. SNPs were detected by PCRSSP (Polymerase chain reaction-sequence specific priming) technique. Latex agglutination test and Enzyme linked Immunosorbent assay for IgG and IgM concluded that 34% of samples were confirmed for IgG and IgM anti-Toxoplasma antibodies.

This study observed that there is no significant positive or negative relationship between the SNPs of mentioned interleukins and toxoplasmosis in the cases of aborted women in Sulaimania city/north of Iraq.

Keywords: Polymorphisms; TNF- α ; Cytokine; Ethidium Bromide; Agarose gel; IL-1 β ; SNPs

Introduction

Toxoplasma gondii is an obligate intracellular parasite that infects at least one third of the world's population with toxoplasmosis [1].

The diversity of the disease, it's more severe in immunocompromised people and pregnant women, while asymptomatic form of the infection is more prevalent in most immunocompetent people, transmission recurrence and disease seriousness are oppositely related. The transmission in (first and second trimester) may result in severe congenital infection and can result in death and spontaneous abortion, while the transmission in the third trimester usually results in abnormal newborns.

Cytokines have a vital role in pathogenesis of disease and pregnancy miscarriage; however and similarly imperative, polymorphisms of genes that encode for cytokines have appeared to interpose with the expression of these particles and may have a vital role in gene expression like resistance or sensibility to infections such as toxoplasmosis, genetic polymorphism are variation in DNA sequence among people that may cause the differences in response to the infection, hereditary varieties happening in over 1% of individuals.

The immunological changes at the maternal-foetal interface, for example, unevenness in the generation of T helper (Th1 and Th2) lymphocytes related cytokines may increase the danger of foetal disease; distinctive cytokines have been mentioned to be engaged beside effectors in susceptible reaction against the parasite [2]. Cytokines had been appeared to impact all steps of generation, and assume a key part in pregnancy result, cytokines such as, IL-4, IL-6 and IL-10 are support pregnancy achievement while others, for example, TNF- α and interferon (IFN)- γ , are deleterious [3].

The ordinary placenta secretes IL-4, IL-6 and IL-10, while TNF- α and IFN- γ have been related with a weakness of human trophoblastic cell development and caused premature birth in mice [4]. Moreover, transforming growth factor (TGF)- β has been associated with placental action and foetal growth, and lymphocyte emitting TGF- β have been causally connected with pregnancy accomplishment in mice, while they are mentioned to be reduced in case of pregnant loss. Besides, successful pregnancy gives off an impression of being associated with type Th2 cell maternal immunity; conversely, maternal Th1 immunity can be dangerous to foetal growth, moreover demonstrated that CD₄+CD₂₅⁺ regulatory T (Treg) cells are fundamental for the upkeep of pregnancy.

The ability of pregnancy to influence the immune system and, thus, the immune system to influence pregnancy, has been recommended to have two outcomes for parasitic infection. Firstly, pregnancy may prefer the survival of numerous parasites that require Th1 reaction to control them. The second is parasitic diseases that encourage a powerful Th1 reaction may negatively influence pregnancy [5].

The inflammatory cytokines, such as IFN- γ and TNF- α , which are mentioned harmful for pregnancy. In intense *T. gondii* disease and may affect the maternal-foetal immunological unevenness. The two cytokines have been known to restrain the outgrowth of human trophoblast cells *in vitro* and synergistically induce apoptosis of human essential villous trophoblast cells. Moreover, actuated macrophages may harm the ideas by means of production of nitric oxide (NO) and TNF- α [6].

Single Nucleotide Polymorphisms (SNPs) in the promoter part of cytokine genes have been shown to be related with the improvement of toxoplasmosis; For example, IFN- γ +874 T/A SNP of IFN- γ gene has been demonstrated their role in clinical signs of toxoplasmosis [7]. Moreover suggested that IL6-174 SNP genotypes related with

minimum ration of production of IL-6 might be associated with the occurrence of toxoplasmic retinochoroiditis [8].

Patients and Methods

90 sera samples were used for detection of specific antibody of *T. gondii* from this:

50 samples of them from patients were admitted to Maternity Teaching Hospital/Sulaimania health department and private clinics during the period Jan. 2017 – Mar. 2017 to end their pregnancy (prematurely embryo). Their sera were positive for anti-Toxoplasma antibody by using two laboratory methods, the age range was 20-45 years.

40 samples as control which included healthy female (University staff and health staff) and their sera were negative for anti-Toxoplasma antibody by both diagnostic kits, they also match patients for age and mean.

From each participating subject, 5 ml of venous blood was drawn using disposable syringe, and distributed into two aliquots. The first aliquot (3 ml) was dispensed into a plain tube and left in the refrigerator (4°C) for 15 minutes to clot, and then it was centrifuged (10 minutes at 5000 rpm). The separated serum was distributed into

aliquots (0.5 ml) in Eppendorf tubes, which were frozen at -30°C. The second aliquot (2 ml) was transferred to EDTA tube and frozen at -20°C until DNA extraction for genotyping of cytokine gene polymorphisms.

The antibody of *T. gondii* was achieved by using by two laboratory methods, the first was Latex antibody test (LAT), whereas the second confirmatory test included testing the sera for IgG and IgM antibodies by two Enzyme linked Immunosorbent assay kits (ELISA-IgG and IgM; ACON Laboratories Inc., USA). After confirmation, only the positive samples with two tests were included in this study.

Genomic DNA extraction

The genomic DNA was separated from EDTA blood using the AccuPrep® Genomic DNA Extraction Kit (Bioneer Corporation, Korea), which was designed to a fast and simple technique for the extract of purified DNA from mammalian blood. The DNA yield was spectrophotometrically assessed by using a Nano Drop, in which the sample was read at two wavelengths (260 and 280 nm). If the yield approximately 1.9 the sample was considered free of contamination and having a sufficient amount of DNA for a further analysis [9].

	1	2	3	4	5	6	7	8	9	10	11	12
A	Lane 1 (Pis.1-8)	Lane 1 (Pis.9-16)	Lane 1 (Pis.17-24)	Lane 2 (Pis.25-32)	Lane 2 (Pis.33-40)	Lane 2 (Pis.41-48)	Lane 3 (Pis.49-56)	Lane 3 (Pis.57-64)	Lane 3 (Pis.65-72)	Lane 4 (Pis.73-80)	Lane 4 (Pis.81-88)	Lane 4 (Pis.89-96)
B												
C	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
D												
E												
F												
G												
H												

Figure 1: Cytokine genotyping PCR tray showing the sequence of PCR Reaction Mix adding. The word "Lane" refers to a well's corresponding gel lane.

Genotyping of cytokine polymorphisms

The Cytokine CTS-PCR-SSP Tray Kit was used to detect cytokine gene polymorphisms. The kit was developed in the laboratories of Department of Transplantation Immunology, University Clinic Heidelberg (Heidelberg, Germany), and it was used as the official reagent set for the Cytokine Component of the 13th International Histocompatibility Workshop. It enabled the user to detect some of the polymorphisms described in the promoter regions of IL1A, IL1B,

IL1R1, IL1RN, IL2, IL4, IL6, IL10, IL12B, IFNG, TNF genes, as well as some polymorphisms in the translated regions of TGFB1 and IL4R genes. The method of detection was a PCR-SSP (polymerase chain reaction-sequence specific priming) assay, which allowed the definition of the polymorphic variants of the genes that were present in the individual under test. The first step in the assay included a preparation of PCR Reaction Mix, which consisted of 138 μ l master mix (CYT), 2.8

μ l Taq DNA polymerase (5 Unit/ μ l), 329 μ l deionized distilled water and 50 μ l DNA sample (0.10-0.15 μ g/ μ l). The mix was mixed by vortex thoroughly and kept on ice. This was followed by taking the PCR tray and placing it inside a sample holder, and the adhesive seal was carefully removed from the tray. To each well of the first 48 wells, 10 μ l of the PCR Reaction Mix was added in the sequence given in Figure 1.

The tray was placed in the thermal cyclor and the thermal cycling profile was optimized and validated for the use with the CTS-PCR-SSP TRAY KITS, and as shown in Table 1.

Stage	Temp	Duration/sec	Cycles
Initial denaturation	94°C	120	10
Denaturation	94°C	15	
Annealing+Extension	65°C	60	
Followed by			
Denaturation	94°C	15	20
Annealing	61°C	50	
Extension	72°C	30	
Hold	4°C	900	

Table 1: Optimization and validation of thermo-cycling for the use with the CTS-PCR-SSP tray kits.

Agarose gel electrophoresis

After thermo-cycling, absence or presence of PCR products was visualized by submarine Agarose gel electrophoresis.

Preparation of agarose gel: The Agarose gel was prepared at a concentration of 2% Agarose, by mixing 7 grams of Agarose, 7 ml of 50x TAE (Tris-acetate-EDTA) buffer and 350 ml of deionized distilled water in a 500 ml conical flask. The mixture was boiled to dissolve the Agarose, using a magnetic stirring hotplate or a microwave oven. After that, it was cooled to 60°C, and by then 17 μ l of Ethidium bromide (10 mg/ml) was added and well-mixed. The gel was poured on an electrophoresis plate (25 x 25 cm) fixed on an even surface, and then four combs (each with 24 teeth) were placed and the gel was allowed to set for one hour at room temperature [10].

Running electrophoresis: When the thermo-cycling was terminated, the PCR tray was handled outside the thermo-cycler carefully and the strip caps that covered the wells were removed from the tray. This was followed by removing the combs from the gel, which was covered with running TAE buffer (approximately 2-3 mm above the gel surface). Then, 10 μ l of each PCR product was loaded onto the respective gel wells in a sequence that was given in Figure 1. The electrophoresis was run for 20 minutes at 170 volts.

Interpretation of electrophoresis patter: After the end of electrophoresis, the power was turned off, electrodes were disconnected and the gel was removed and place on UV light trans-illuminator (312 nm) to visualize the bands. Finally a picture was taken, and the genotype of each cytokine was recorded according to the chart provided by the supplier of the genotyping kit (Figure 2).

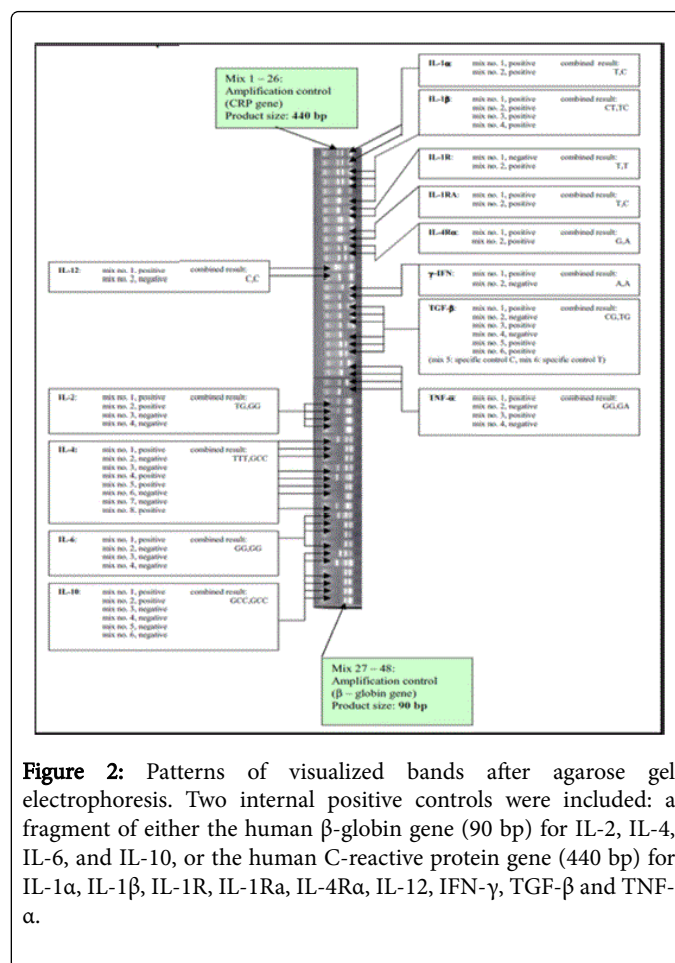


Figure 2: Patterns of visualized bands after agarose gel electrophoresis. Two internal positive controls were included: a fragment of either the human β -globin gene (90 bp) for IL-2, IL-4, IL-6, and IL-10, or the human C-reactive protein gene (440 bp) for IL-1 α , IL-1 β , IL-1R, IL-1Ra, IL-4R α , IL-12, IFN- γ , TGF- β and TNF- α .

Statistical analysis: Genotypes of cytokines were presented as percentage frequencies, and significant differences between their distributions in toxoplasmosis patients and controls were assessed by two-tailed Fisher's exact probability (P). The Relative Risk (RR), Etiological Fraction (EF) and Preventive Fraction (PF) were also estimated to define the association between a genotype with the disease. If the association was positive, the EF was calculated, whereas if it was negative, the PF was given [11].

Results and Discussion

Diagnostic test toxoplasmosis

Anti-Toxoplasma Abs	Positive cases			
	LAT		ELISA	
	No.	%	No.	%
IgG only	17	34%	28	56%
IgM only	16	32%	5	10%
IgG+IgM	17	34%	17	34%
Total	50	100	50	100

D.F.=2; P value \leq 0.01

Table 2: Results of serum-testing in toxoplasmosis patients by rapid test cassette and enzyme immunoassay.

The current study investigated SNPs of 3 cytokines gene (IL1B, IL10 and TNF) in 50 toxoplasmosis-aborted women. These SNPs were ascertained by PCR-SSP (polymerase chain reaction-sequence specific priming) method, and 40 apparently healthy women were included as controls.

Both Sero-diagnostic tests (Latex agglutination test and Enzyme linked Immunosorbent assay for IgG and IgM) revealed that 34% of cases were positive for IgG and IgM anti-Toxoplasma antibodies; however, the cases positive for one type of antibodies showed some differences. In the LAT, 34% of cases were positive for IgG antibody, whereas it was 56% in the ELISA for IgM antibody, 32% were positive by LAT and 10% by ELISA. Such differences were significant ($P \leq 0.01$) (Chi-square) (Table 2).

Cytokine gene polymorphisms

The study deals with SNPs of 13 cytokine and cytokine receptor genes (IL1A, IL1B, IL1R1, IL1RN, IL2, IL4, IL4R, IL6, IL10, IL12B, IFNG, TNF and TGFB1) in order to define the type of relationship (positive, negative or no correlation) between these SNPs and toxoplasmosis in aborted women. These SNPs were ascertained by PCR-SSP method, in which different PCR mixes were used to define these polymorphisms at different positions, which were revealed after agarose-gel electrophoresis of the PCR amplicons, and their patterns of migration in the gel were given in Figure 3.

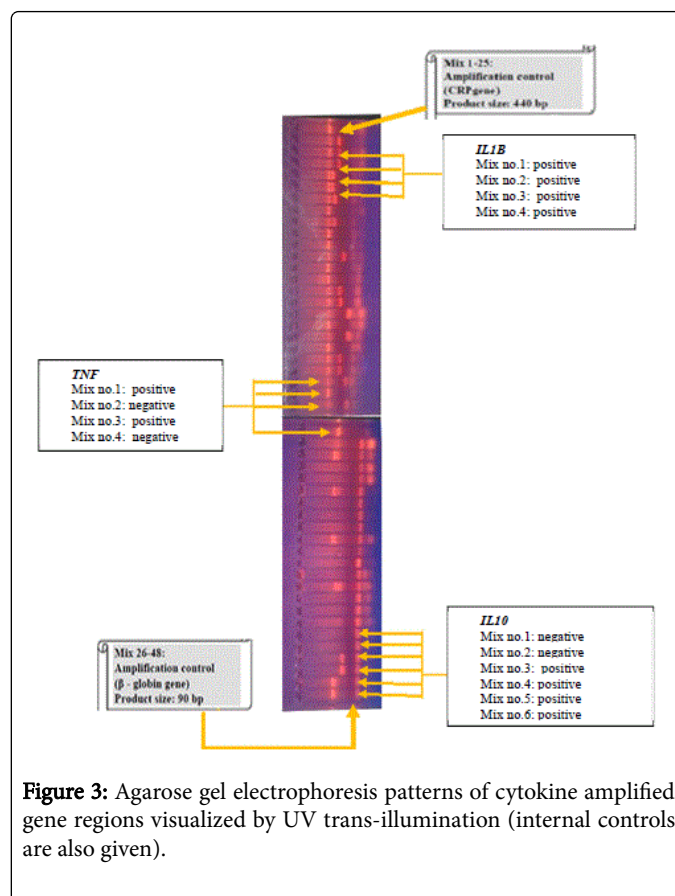


Figure 3: Agarose gel electrophoresis patterns of cytokine amplified gene regions visualized by UV trans-illumination (internal controls are also given).

Geno type or Allele	Patients (N. =50)		Controls (N. =50)		Odds Ratio	95% Confidence Interval	EF or PF	P
	No.	%	No.	%				
TT	12	24	15	37.5	0.53	0.21 - 1.30	0.18	NS
TC	18	36	12	30	1.31	0.54 - 3.16	0.09	NS
CC	20	40	12	32.5	1.38	0.59 - 3.27	0.11	NS
T	42	42	42	52.5	0.66	0.36 - 1.18	0.18	NS
C	58	58	38	47.5	1.53	0.85 - 2.75	0.2	NS

Abbreviations: EF: Etiological Fraction; PF: Preventive Fraction; p: Probability; NS: Not Significant ($p > 0.05$)

Table 3: Observed numbers and percentage frequencies and Hardy-Weinberg (H-W) equilibrium of IL1B₊₃₉₆₂ genotypes and alleles in toxoplasmosis patients and controls.

Interleukin-1 beta gene (IL1B₊₃₉₆₂)

The genotypes of IL1B₊₃₉₆₂ SNP in both patients and controls were in agreement with H-W equilibrium and there is no departure from Hardy-Weinberg (H-W) equilibrium could be established, However, no significant differences were observed between patients and controls in the distribution of alleles or genotypes frequencies of TT, TC and CC (Table 3).

Interleukin-10 gene (IL10₋₅₉₂)

The frequencies of IL-10₋₅₉₂ genotypes were in a good agreement with H-W equilibrium in patients and controls. However, Genotype and allele frequencies demonstrated no significant differences between patients and controls at position ₋₅₉₂ (Table 4).

Geno type or Allele	Patients (N. =50)		Controls (N. =50)		Odds Ratio	95% Confidence Interval	EF or PF	P
	No.	%	No.	%				
CC	10	20	10	25	0.75	0.28-2.01	0.06	NS
CA	22	44	13	32.5	1.63	0.69-3.84	0.17	NS
AA	18	36	17	42.5	0.76	0.33-1.77	0.1	NS
C	42	42	33	41.3	1.03	0.57-1.87	0.01	NS
A	58	58	47	58.7	0.97	0.54-1.75	0.02	NS

Abbreviations: EF: Etiological Fraction; PF: Preventive Fraction; p: Probability; NS: Not Significant (p>0.05)

Table 4: Observed numbers and percentage frequencies and Hardy-Weinberg (H-W) equilibrium of IL10₋₅₉₂ genotypes and alleles in toxoplasmosis patients and controls.

Tumor Necrosis Factor-Alpha (TNF- α); TNF- α ₋₃₀₈

Genotype frequencies of TNF- α at position ₋₃₀₈ showed no deviation from H-W equilibrium in patients. However, the genotype or allele frequencies of TNF- α ₋₃₀₈ showed no significant difference between patients and controls at this position.

The role of IL-10 in pathogenesis of toxoplasmosis and the associated abortion might be suggested because these SNPs showed a strong effect on the transcription of IL10 gene and its production. IL-10 has been considered an essential cytokine for maintaining a normal pregnancy [12].

A study conducted in 2006 has demonstrated a correlation between IL10₋₅₉₂ SNPs and early spontaneous abortions; another study demonstrated the existence of the correlation only between the polymorphism IL10₋₅₉₂ and recurrent abortions [13,14].

Conclusion

There have also been studies that concluded that there are no correlations between any of the SNPs and etiologic of spontaneous miscarriages. Therefore, in this recent study, we concluded that there are no susceptibility and protective effects suggested in Kurdish women and no more investigation can confirm these findings.

References

1. Alegria OLB, Ortega CH, Solares CI, Torres RCP, Ruiz SA, et al. (2010) Congenital toxoplasmosis: Candidate host immune genes relevant for vertical transmission and pathogenesis. *Genes Immun* 11: 363-373.
2. Abbas AK, Murphy KM, Alan S (1996) Functional diversity of helper T Lymphocytes. *Nature* 383: 787-793.
3. Chaouat G, Meliani AA, Martal J, Raghupathy R, Elliott JF, et al. (1995) IL-10 prevents naturally occurring fetal loss in the CBA x DBA/2 mating combination, and local defect in IL-10 productions in this abortion-prone combination is corrected by in vivo Injection of IFN-tau. *J of Immunol* 154: 4261-4268.
4. Gimenez GC, Reig AJ (2015) Recurrent miscarriage: Causes, evaluation and management. *Postgrad Med J* 91: 151-162.
5. Wujcicka W, Wilczyński J, Nowakowska D (2014) Do the placental barrier, parasite genotype and Toll-like receptor polymorphisms contribute to the course of primary infection with various *Toxoplasma gondii* genotypes in pregnant women? *Eur J Clin Microbiol Infect Dis* 33: 703-709.
6. Meira CS, Chioccola PVL, Vidal JE, de Mattos CC, Motoie G, et al. (2014) *Toxoplasma* groups. Cerebral and ocular toxoplasmosis related with IFN- γ , TNF- α , and IL-10 levels. *Front Microbiol* 13:492.
7. Neves Ede S, Albuquerque MC, Silva PCS, Silva LB, Bueno WF, et al. (2012) Genetic polymorphism for IFN γ +874T/A in patients with acute toxoplasmosis. *Rev Soc Bras Med Trop* 45: 757-760.
8. Cordeiro CA, Moreira PR, Bessa TF, Costa GC, Dutra WO, et al. (2013) Interleukin-6 gene polymorphism (-174 G/C) is associated with toxoplasmic retinochoroiditis. *Acta Ophthalmol* 91: e311-314.
9. Frank TC, Tait BD (2012) Immunogenetics : Method and application in clinical practice. *Method in Molecular Biology* p:882.
10. Walker P, Lerski RA, Mathur-De Vre R, Binet J, Yane F, et al. (1988) Preparation of agarose gels as reference substances for NMR relaxation time Measurement. *Elsevier* 6: 215-222.
11. Ad'hiah AH (1990) Immunogenetic studies in selected human diseases. Ph.D. Thesis, Department of Human Genetics, University of Newcastle upon Tyne. U.K.
12. Raghupathy R (2001) Pregnancy: Success and failure within the Th1/Th2/Th3 paradigm. *Semin Immunol* 13:219-228.
13. Sarvestani KE, Zolghadri J, Fard GB, Sarvari J (2005) Cytokine gene polymorphisms and susceptibility to recurrent pregnancy loss in Iranian women. *J Reprod Immunol* 65: 171-178.
14. Bohiltea LC, Radoi EV (2014) Interleukin-6 and interleukin-10 gene polymorphisms and recurrent pregnancy loss in Romanian population. *Iran J Reprod Med* 12: 617-22.