Online ISSN: 2588-3526

Journal of Wildlife and Biodiversity

Volume 7 (Special Issue): 186-195 (2023) (http://www.wildlife-biodiversity.com/)

Research Article

Impact of IL-6-174G/C, IL-10-1082A/G Gene Polymorphisms on *Toxoplasma gondii* infections

Hama Hussein Hama Hussein[1], Samir Jawdat Bilal[2], Sarhang Hasan Azeez[3]*, Khalid Jabar Aziz[4], Rahim Abdulkarimi[5]

[1]Ministry of Education, General-Directorate Education in Raparin, Educational Department, Sub-Directorate of Pshdar, Qaladiza Scientific High School
[2]Salahaddin University- Erbil, College of Agricultural Engineering Sciences, Department of Fish Resources and Aquatic Animals
[3]Salahaddin University-Erbil, College of Education, Biology Department
[4]Salahaddin University-Erbil, College of Veterinary Medicine, Basic Science Department
[5]Department of Environment, Boukan Office, West Azerbaijan Province, Iran
*Email: sarhang.azeez@su.edu.krd

Received: 16 August 2023 / Revised: 26 November 2023 / Accepted: 27 November 2023/ Published online: 28 November 2023.

How to cite: Hama Hussein, H.H., Bilal, S.J., Hasan Azeez, S., Jabar Aziz, K., Abdulkarimi, R. (2023). Impact of IL-6-174G/C, IL-10-1082A/G Gene Polymorphisms on *Toxoplasma gondii* infections, Journal of Wildlife and Biodiversity, 7 (Special Issue), 186-195. **DOI**: https://doi.org/10.5281/zenodo.10212098

Abstract

Toxoplasma gondii is one of the most common intrauterine infections worldwide and the major cause of perinatal morbidity and mortality. This study aims to determine the genetic susceptibility of certain types of cytokine single nucleotide gene polymorphism to toxoplasmosis. Materials: the study involved 40 females with confirmed seropositive *Toxoplasma gondii* infection and 30 healthy females as the control group. Single nucleotide gene polymorphisms of IL-16-174G/C, IL-10-1082A/G, and TNF-α-308G/C were determined. IL-6-174G/C results showed no relation between GG and the disease, the protective effect of GC genotype, and CC as a risk factor with a 3-fold effect OR:3.22. C allele as a risk for the infection. For IL-10-1082A/G genotypes, the wild and mutant homozygotes were associated with the disease with almost 1.5 folded effects, OR: 1.46 and 1.56 respectively. While the mutant heterozygote has a protective

effect against the disease, OR: 0.57. Conclusion: the study found a positive relation between the IL-6-174 CC genotype with 3 folded effects, and for IL-10-1082A/G the AA and AG were associated with the disease by 1.5 fold.
Keywords: Toxoplasmosis, SNP, Cytokines, IL-6-174G/C, IL-10-1082A/G, TNF-α-308G/C.

Introduction

Toxoplasmosis is an infection caused by a parasite with the protozoan *Toxoplasma gondii*. The infection results in different types of proven syndromes in humans, environmental mammals, and many bird classes (Rasyan and Ray, 2004). There has been progress throughout time about toxoplasmosis and they have been mentioned below in sequential order. *T. gondii* was first detected in North Africa by a rodent (Nicolle and Manceaux, 1909). Two of the main forms of the main parasite are tissue cysts and oocysts which play important roles in the transmission. A study by Jones in 2003 shows that hot areas with low altitudes and moist environments help to increase the infection of *T. gondii*. Human Infection can arise via: (i) Tissue absorption cysts in un-cooked meat. (ii) Food absorption or polluted water with mature oocysts fecal-orally. (iii) Mother-to-fetus transmission is called Transplacental or vertical transmission. (iv) Donors can also rarely transmit the infection via needle stick wounds, blood transfusion or orange transplantation. Approximately one in three people in the world may get infected by Toxoplasmosis (Ayeh-Kumi et al., 2010, Monotoya et al., 2004). Ayeh-Kumi (2010) claims that between 30% and 65% of people are already infected by toxoplasmosis. Toxoplasmosis is transmitted to humans by oral ingestion of cysts in infected animal tissues or sporocysts in their extracts, produced by *Toxoplasma gondii* protozoa. Reticuloendothelial system, muscle, eye, and brain tissue, especially in the formation of cysts in many tissues or manifests with acute infection, it is an infectious disease that can transplacentally pass from infected pregnant to the fetus resulting in congenital infection, anomalies, and abortion (Desmonts et al., 1974).

In immune-competent hosts, the infection is controlled due to the elicitation of a strong innate and adaptive immune response that leads to the elimination of the majority of parasites (Sasai et al., 2018). Although Toxoplasma infection induces robust immunity, the parasite has evolved strategies to evade this response and persist as a chronic infection (Lima and Lodoen., 2019). Innate immunity provides a first line of defense against the invading pathogen and this response has been extensively studied. Notwithstanding, the indispensable role of innate immunity, the adaptive immune response is essential for the ultimate survival of the infected host. Development

and maintenance of a robust CD8 T cell immunity, facilitated by helper CD4 T cell response, is crucial for keeping the parasite in a chronic state and preventing the reactivation of the latent infection (Khan et al., 2019).

Proinflammatory cytokines, including IL1, IL6, IL12, and TNF-α, as well as anti-inflammatory cytokine IL10, were reported to be involved in the development of immune responses after infection with *T. gondii* (Carneiroet al., 2016; Liu et al., 2014). Considering genetic alterations located within genes encoding cytokines, *IL6* -174 G>C and *IL10* -1082 G>A single nucleotide polymorphisms (SNPs) were reported to be associated with toxoplasmic retinochoroiditis (TR) (Cordeiro et al., 2008; Cordeiro et al., 2013). Moreover, the GC heterozygotic status within *IL6* −174 G>C SNP, as well as the presence of C alleles in *IL6* and *IL1B* +3954 C>T polymorphisms, were reported to be associated with congenital toxoplasmosis (Wujcicka et al., 2015).

Martial and methods

Sampling

For this study, 40 patients were involved between November 2022 and May 2023, in Maternity Teaching Hospital, Erbil, Iraq with seropositive toxoplasmosis. A questionnaire form was prepared including demographic data about the patients like, age, address, socioeconomic status, etc. consent was taken for each patient. 30 healthy females were obtained as the control group with no history of toxoplasmosis**.**

Blood samples

Venous blood of 5 ml was taken for each participant of the study. Blood was divided into two aliquots, the first aliquot (3ml) was dispensed into a plain tube and it was centrifuged (15 minutes at 3000 rpm) in a temperature-controlled centrifuge (4°C). The separated serum was distributed into 3 aliquots in Eppendorf tubes, which were frozen at -20℃ until an assessment of hormones and cytokine serum levels. The second aliquot (2 ml) was transferred to an EDTA tube and frozen at -20℃ until DNA extraction for genotyping of cytokine gene polymorphisms.

DNA extraction and genotyping

The genomic DNA was isolated and extracted from the venous blood of the studied samples according to the manufacturer s protocol. The amplification refractory mutational system method (ARMS-PCR) was utilized. The primer sequences were as follows IL-6 generic primer 5 -GCC

TC G G C TC CC GTC C-3 IL-6 G allele Primer 5 -CCC CT GTT GTG TCT TGC G-3 and IL -6 C Allele Primer 5 -CCC CT GTT GTG TCT TGC C-3. The PCR reaction was carried out in a thermal cycler (PX2) with the following program for IL-6-174. The samples were placed in a 20 µ reaction volume containing 40 ng genomic DNA, 1.5 mM dNTPs, 25 mM MgCl2 1 µ of 10 pmol of each primer, and 0 4 units of Taq polymerase (Fermentas, Maryland, US in 1 Reaction uffer Cycling conditions were as follows: 1 minute at 95 C followed by 10 cycles of 15 seconds at 95 C 50 seconds at 58 C 40 seconds at 72 C followed by 20 cycles of 20 seconds at 95 C, 50 seconds at 54°C and 50 seconds at 72°C with 5 minutes at 72 C as the final extension, 230bp.

For the IL-10 genotype at (-1082 G/A) (rs1800896), the assays were performed in a 20 µL reaction volume containing 40 ng genomic DNA, 1.5 mM dNTPs, 25 mM MgCl2 , 1 µL of 10 pmol each primer and 0.4 units of Taq polymerase (Fermentas, Maryland, USA) in 1X Reaction Buffer. The primer sequences were as follows: IL-10 generic primer, 5′-CAGTGCCAACTGAGAATTTGG-3′, IL-10 (G) Allele Primer 5′-CTACTAAGGCTTCTTTGGGAG- 3, and IL-10 (A) Allele Primer 5′-ACTACTAAGGCTTCTTTGGGAA-3. The PCR reaction was carried out in a thermal cycler (PX2) with the following cycling conditions: 95°C for 3 minutes, followed by 35 cycles at 95°C for 45 seconds, 58°C for 40 seconds, 72°C for 1 minute, and finally a 7-minute extension at 72°C. The amplicon size was 254bp. The amplified products were analyzed on 2% agarose gel.

Statistical analysis

Statistical Analysis All statistical analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Normally distributed variables were expressed as mean ± SD as appropriate. The level of statistical significance was set at $P<0.05$. Descriptive data were presented as mean ± standard deviation (SD). Genotype and allele frequencies were compared between the groups using a (χ^2)-test of independence with 2 x 2 contingency tables and z statistics. Statistical significance of the variables was established at the level $P<0.05$.

Results

Regarding the IL-6-174G/C, higher producer genotype GG was non-significant and non-associated with the disease, OR: 1.07, C.I95%: 0.37-3.07. While the intermediate producer GC has a protective effect to get the disease, OR: 0.7, C.I95%: 0.24-2.08. The low producer CC genotype has more than 3 folded risks for having the disease, OR: 3.22, C.I95%:0.29-82.29. The

G allele has a protective effect against the disease OR: 0.88, while the *C* allele was most preferable for getting the disease OR: 1.14. The dominance allele showed a protection effect with OR: 0.31, and the recessive one had risk with OR: 0.94 (Table 1).

IL-10-1082A/G showed that wild homozygote (high producer) AA associated with the disease with 1.5 folded risk, OR: 1.46, C.I95%: 0.5-4.24, the mutant heterozygote (intermediate producer) AG has protective effect against the disease, OR: 0.57. The mutant homozygote (low producer) GG also has the 1.5 folded effects for having the disease, OR: 1.56, C.I95%: 0.2-18.25 (Table 2). Both alleles have non-significant effects for getting the disease with the OR: 1.17 and 0.86 respectively. Both dominancy and recessive traits have the same effect on the disease pathogenesis (Table 2).

Table 1. Genotypes and allele frequency with dominancy effect of the IL-6, IL-10 and TNF-α SNP of the patients and the control.

Genotypes	Patients N (%)	Control N(%)	OR(95%C.I)	P value
IL-6				
GG	22(55)	16(53.33)	1.07(0.37 to 3.07)	0.54
GC	14(35)	13(43.33)	0.70(0.24 to 2.08)	0.32
CC	4(10)	1(3.33)	3.22(0.29 to 82.29)	0.28
G	58(72.5)	45(75)	0.88(0.38 to 2.01)	0.44
C	22(27.5)	15(25)	1.14(0.50 to 2.65)	0.44
dominance				
GG+GC	36	29	0.31(0.01 to 3.41)	0.28
recissive				
GC+CC	18	14	0.94(0.33 to 2.69)	0.54

Table 2. Genotypes and allele frequency with dominancy effect of the IL-10-1082A/G SNP of the patients and the control.

IL-10				
AA	25(62.5)	16(53.33)	1.46(0.50 to 4.24)	0.29
AG	11(27.5)	12(40)	0.57(0.18 to 1.76)	0.19
GG	4(10)	2(6.67)	1.56(0.20 to 18.25)	0.48
A	61(76.25)	44(73.33)	1.17(0.50 to 2.70)	0.42
G	19(23.75)	16(26.67)	0.86(0.37 to 2.00)	0.42
dominance				
AA+AG	36	28	0.64(0.05 to 4.89)	0.48
recessive				
AG+GG	15	14	0.69(0.24 to 2.00)	0.29

Discussion

Toxoplasma gondii is one of the most common intrauterine infections worldwide and the major cause of perinatal morbidity and mortality (Rasyan and Ray, 2004). Congenital infections with *T. gondii* are diagnosed in about 0.07–2.9 % of live newborns, leading to asymptomatic as well as symptomatic disease, sometimes with fatal course (Nicolle and Manceaux, 1909). Considering the immune response against *T. gondii*, several studies have shown the role of proinflammatory cytokines, including interleukin (IL) 6 and IL1 (Cordeiro et al., 2008; Cordeiro et al., 2013). Among women infected with *T. gondii*, IL6 expression was estimated to be twice as high

compared to the control cases. In another study, the development of toxoplasmosis-related ocular lesions among *T. gondii*-infected patients was reported to be associated with high IL1 and TNF-α levels (Cordeiro et al., 2008). Considering a possible participation of the genetic alterations, located at IL1 and IL6 molecule encoding genes, the −174 G>C single nucleotide polymorphism (SNP) from the IL6 gene was reported to be correlated with toxoplasmic retinochoroiditis (TR). The prevalence rates of genotypes and alleles at the IL6 −174 G>C SNP differed significantly between the patients with TR and the healthy blood donors with positive serology for *T. gondii* infection and without retinal symptoms of the previous disease. In turn, another study reported no correlation of either IL1A −889 C>T or IL1B C>T SNPs with the occurrence of TR (Wujcicka et al., 2013). So far, no study has been performed to seek for a possible involvement of IL1 and IL6 SNPs in the development of congenital infection with *T. gondii*. In the reported study, we aimed to analyze and describe a possible influence of the polymorphisms located at the IL1A, IL1B, and IL6 genes on the occurrence of congenital *T. gondii* infection in fetuses and neonates. The haplotype prevalence rates were also estimated for IL1 SNPs. A multiple-SNP analysis was performed to estimate the assumed simultaneous influence of IL1 and IL6 SNPs on the occurrence of the disease (Naranjo-Galvis et al., 2018).

Contrary to our results, the GC heterozygotic status within IL6 −174 G>C SNP, as well as the presence of C alleles in IL6 was reported to be associated with congenital toxoplasmosis (Wujcicka et al., 2013).

The significantly higher prevalence rate of the C allele versus the G allele observed in our study among the *T. gondii*-infected offspring as compared to the control cases was also estimated for TR patients which was consistent with the same previous study. Previously, the IL6 −174 G>C polymorphism was determined as being associated with altered expression levels of the encoded cytokine as well (Rai et al., 2021). Taking into account the papers reporting increased IL6 levels to be correlated with *T. gondii* infection in various patient groups, it seems possible that the GC heterozygotic status at the IL6 −174 G>C SNP may alter the immune response against the parasite through higher IL6 cytokine production. However, a detailed description of IL6 molecular changes, possibly associated with congenital toxoplasmosis development, would be a challenge. So far, the C allele at the IL6 −174G>C SNP was reported to generate new binding sites for NF-1 and Smad4 transcription factors, which are not observed in the presence of the G allele (Castellucci et al., 2006; Schaaf et al., 2005). In addition, a variable AnTn tract, located at

the nucleotides from −392 up to −373 within the IL6 gene promoter, was also reported to affect the transcription of −174 G allele and −174 C allele-related haplotypes (Terry et al., 2000).

Considering IL6 -174 G>C polymorphism, similarly to our findings the C allele was also previously reported to be significantly associated with congenital parasitic infection among foetuses and neonates, as well as with TR among adult patients (Wujcicka et al., 2013; Terry et al., 2000). Taking into account IL10 –1082 G>A SNP, the study agreed with our results that the presence of A allele was shown to be related to the occurrence of TR (Cordeiro et al., 2013). The same study found that TR patients with AA and AG genotypes were determined to be at increased risk of the disease, as compared to the individuals with GG genotypes, while ours only AA was increased risk to the disease and the AG was protective (Cordeiro et al., 2013). Similarly, in the current study, the AG heterozygotic pregnant women within IL10 -1082 G>A polymorphism, were significantly more susceptible to congenital transmission of the parasite to their fetuses. Among a larger cohort of patients, the lack of significance observed for the relationship between AG genotypes within IL10 -1082 G>A polymorphism, and congenital infection, might result from different structures of randomly selected groups of pregnant women, regarding parasitic transmission to their fetuses confirmed by previous studies.

Conclusion

Considering IL-6-174G/C the CC genotype and C allele were determined as an increased risk to the TR, and G allele was most likely not associated with the disease. Whilst for the IL-10 the mutant heterozygote AG genotype was considered as a protective factor for the disease, both wild and mutant homozygotes were 1.5 folded at increased risks for the toxoplasmosis.

References

Ayeh-Kumi, Opoku, A. G., Kwakye, N., Dayie, N., Asmah, R., Obeng, N & Kretchy, J. (2010). Seroprevalence of toxoplasmosis among patients visiting the Korle-Bu Teaching Hospital. Accra, Ghana: Reviews in Infection, 1(3),147-150.

Carneiro, A.C., Machado, A.S., Bela, S.R., Costa, J.G., Andrade, G.M., Vasconcelos-Santos, D.V., Januario, J.N., Coelho-Dos-Reis, J.G., Ferro, E.A., TeixeiraCarvalho, A., Vitor, R.W & Martins-Filho, O.A.(2016). Cytokine Signatures Associated With Early Onset, Active Lesions and Late Cicatricial Events of Retinochoroidal Commitment in Infants With Congenital Toxoplasmosis. Journal of Infection Disease, 213 (12), 1962-1970.

Castellucci, L., Menezes. E., Oliveira, J., Magalhaes, A., Guimaraes, L.H., Lessa, M., Ribeiro, S., Reale, J., Noronha, E.F., Wilson, M.E., Duggal, P., Beaty, T.H., Jeronimo, S., Jamieson, S.E., Bales,

A., Blackwell, J.M., de Jesus, A.R & Carvalho EM.(2006). IL6 –174 G/C promoter polymorphism influences susceptibility to mucosal but not localized cutaneous leishmaniasis in Brazil. J Infect Dis, 194(4), 519–527.

Cordeiro, C.A., Moreira, P.R., Andrade, M.S., Dutra, W.O., Campos, W.R., Orefice, F & Teixeira, A.L. (2008). Interleukin-10 gene polymorphism (-1082G/A) is associated with toxoplasmic retinochoroiditis. Invest Ophthalmol Vis Sci, 49 (5), 1979-1982.

Cordeiro, C.A., Moreira, P.R., Bessa, T.F., Costa, G.C., W.O. Dutra, W.R. Campos, F. Orefice, L.H., Young, L.H & Teixeira A.L. (2013). Interleukin-6 gene polymorphism (-174 G/C) is associated with toxoplasmic retinochoroiditis, Acta Ophthalmol. 91 (4) (2013) e311-e314.

Desmonts, G., Gouvreur, J., & Congenital. (1974). A Prospective Study of 378 Pregnancies (Vol. 290).New England Journal of Medicine, 290(20),1110-6. https://doi: 10.1056/NEJM197405162902003.

Khan, I.A., Hwang, S & Moretto, M.(2019). Toxoplasma gondii: CD8 T Cells Cry for CD4 Help. Front Cell Infect Microbiol , 9:136.

Lima, T.S & Lodoen, M.B. (2019). Mechanisms of Human Innate Immune Evasion by Toxoplasma gondii. Front Cell Infect Microbiol, 9:103.

Liu, X., Zhao, M., Yang, X., Han, M., Xu, X., Jiang, Y & Hu, X. (2014). Toxoplasma gondii infection of decidual CD1c(+) dendritic cells enhances cytotoxicity of decidual natural killer cells. Inflammation, 37 (4),1261-1270.

Monotoya, J., & Liesenfeld, O. (2004). Toxoplasmosis. Lancet, 363(9425), P1965-1976. https://doi.org/10.1016/S0140-6736(04)16412-X

Mousa, N.M., Jasim, H.M. (2021). Association of IL-1β +3954 G>A and IL-6 -174 G/C polymorphisms in congenital toxoplasmosis. Vacci Monitor, 30(3), 125-132.

Naranjo-Galvis, C.A., de-la-Torre, A., Mantilla-Muriel, L.E., Beltrán-Angarita. L., Elcoroaristizabal-Martín, X., McLeod, R., Alliey-Rodriguez, N., Begeman, I.J., López de Mesa, C., Gómez-Marín, J.E & Sepúlveda-Arias, J.C. (2018). Genetic Polymorphisms in Cytokine Genes in Colombian Patients with Ocular Toxoplasmosis. Infect Immun, 86(4):e00597-17. doi: 10.1128/IAI.00597-17.

Nicolle, & Manceaux. (1909). Sur un protozoaire nouveau du gondi. Paris, France: C R Soc Biol.

Rai, H., Colleran, R., Cassese, S., Joner, M., Kastrati, A & Byrne, R.A. (2021). Association of interleukin 6 -174 G/C polymorphism with coronary artery disease and circulating IL-6 levels: a systematic review and meta-analysis. Inflamm Res Dec, 70(10-12), 1075-1087. doi: 10.1007/s00011-021-01505-7.

Rasyan, & Ray. (2004). Sherris Medical Microbiology (4 ed.). New York, USA: McGraw.

Sasai, M., Pradipta, A & Yamamoto, M. (2018). Host immune responses to Toxoplasma gondii. International Immunology, 30(3), 113-119. https:// doi: 10.1093/intimm/dxy004.

Schaaf, B., Rupp, J., Müller-Steinhardt, M., Kruse, J., Boehmke, F., Maass, M., Zabel, P & Dalhoff K. (2005). The interleukin-6 -174 promoter polymorphism is associated with extrapulmonary bacterial dissemination in Streptococcus pneumoniae infection. Cytokine, 31(4),324–328.

Smith, J.D., Khan, V., Zhang, H., Williams, T., Garcia, J., Sato, Y., Thompson, D., Taylor, F. G., McDonald, L., Laine, C., Arnott, A., Fisher, V., Derryck, W., Young, N., Creswell, I., Greene, P., Walsh, Dubois, T., Wright, B., … Roach, C. (2009). Māori voices: Approaches to bilingual research. Journal of Academic Methodologies, 38(3), 17–28.

Terry, C.F., Loukaci, V & Green FR. (2000). Cooperative influence of genetic polymorphisms on

interleukin 6 transcriptional regulation. J Biol Chem, 275(24),18138–18144.

W. Wujcicka, Z. Gaj, J. Wilczynski, D. Nowakowska, Contribution of IL6 -174 G>C and IL1B +3954 C>T polymorphisms to congenital infection with *Toxoplasma gondii*. (2015). Eur J Clin Microbiol Infect Dis, 34 (11), 2287-2294.

Wujcicka, W., Wilczyński, J & Nowakowska, D. (2013). SNPs in tolllike receptor (TLR) genes as new genetic alterations associated with congenital toxoplasmosis? Eur J Clin Microbiol Infect Dis, 32(4),503–511