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# Potential effects of toxoplasmosis on Toll-like receptor-5 gene polymorphisms (rs5744168, rs2072493) in a sample of patients undergoing hemodialysis

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**Abstract.** *Toxoplasma gondii* (*T. gondii;* toxoplasmosis) causes latent infection that is activated in immunocompromised patients, such as in patients undergoing hemodialysis. The aim of the present study was to examine the association between TLR-5 gene polymorphisms in a sample of patients undergoing hemodialysis who were positive for toxoplasmosis determined using quantitative-polymerase chain reaction techniques. The present case-control study was performed on 100 patients undergoing hemodialysis. The study included 50 seropositive patients [immunoglobulin (Ig)M and IgG] for *T. gondii* (group1) and a control group of 50 patients undergoing hemodialysis who were seronegative (IgM and IgG) for *T. gondii* (group 2). Toxoplasma antibodies were detected using ELISA, and Toll-like receptor (TLR)-5 gene polymorphisms (rs5744168 and rs2072493) were detected using qPCR. Group 1 included 18 patients who had a higher heterozygous genotype (GA) frequency (36%) of the TLR-5 gene polymorphism (rs5744168) than group 2 10 (20%). However, the difference was not statistically significant (odds ratio, 2.25; 95% confidence interval (CI), 0.91 to 5.54; P=0.0781). The same was true for the frequency of the mutant allele (allele A) in groups 1 and 2 (9 vs. 10%, respectively), with no significant differences (OR, 0.89; 95% CI, 0.34 to 2.29; P=0.80). However, the frequency of the heterozygous genotype (TC) of the TLR-5 gene polymorphism (rs2072493) remained similar in group 1 and group 2 (both 20 (40%)) with no statistically significant differences (OR, 1.00; 95% CI, 0.44 to 2.22; P>0.999). The frequency of the mutant allele (allele C) appeared in both groups (group 1, 30%; group 2, 20%), exhibiting no statistically significant differences (OR, 1.71; CI, 0.89 to 3.28; P=0.1043). All these values were at the allelic level. A positive correlation was observed for rs5744168 in groups 1 and 2 with positively correlated, whereas a negative correlation was observed for rs2072493 in groups 1 and 2. On the whole, the present study demonstrates that is no statistically significant association between toxoplasmosis and mutant alleles (alleles A and C) in patients undergoing hemodialysis.

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*Key words: Toxoplasma gondii*, patients undergoing hemodialysis, Toll-like receptor-5, immunoglobulin M, PCR

## Introduction

The human body maintains homeostasis through the filtration of blood via the kidneys. Any dysfunction in kidney function poses a substantial threat to the life of an individual, potentially leading to renal failure (1). Over the past two decades, there has been a notable increase in patients with renal failure requiring hemodialysis (2). These patients are inherently immunocompromised due to dysfunctions in their immune responses, rendering them more susceptible to opportunistic infections, such as the *Toxoplasma gondii (T. gondii)* protozoal parasite (3).

*T. gondii,* a protozoal parasite discovered in 1908, exhibits both sexual and asexual life cycles and has a broad host range that includes humans, pets and wildlife. During the emergence of AIDS in the 1980s, this parasite emerged as an opportunistic pathogen, presenting a life-threatening risk to immunocompromised individuals (4-6). Infection with *T. gondii* activates the immune response of the host, which often fails to control the infection. Host immune cells recognize *T. gondii*-derived ligands, initiating acquired immunity and ultimately curbing the proliferation of the parasite *in vivo*. Innate immunity plays a pivotal role in the immediate defense against pathogens, with the Toll-like receptor (TLR) family serving as a vital component in detecting pathogen-associated molecular patterns via its extracellular domain and subsequently triggering inflammatory signaling pathways through its intracellular domains (7).

Of particular interest, TLR-5 is considered to mediate the recognition of *T. gondii* profilin. The ectodomain of human TLR-5 harbors binding sites shared between flagellin and

profiling (8). The TLR-5 gene is encoded by six exons and is located on the long arm of human chromosome 1 (hCh1q). Notably, there are currently nine reported polymorphisms in the promoter and coding regions of the gene (9). Functional TLR gene polymorphisms, such as rs2072493 and rs5744168, are found within the exon regions (10) and have significant immunological implications (11).

The present study aimed to explore the significance of TLR-5 gene polymorphisms in individuals undergoing hemodialysis and who are afflicted with toxoplasmosis. The present study aimed to investigate the association between TLR-5 gene polymorphisms that render individuals more susceptible to toxoplasmosis in a sample of patients undergoing hemodialysis. Quantitative-polymerase chain reaction (qPCR) techniques were employed to discern these genetic associations.

## Patients and methods

*Recruitment of participants*. All study participants were recruited from the Department of Hemodialysis at Balad General Hospital, Salah Al-Din, Iraq. Data collection was performed between January, 2022 and June, 2022. Ethics approval for the study was granted by the Institutional Review Board of the College of Medicine, AL-Nahrain University (Approval no. 20221152, Ref: IRB/221 on November 29, 2022). All patients provided written informed consent to participate in the study.

*Study design*. The present case-control study included a total of 100 participants undergoing hemodialysis. Among the participants 50 patients were classified as seropositive for *T. gondii*. [immunoglobulin (Ig)M- and IgG]-positive, group 1], while the remaining 50 patients served as the control group and were seronegative for *T. gondii* (IgM- and IgG-negative, group 2).

*Blood sample collection*. A total of 5 ml whole venous blood was collected from each participant at the laboratory facility of the hospital. Patient demographic information was recorded. Of the collected blood, 2 ml were transferred to ethylenediaminetetraacetic acid (EDTA) tubes and stored at -20˚C until further processing. The remaining 3 ml were preserved in plain gel tubes for serum preparation.

*Serological testing*. For the detection of human *T. gondii*, the blood samples were tested for IgM and IgG antibodies using the enzyme-linked immunosorbent assay (ELISA) method, following the manufacturer's instructions (cat. no. ABIN367461, Biocompare).

*Genetic analysis*. The quick protocol SYNCTM DNA extraction kit (Geneaid) was applied to obtain highly pure genomic DNA using venous ethylene diamine tetra-acetic acid anticoagulated blood samples. The absolute quantification real-time PCR system and the commercially available TaqMan SNP Genotyping Assays (Applied Biosystems; Thermo Fisher Scientific, Inc.) were used for the genotype analysis. Real-time amplification was performed under the following conditions: Denaturation at 95˚C for 10 min, followed by 40 cycles of denaturation at 95˚C for 15 sec and annealing/extension at 60˚C for 60 sec. Allelic discrimination was performed using SDS software (v.2.3).

The selected single nucleotide polymorphisms (SNPs) in the TLR-5 gene (rs2072493 and rs5744168) were identified using qPCR with specific primers. The primers used for TLR-5 SNPs detection in present study are listed in Table I, that amplify the SNP region. These primers are able to amplify wide spectrum of DNA of TLR-5 genotypes that extracted from blood. The pair of primers for SNP (rs2072493) flanks have a sequence of approximately 140 bp, while the pair of primers for SNP (rs5744168) flanks have a sequence of approximately 187 bp. The primer sequences were designed based on the SNP site of the TLR-5 gene, as sourced from the National Center for Biotechnology Information (NCBI) and designed using the Primer3 program. The sequences of the primers used are listed in Tables I and II (12). Gene expression was analyzed using the Livak method (13). The comparative CT ($\Delta\Delta$Cq) approach was utilized to assess the relative expression of two genes.

*Statistical analysis*. Statistical analysis was conducted using GraphPad Prism version 7. To compare observed parameters and SNP numbers between subgroups, one-way ANOVA analysis with Tukey's post hoc test was employed. Descriptive statistics are presented as the mean ± standard error, and Chi-squared tests were used to analyze as nominal variables. Frequencies and percentages (%) were recorded and compared. Odds ratios (OR), 95% confidence intervals (CI) and P-values were calculated using MedCalc to demonstrate the nominal regression results. A value of $P<0.05$ was considered to indicate a statistically significant difference. Pearson's correlation analysis was used to determine the correlation of the polymorphisms between the groups. Correlation coefficients were computed to determine the correlations between markers using MegaStat (version v 10.12) for Excel 2010.

## Results

*Patient demographics*. The mean age of the patients in group 1 was 43.42 years, while that of the subjects in group 2 was 42.50 years, with a standard deviation (SD) of 12.85. The ages of the participants ranged from 19 to 68 years. The sex of the study groups was as follows: Males, n=25 (50%); and females, n=25 (50%).The number of patients with other diseases was 39 (78%) in group 1 and 31 (62%) in group 2. Moreover, the number of patients with no other diseases was 11 (22%) in group 1 and 19 (38%) in group 2. A large percentage of patients did not have pets: 40 (80%) patients in group 1 and 42 (84%) patients in group 2. Consequently, the remaining patients had pets: 10 (20%) in group 1 and 8 (16%) in group 2. The results also revealed that the disease duration of the patients ranged from 1 month to 1 year: In group 1, there were 29 patients (42%), while group 2 had 33 patients (66%). On the other hand, the duration from 1 to 3 years was observed in 21 patients (42%) in group 1 and 29 patients (38%) in group 2.

As regards the type of residence, 17 (34%) patients in group 1 had an urban residence and 33 (66% had a rural

Table I. Primers for SNP2072493 (annealing temperature, 60˚C).

| Primer name | Sequence |
|---|---|
| rs2072493-F | 5'-GAGGCCCAGCTATAGTGACATTG-3' |
| rs2072493-R | 5'-CCAGCTCCTAGCTCCTAATCCT-3' |
| rs2072493-P/C | Hex-5'-TGTGATTAAGCCAACTGATA-3 |
| rs2072493-P/T | Fam-5'-TGTGATTAAGCCAATTGATAA-3' |

F, forward; R, reverse; P, probe; C and T, nucleotides.

Table II. Primers for SNP5744168 (annealing temperature, 60˚C).

| Primer name | Sequence |
|---|---|
| rs5744168-F | 5'-TTATTGCCACTCAAGAAGATATCGG-3 |
| rs5744168-R | 5'-ACAGTTCGAATTTCTATGGACTACC-3' |
| rs5744168-P/A | Hex-5'-TAAGAGCATTGTCTCAGAGAT-3' |
| rs5744168-P/G | Fam-5'-AGCATTGTCTCGGAGATC-3' |

F, forward; R, reverse; P, probe; C and T, nucleotides.

Table III. Demographic data of the study groups.

| Parameter | Group 1 | Group 2 |
|---|---|---|
| Age, years; median (5-95 percentile) | 43.42 (19 to 68) | 42.50 (20-65) |
| Sex | | |
| Female | 25 (50%) | 25 (50%) |
| Male | 25 (50%) | 25 (50%) |
| Other diseases | | |
| Yes | 39 (78%) | 31 (62%) |
| No | 11 (22%) | 19 (38%) |
| Pet ownership | | |
| Yes | 10 (20%) | 8 (12%) |
| No | 40 (80%) | 42 (84%) |
| Residence | | |
| Urban | 17 (34%) | 31 (62%) |
| Rural | 33 (66%) | 19 (38%) |
| Hemolysis duration | | |
| From 1 month to 1 year | 29 (48%) | 33 (66%) |
| From 1 to 3 years | 21 (42%) | 29 (48%) |
| Total | 50 | 50 |

residence. In group 2, 31 (62%) of the participants had an urban residence and 19 (38%) had a rural residence (Table III).

*TLR-5 gene polymorphism rs5744168*. In the case of the TLR-5 gene polymorphism rs5744168, only two genotypes (GA and GG) were observed in the study groups, as illustrated in Fig. 1. The homozygous genotype GG had a frequency of 41 (82%) in group 1, and this frequency was not statistically significant (P=0.7989) when compared to group 2, which had 40 (80%) patients with this genotype. Conversely, the heterozygous genotype GA was more prevalent in group 1, accounting for 18 (36%) individuals, in contrast to group 2 with 10 (20%) individuals. This difference was not statistically significant (P=0.0781). Further analysis revealed an OR of 2.25 with a 95% CI ranging from 0.91 to 5.54 (Table IV). At the allelic level, the frequency of the normal allele (allele G) in both groups was comparable, with group 1 and group 2 displaying frequencies of 91 and 90%, respectively, with no significant difference (P=0.80). Similarly, the frequency of the mutant allele (allele A) in groups 1 and 2 was 9 and 10%, respectively, with no significant differences (P=0.80). The OR for this comparison was 0.89, with a 95% CI ranging from 0.34 to 2.29 (Table IV).

*TLR-5 gene polymorphism rs2072493*. The PCR amplification of the TLR-5 gene polymorphism rs2072493 is illustrated in Fig. 2. This polymorphism displayed three genotypes (TT, TC and CC). In comparison to group 2, which had a homozygous genotype (TT) frequency of 25 (50%) patients, group 1 had a homozygous genotype (TT) with a frequency of 30 (60%) patients. This difference was not statistically significant (P=0.3157), with an OR of 0.66 and a 95% CI ranging from 0.30 to 1.47. The frequency of the heterozygous genotype (TC) was 20 (40%) patients in group 1, the same as group 2, with no significant differences (P=1.000). Additionally, the homozygous genotype CC had a frequency of 5 (10%) patients in group 1, whereas it was absent in group 2 (0%). This discrepancy was significant (P=0.09), with an OR of 12.20 and a 95% CI ranging from 0.65 to 226.97 (Table V).

At the allelic level, the frequency of the wild-type allele (allele T) reached 70% in group 1 and 80% in group 2, with no significant difference (P=0.1043) and an OR of 0.58 and a 95% CI ranging from 0.30 to 1.11. Conversely, the mutant allele (allele C) had a frequency of 30% in group 1, as opposed to 20% in group 2, indicating no significant difference (P=0.1043), with an OR of 1.7143 and a 95% CI ranging from 0.89 to 3.28 (Table V).

*Correlation of rs5744168 between the two groups*. As shown in Table VI, a positive correlation was found for rs5744168 between the groups, with a significant difference (P<0.001) between the groups.

*Correlation of rs2072493 between the two groups*. As shown in Table VII, a negative correlation was found for rs2072493 between the groups, with no significant difference (P<0.660) between the groups.

## Discussion

*Demographic data*. The present case control study revealed no differences in the mean age between the patients and controls as the age of the control group was selected according to patients group also the number of participants.

Table IV. TLR-5 gene polymorphism rs5744168 genotypes and alleles in groups 1 and 2.

| rs5744168 | Group 1 (n=50) | Group 2 (n=50) | OR (95% CI) | P-value |
|---|---|---|---|---|
| Genotypes | | | | |
| GG | 41 (82%) | 40 (80%) | 1.13 (0.41 to 3.09) | 0.7989 |
| GA | 18 (36%) | 10 (20%) | 2.25 (0.91 to 5.54) | 0.0781 |
| AA | 0 (0.0%) | 0 (0.0%) | | |
| Alleles | | | | |
| G | 91 (91%) | 90 (90%) | 1.12 (0.43 to 2.89) | 0.80 |
| A | 9 (9%) | 10 (20%) | 0.89 (0.34 to 2.29) | 0.80 |

TLR, Toll-like receptor; OR, odds ratio; CI, confidence interval.

Table V. TLR-5 gene polymorphism rs2072493 genotypes and alleles in groups 1 and 2.

| rs2072493 | Group 1 (n=50) | Group 2 (n=50) | OR (95% CI) | P-value |
|---|---|---|---|---|
| Genotypes | | | | |
| TT | 25 (50%) | 30 (60%) | 0.66 (0.30 to 1.47) | 0.3157 |
| TC | 20 (40%) | 20 (40%) | 1.00 (0.44 to 2.22) | 1.000 |
| CC | 5 (10%) | 0 (0.0%) | 12.2 (0.65 to 226.97) | 0.0934 |
| Alleles | | | | |
| T | 70 (70%) | 80 (80%) | 0.58 (0.30 to 1.11) | 0.1043 |
| C | 30 (30%) | 20 (20%) | 1.71 (0.89 to 3.28) | 0.1043 |

Correlation was not significant (P=1). TLR, Toll-like receptor; OR, odds ratio; CI, confidence interval.

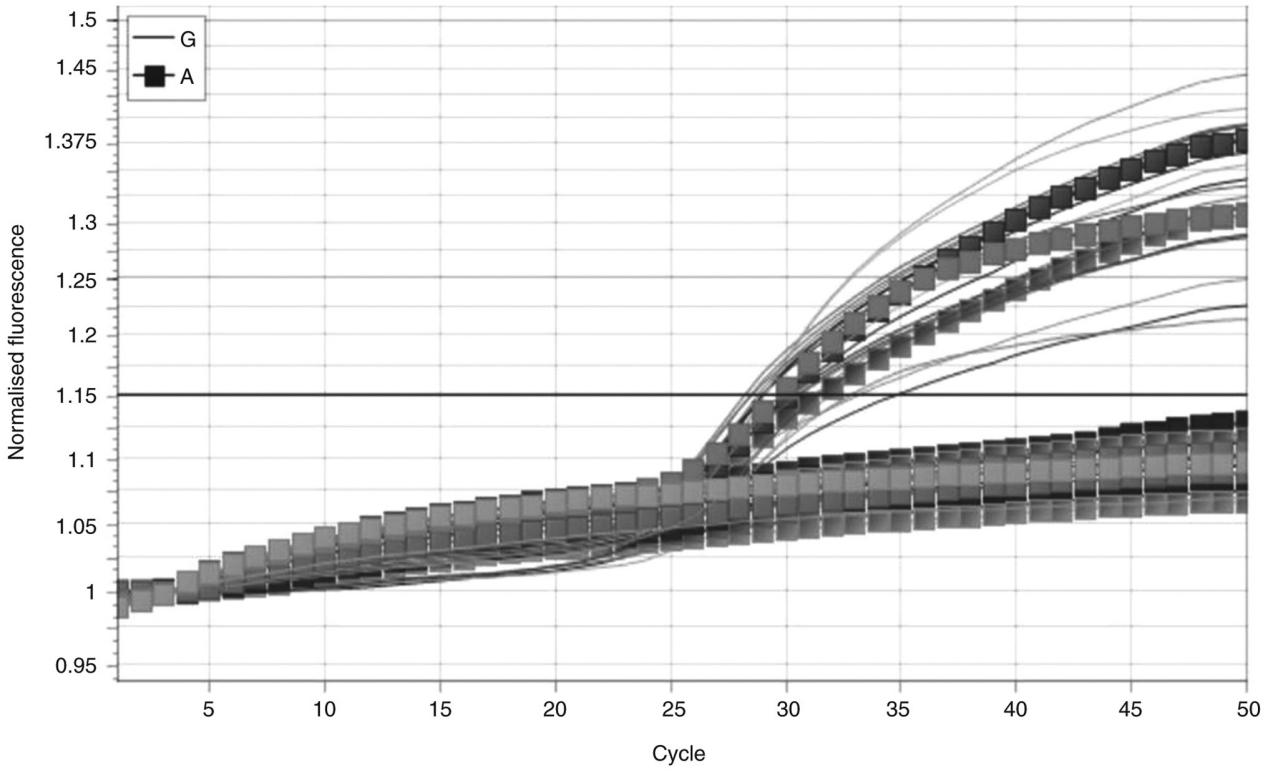


Figure 1. Quantitative PCR amplification of the rs5744168 polymorphism of the TLR-5 gene. In the case of the TLR-5 rs5744168 gene polymorphism, only two genotypes (GA and GG) were observed in the study groups. The homozygous genotype GG had a frequency of 41 (82%) in group 1, and this frequency was not statistically significant (P=0.7989) when compared to the second group, which had 40 (80%) with this genotype. Conversely, the heterozygous genotype GA was more prevalent in the first group, accounting for 18 (36%) individuals, in contrast to the second group with 10 (20%) individuals (Table (IV).

Table VI. Correlations of rs5744168 between groups 1 and 2.

| | Group 1 (n=50) | | Group 2 (n=50) | |
|---|---|---|---|---|
| rs5744168 | R value (Pearson's correlation) | P-value | R value (Pearson's correlation) | P-value |
| Group 1 | 1 | | 0.677[a] | 1[b] |
| Group 2 | 0.677[a] | 0.001 | 1 | |

[a]Correlation was significant (P=0.01); [b]correlation was not significant (P=1).

Table VII. Correlations of rs2072493 between groups 1 and 2.

| | Group 1 (n=50) | | Group 2 (n=50) | |
|---|---|---|---|---|
| rs207249 | R value (Pearson's correlation) | P-value | R value (Pearson's correlation) | P-value |
| Group 1 | 1 | | -0.064 | 0.66 |
| Group 2 | -0.064 | 0.66 | 1 | |

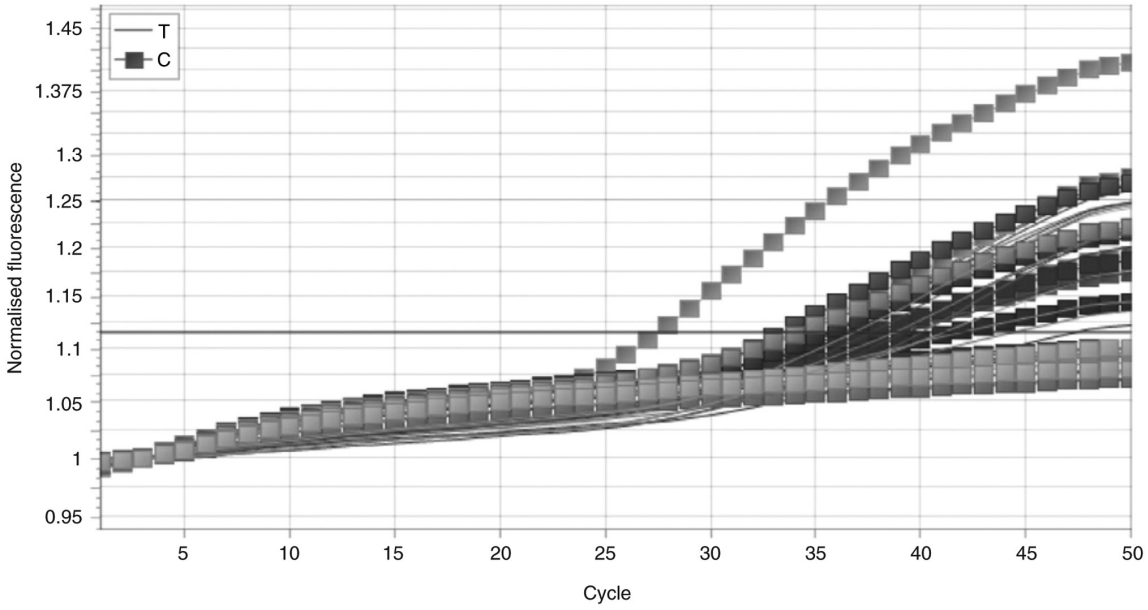


Figure 2. Quantitative PCR amplification of the rs2072493 polymorphism of the TLR-5 gene. The TLR-5 rs2072493gene polymorphism displayed three genotypes (TT, TC and CC). In comparison to Group 2, Group 1 had a homozygous genotype (TT) frequency of 25 (50%), Group 2 had a homozygous genotype (TT) with a frequency of 30 (60%) (Table V).

In group 1 the number of rural residents was higher than that of urban residents as they are more likely to have a lower quality of life and a substantial financial burden (14). The demographic data demonstrated that the patients had other diseases and that many of them did not have pets. However, the seropositivity in the patients may have been due to the long duration of medication and other sources of infection, rather than having pets, as the majority of patients did not have pets.

*TLR-5 gene polymorphisms*. SNPs are prevalent in the human genome, and they exert a significant influence on innate immune responses by altering the amplitude and quality of intracellular signaling cascades. These genetic variations can affect susceptibility to infections and disease outcomes, and are crucial in understanding the etiology of human diseases, clinical characteristics, drug development and treatment strategies (15).

In the present study, the TLR-5 gene polymorphism rs5744168 was not significantly associated with susceptibility to toxoplasmosis in group 1 (P>0.05). While there is a paucity of studies directly comparable to the findings presented herein, SNP rs5744168 has been linked to other infections, such as *Helicobacter pylori*, as well as certain diseases such as breast cancer, Crohn's disease and systemic lupus erythematosus (16). The results of the present study align with existing research,

which generally suggests that the gene polymorphism rs5744168, characterized by a stop codon and low mutant allele frequencies, can reduce the interaction between TLR-5 and pathogen-associated molecular patterns of *T. gondii*, thereby limiting the immune response. This occurs due to the truncation of TLR5 transmembrane signaling, inhibiting TLR5 homodimer assembly and localization, which, in turn, restricts the immune response (17).

In the present study, as regards susceptibility to toxoplasmosis, the TLR-5 gene polymorphism rs2072493 (P>0.05) did not exhibit any significant associations. However, to the best of our knowledge, there are a limited number of similar studies available for a direct comparison. Globally, studies on TLR-5 gene polymorphism and toxoplasmosis are relatively rare. The allele frequency polymorphism for rs2072493 the present study (30% in group 1) is in contrast to allele frequency percentages in various populations, such as Caucasians (15%), Chinese (26%) and Northern Indians (12%) (9,18,19). These discrepancies may be attributed to differences in the study population and genetic heterogeneity across diverse ethnicities. Moreover, SNP rs2072493 has been linked to various diseases, including colorectal cancer, Graves' disease and hepatitis B virus infections (20-22).

*Correlations of rs5744168 and rs2072493 between groups 1 and 2. T. gondii* has been implicated in glomerular lesions and urinary abnormalities, often leading to renal failure, as indicated by elevated creatinine levels in urine (23). The present study confirmed positive correlations of rs5744168 between groups 1 and 2. TLR-5 ligation triggers the production of pro-inflammatory cytokines, such as TNF-α and IL-6, through the NF-κB pathway (24), which is consistent with elevated pro-inflammatory responses observed in subjects undergoing hemodialysis (25).

The transmission of the parasite relies on its ability to establish long-lasting chronic infections. High levels of *T. gondii*-specific IgG and cytokines in sera play a pivotal role in limiting parasite growth, thereby perpetuating the immune response to *T. gondii* through chronic infections (26). The present study observed negative correlations of rs2072493 between groups 1 and 2. The ability of *T. gondii* to stimulate Th2-cytokines, including IL-10, may explain the significant increase in IL-10 levels. Additionally, IL-10 plays a critical role in suppressing the release of various cytokines produced by T-cells, such as IL-12, IL-6 and IFN-γ. It also inhibits natural killer cells from producing cellular cytokines (27,28). To the best of our knowledge, there are no prior studies investigating the correlations of the TLR-5 gene polymorphisms, rs5744168 and rs2072493, between patients infected with toxoplasma undergoing hemodialysis.

In conclusion, the present study examined the TLR-5 gene polymorphisms (rs5744168 and rs2072493) and their association with susceptibility to toxoplasmosis in patients undergoing hemodialysis and yielded several key findings. Firstly, no statistically significant differences were observed in the susceptibility to toxoplasmosis between individuals with different TLR-5 gene polymorphisms.

Furthermore, the present study revealed a notable positive correlation between patients undergoing hemodialysis carrying the rs5744168 polymorphism, both among seropositive and seronegative individuals. By contrast, those with the rs2072493 polymorphism exhibited a negative correlation with susceptibility to toxoplasmosis. These notable correlations point to the intricate association between TLR-5 gene polymorphisms and the immune response to toxoplasmosis in patients undergoing hemodialysis. Further research in this area may shed light on the underlying mechanisms.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Authors' contributions**

HDAM was involved in the conceptualization of the study, as well as in the study methodology, formal analysis, in the writing of the original draft of the manuscript, and in the writing, reviewing and editing of the manuscript. KARM, ANAB and NHM made substantial contributions to the conception and design of the study, were responsible for the writing the of the manuscript, and were involved in data curation, investigation and data analysis. KARM, ANAB and NHM confirm the authenticity of all the raw data All authors have read and approved the final manuscript.

**Ethics approval and consent to participate**

Ethics approval for the study was granted by the Institutional Review Board of the College of Medicine, AL-Nahrain University (Approval no. 20221152, Ref: IRB/221 on November 29, 2022). All patients provided written informed consent to participate in the study.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**References**

1. Elmore SA, Jones JL, Conrad PA, Patton S, Lindsay DS and Dubey JP: Toxoplasma gondii: Epidemiology, feline clinical aspects, and prevention. Trends Parasitol 26: 190-196, 2010.
2. Mcleod R, Van Tubbergen C, Montoya J and Petersen E: Human toxoplasma infection. In: Toxoplasma Gondii: The Model Apicomplexan-Perspectives and Methods. Elsevier, Amsterdam, 2013.
3. Sasai M and Yamamoto M: Innate, adaptive, and cell-autonomous immunity against Toxoplasma gondii infection. Exp Mol Med 51: 1-10, 2019.

4. Kato S, Chmielewski M, Honda H, Pecoits-Filho R, Matsuo S, Yuzawa Y, Tranaeus A, Stenvinkel P and Lindholm B: Aspects of immune dysfunction in end-stage renal disease. Clin J Am Soc Nephrol 3: 1526-1533, 2008.
5. Omrani VF, Fallahi S, Rostami A, Siyadatpanah A, Barzgarpour G, Mehravar S, Memari F, Hajialiani F and Joneidi Z: Prevalence of intestinal parasite infections and associated clinical symptoms among patients with end-stage renal disease undergoing hemodialysis. Infection 43: 537-544, 2015.
6. Tamura MK: Incidence, management, and outcomes of end-stage renal disease in the elderly. Curr Opin Nephrol Hypertens 18: 252-257, 2009.
7. West AP, Koblansky AA and Ghosh S: Recognition and signaling by toll-like receptors. Annu Rev Cell Dev Biol 22: 409-437, 2006.
8. Gonzalez RMS, Shehata H, O'connell MJ, Yang Y, Moreno-Fernandez ME, Chougnet CA and Aliberti J: Toxoplasma gondii-derived profilin triggers human toll-like receptor 5-dependent cytokine production. J Innate Immun 6: 685-694, 2014.
9. Zeng HM, Pan KF, Zhang Y, Zhang L, Ma JL, Zhou T, Su HJ, Li WQ, LI JY, Gerhard M, *et al*: Genetic variants of toll-like receptor 2 and 5, helicobacter pylori infection, and risk of gastric cancer and its precursors in a Chinese population. Cancer Epidemiol Biomarkers Prev 20: 2594-2602, 2011.
10. Seok H, Lee BC, Kim YO and Chung JH: Association between Exonic SNPs of TLR5 and Benign Prostate Hyperplasia in Korean Population. Korean J Str Res 21: 331-337, 2013.
11. Rutkowski MR, Stephen TL, Svoronos N, Allegrezza MJ, Tesone AJ, Perales-Puchalt A, Brencicova E, Escovar-Fadul X, Nguyen JM, Cadungog MG, *et al*: Microbially driven TLR5-dependent signaling governs distal malignant progression through tumor-promoting inflammation. Cancer Cell 27: 27-40, 2015.
12. Ghasemali S, Nejati-Koshki K, Tafsiri E, Rahmati-Yamchi M, Akbarzadeh A, Alizadeh E, Abbasi M, Barkhordari A, Tozihi M, Kordi S and Zarghami N: Inhibitory effects of beta-cyclodextrin-helenalin complexes on H-TERT gene expression in the T47D breast cancer cell line-results of real time quantitative PCR. Asian Pac J Cancer Prev 14: 6949-6953, 2013.
13. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
14. Jha V, Al-Ghamdi SMG, Li G, Wu MS, Stafylas P, Retat L, Card-Gowers J, Barone S, Cabrera C and Sanchez JJ: Global economic burden associated with chronic kidney disease: A pragmatic review of medical costs for the Inside CKD research programme. Adv Ther 40: 4405-4420, 2023.
15. Robert F and Pelletier J: Exploring the impact of single-nucleotide polymorphisms on translation. Front Genet 9: 507, 2018.
16. Shuang C, Weiguang Y, Zhenkun F, Yike H, Jiankun Y, Jing X, Xinghan L, Yue L and Dalin L: Toll-like receptor 5 gene polymorphism is associated with breast cancer susceptibility. Oncotarget 8: 88622-88629, 2017.
17. Chaichana P, Chantratita N, Brod F, Koosakulnirand S, Jenjaroen K, Chumseng S, Sumonwiriya M, Burtnick MN, Brett PJ, Teparrukkul P, *et al*: A nonsense mutation in TLR5 is associated with survival and reduced IL-10 and TNF-α levels in human melioidosis. PLoS Negl Trop Dis 11: e0005587, 2017.
18. Hawn TR, Verbon A, Lettinga KD, Zhao LP, LI SS, Laws RJ, Skerrett SJ, Beutler B, Schroeder L, Nachman A, *et al*: A common dominant TLR5 stop codon polymorphism abolishes flagellin signaling and is associated with susceptibility to legionnaires' disease. J Exp Med 198: 1563-1572, 2003.
19. Meena NK, Ahuja V, Meena K and Paul J: Association of TLR5 gene polymorphisms in ulcerative colitis patients of North India and their role in cytokine homeostasis. PLoS One 10: e0120697, 2015.
20. Klimosch SN, Foersti A, Eckert J, Knežević J, Bevier M, von Schoenfels W, Heits N, Walter J, Hinz S, Lascorz J, *et al*: Functional TLR5 genetic variants affect human colorectal cancer SurvivalTLR variants affect colorectal cancer survival. Cancer Res 73: 7232-7242, 2013.
21. Xiao W, Liu Z, Lin J, Xiong C, Li J, Wu K, Ma Y, Gong Y and Liu Z: Association of TLR4 and TLR5 gene polymorphisms with Graves' disease in Chinese Cantonese population. Hum Immunol 75: 609-613, 2014.
22. Cao L, Zhang T, Zhu J, Li A, Zheng K, Zhang N, Su B, Xia W, Wu H, Li N and He Q: Polymorphism of TLR 5 rs5744174 is associated with disease progression in Chinese patients with chronic HBV infection. APMIS 125: 708-716, 2017.
23. Gharadaghi Y, Shojaee S, Khaki A, Fathiazad F, Khaki AA, Ghdamkheir E and Rouhaninia M: Antiprotozoal effect of Allium cepa on acute renal failure caused by Toxoplasma gondii. Afr J Pharm Pharmacol 6: 771-777, 2012.
24. Dabagh-Gorjani F, Anvari F, Zolghadri J, Kamali-Sarvestani E and Gharesi-Fard B: Differences in the expression of TLRs and inflammatory cytokines in pre-eclamptic compared with healthy pregnant women. Iran J Immunol 11: 233-245, 2014.
25. Tbahriti HF, Meknassi D, Moussaoui R, Messaoudi A, Zemour L, Kaddous A, Bouchenak M and Mekki K: Inflammatory status in chronic renal failure: The role of homocysteinemia and pro-inflammatory cytokines. World J Nephrol 2: 31-37, 2013.
26. Zhao XY and Ewald SE: The molecular biology and immune control of chronic Toxoplasma gondii infection. J Clin Invest 130: 3370-3380, 2020.
27. Meira CS, Pereira-Chioccola VL, Vidal JE, de Mattos CCB, Motoie G, Costa-Silva TA, Gava R, Frederico FB, de Mattos LC and Groups T: Cerebral and ocular toxoplasmosis related with IFN-γ, TNF-α, and IL-10 levels. Front Microbiol 5: 492, 2014.
28. Salem MS, Shaker MJ and Mohammed NK: Impact of toxoplasmosis in im-mune respons in hemodialysis patients. Diyala J Medicine 22: 1-11, 20222.