Immunological and Molecular Evaluation of *Toxoplasma Gondii* Infection and Determined Impact on Renal and Liver Function Tests in Hemodialysis Patients in Kirkuk City, Iraq

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Abstract

Background: *Toxoplasma gondii* is the etiological agent of toxoplasmosis. Patients with chronic renal failure are at elevated risk for parasitic infections, particularly *Toxoplasma gondii*, due to immune system dysfunction, which can result in severe symptoms and occasionally lead to mortality. This study aimed to identify *Toxoplasma gondii* infection both serologically and molecularly in haemodialysis patients, as well as to assess the impact of toxoplasmosis on renal and liver function tests in these individuals. **Materials and Methods:** A total of 180 individuals participated in the present study, comprising 130 haemodialysis patients and 50 healthy persons. Serological detection of toxoplasmosis was performed on all 180 samples using the ELISA technique, and all positive ELISA results for *Toxoplasma gondii* antibodies were subsequently subjected to Real-Time PCR detection. Additionally, blood urea, serum creatinine, serum alanine aminotransferase, serum aspartate aminotransferase, and serum alkaline phosphatase levels were quantified using NeoChem 20. **Results:** Seropositivity was detected in 39.2% of haemodialysis patients and 28% of the healthy cohort. The prevalence rates of anti-toxoplasmosis IgM and IgG antibodies, as well as the *Toxoplasma gondii* B1 gene in haemodialysis patients, were 4.6%, 34.6%, and 27.4%, respectively, while the corresponding values in the healthy group were 0%, 28%, and 0%, respectively. The infection rate in male haemodialysis patients was greater than in females, though not significantly. Moreover, the concentrations of blood urea and serum creatinine were elevated in haemodialysis patients infected with toxoplasmosis compared to haemodialysis patients without toxoplasmosis and the healthy group. Conversely, the concentrations of serum Alanine aminotransferase (S.ALT), serum aspartate aminotransferase (S.AST), and serum alkaline phosphatase (S.ALP) were significantly elevated in haemodialysis patients infected with toxoplasmosis compared to other cohorts in this investigation. **Conclusions:** The implementation of serological and molecular screening tests for *Toxoplasma gondii* in haemodialysis patients, who are frequently exposed to dialysis, is a critical measure to prevent the spread of infection among this population. Infection with *Toxoplasma gondii* adversely affects kidney and liver biomarkers in haemodialysis patients compared to a healthy cohort.

Keyword: *Toxoplasma gondii*, Hemodialysis, ELISA, Real-Time PCR, Renal and Liver Function Tests.

Introduction

The prevalent protozoan parasite responsible for Toxoplasmosis is *Toxoplasma gondii*, which infects around 20 to 90% of the global population. The intricate life cycle of toxoplasmosis involves two hosts: felids, such as cats, which act as the definitive host, and mammals, including humans[1], which are regarded as intermediate hosts. In immunocompetent individuals, the disease may present with influenza-like symptoms or remain silent, however in immunosuppressed individuals, such as those undergoing haemodialysis, it might lead to more severe symptoms due to the reactivation of latent infection. Infection with T. gondii occurs through tachyzoites, bradyzoites, and oocysts, which are the infective stages in its life cycle. Upon entering the host body during the infective stage, T.

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gondii invades various organs, resulting in cellular damage and inflammation, particularly in the kidneys, liver, and central nervous system. The excretion of toxins and waste from the blood is the primary function of the kidneys; thus, the assessment of kidney function tests is a crucial indicator of renal efficiency in healthy individuals. The elevation of blood urea and serum creatinine levels serves as a clinical indicator for chronic renal disease. Patients with renal diseases are at elevated risk of infections from bacteria, viruses, and parasites, particularly toxoplasmosis, due to compromised immune function. Due to the challenges in demonstrating the T. gondii parasite, molecular and serological techniques serve as the definitive diagnosis for toxoplasmosis, particularly in immunocompromised patients, facilitating early detection and management of T. gondii infection.[2,3]

Material and Methods

Study Population

This study was performed at the Al-Amal Dialysis Centre in Kirkuk, Iraq, involving 130 haemodialysis patients (55 females and 75 males, aged 16 to 88 years) and a control group of 50 healthy individuals (25 females and 25 males, aged 21 to 60 years) from September 2024 to December 2024.

Sample Collection

Approximately 5 mL of venous blood was taken from haemodialysis patients and a healthy cohort using a sterile syringe, with 3 mL of the blood transferred into sterile gel tubes. Test tubes were maintained at room temperature for 20 to 30 minutes to facilitate blood coagulation, followed by centrifugation at 3000 rpm for 5 to 10 minutes. The sera were thereafter placed in Eppendorf tubes and stored in a freezer at -20°C until utilised. The remaining blood was placed in EDTA tubes and subjected to the DNA extraction method for Real-Time PCR detection.

Enzyme-linked Immunosorbent Assay (ELISA)

This test was employed to detect anti-Toxoplasmosis IgM and IgG antibodies utilising Sunlong Biotech Co., Ltd. (China) in accordance with the instructions supplied by the kit's manufacturer.

DNA extraction and Real- Time PCR

The positive samples from the ELISA test for both haemodialysis patients and the healthy group underwent DNA extraction. The FavorPrep Tissue Genomic DNA Extraction Mini Kit (Australia) was utilised for this objective. DNA extraction was performed in accordance with the methodology supplied by the kit manufacturer, and the extracted DNA was stored at -20 °C. The master mix was created, containing all needed components (GoTaq® qPCR Master Mix, Promega, USA), and real-time PCR was performed to detect the B1 gene fragment of T. gondii. The primers utilised in this work were synthesised by Macrogenes Ltd, Korea.

Table 1: The Master Mix Components for Detection T. *Gondii B1* Gene.

Materials	Volume
Master Mix	13 µl
Target DNA	5 µl
B1 gene F primer(5′GGTTCCGCCTCCTTCGTCCG′3)(this study)	1 µl
B1 gene R primer(3′CGCCAGCAGAGGGGAGCTCG′5)(this study)	1 µl
Nuclease free water	5 µl
Total volume	25µl

Table 2: The Thermocycle Conditions used for Detection *T.gondii B1* Gene.

Cycle No	Stage	Temperature	Time
1	Initial denaturation	94°C	4 mins
	Denaturation	94°C	10 secs
40	Annealing	66°C	20 secs
	Extention	72°C	50 secs

NeoChem 20

This is a fully automated biochemistry analyser featuring a highly precise optical system composed of fibre optics and high-resolution filters, designed to measure blood urea, serum creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). These measurements are conducted using the Urea Kit, Creatinine Kit, ALT Kit, AST Kit, and ALP Kit, all from BT Products, Turkey, in accordance with the protocols established by the kit manufacturer.

Ethical Approval

At the outset of the study, official ethical permission was secured from the Kirkuk Directorate of Health, Ministry of Health, Iraq. The participants were apprised of the purpose and procedure for collecting information from those attending the Al-Amal Dialysis Centre, as per document number 703, dated September 2024.

Statistical Analysis

The data were analysed with a one-way ANOVA test via

Prism software (Version 7). P-values less than 0.05 were deemed statistically significant.

Results

Among the 180 participants (130 haemodialysis patients and 50 healthy individuals), the findings indicated that seropositivity for anti-Toxoplasma antibodies (IgG and IgM) was present in 39.2% of the haemodialysis patients and 28% of the healthy group, as illustrated in Table 3. Table 4 illustrates that the prevalence rates of anti-toxoplasmosis IgM and IgG antibodies in haemodialysis patients were 4.6% and 34.6%, respectively, whereas the equivalent values in the healthy group were 0% and 28%, respectively.

Table 3: Sero-prevalence of *Toxoplasma Gondii*.

Group	Sample Number	Positivity(%)	Negativity(%)
Hemodialysis patients	130	51(39.23 %)	79(60.76 %)
Healthy Group	50	14(28 %)	36(72 %)
Total	180	65(36.11 %)	115(63.88 %)

Table 4: The Seropositivity of Anti-toxoplasma Antibodies IgG and IgM in both Hemodialysis Patients and Healthy Group using to ELISA Test.

Group	IgM Positivity(%)	IgG Positivity(%)
Hemodialysis patients	6(4.61 %)	45(34.61 %)
Healthy Group	0(0 %)	14(28 %)
Total	6(4.61 %)	59(32.77 %)

The study observed a total seropositivity of Toxoplasmosis antibodies (IgG and IgM) in haemodialysis patients at 49.01% and 50.99%, respectively, for both females and males. The results indicate no significant differences between females and males regarding infection with toxoplasmosis, as detailed in Table 5.

Table 5: The Relationship between Seroprevalence of Anti-toxoplasmosis IgG and IgM Antibodies and Sex in Hemodialysis Patients and Healthy Group using ELISA Assay .

IgG				IgM				Total IgG&Igm
Group	Sex	Positive	%	Group	Sex	Positive	%	
Hemodialysis Patients	Female (N=55)	24	53.33	Hemodialysis Patients	Female (N=55)	1	16.66	25 (49.01%)
	Male (N=75)	21	46.66		Male (N=75)	5	83.33	26 (50.99%)
	Total (N=130)	45	100		Total (N=130)	6	100	51 (100%)
Healthy Group	Female (N=25)	9	64.28		Female (N=25)	0	0	9 (64.28%)
	Male (N=25)	5	35.71	Healthy Group	Male (N=25)	0	0	5 (35.71%)
	Total (N=50)	14	100		Total (N=50)	0	0	14 (100%)

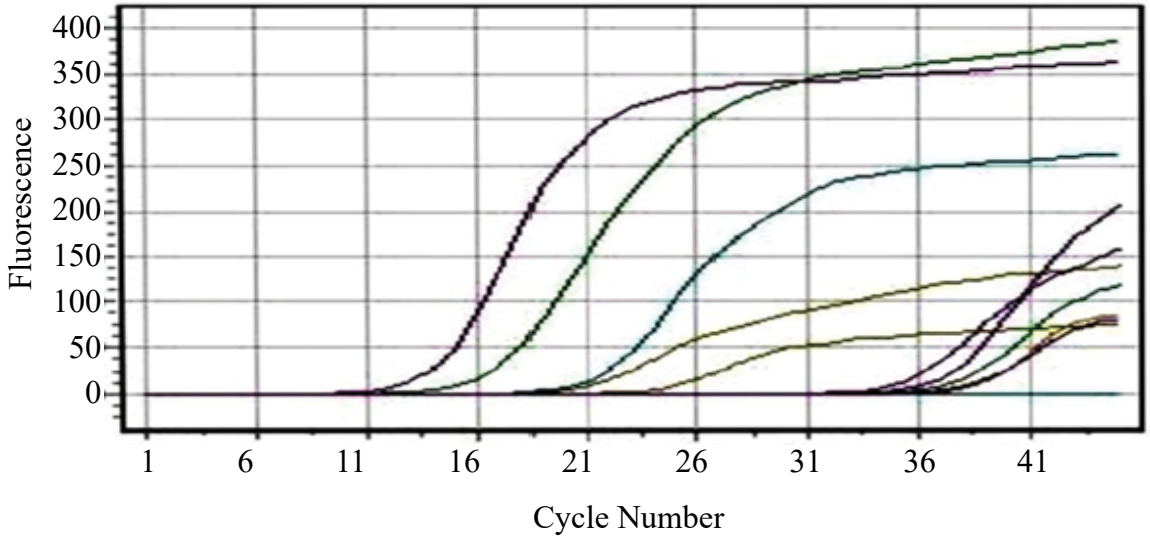

Figure 1: The Real-time PCR Amplification Curve of FAM Channel for *Toxoplasma Gondii* B1 Gene in Hemodialysis Patients.

According to the Real-Time PCR results, out of a total of 65 positive ELISA tests for Toxoplasmosis antibodies (in haemodialysis patients and a healthy group), only 27.45% of haemodialysis patients tested positive for the T. gondii B1 gene, whereas the healthy group showed no detection of the B1 gene of T. gondii, as illustrated in Figure 1.

Table 6 illustrates the mean blood urea level in haemodialysis patients with positive toxoplasmosis, recorded at 121.7±30 mg/dl. In contrast, the mean blood urea levels for haemodialysis patients with negative toxoplasmosis and the healthy group were 111.4±29 mg/dl and 19.46±5.66 mg/dl, respectively. The results indicated a substantial influence of toxoplasmosis on blood urea levels in haemodialysis patients with positive toxoplasmosis compared to other groups.

In the present study, the mean serum creatinine level in haemodialysis patients with positive toxoplasmosis was 11.27±17 mg/dl, whereas the mean serum creatinine levels in both haemodialysis patients with negative toxoplasmosis and the healthy group were 8.13±2 mg/dl and 0.98±0.13 mg/dl, respectively, as illustrated in Table 6. These findings demonstrated the detrimental influence of toxoplasmosis on elevated creatinine levels in haemodialysis patients.

Table 6: The Mean Levels of Blood urea and Serum Creatinine in Hemodialysis Patients and Healthy Group According to Presence of Toxoplasmosis Antibodies.

Parameters	Hemodialysis with Toxoplasmosis +ve	Hemodialysis with Toxoplasmosis –ve	Healthy Group
	Mean±SD	Mean±SD	Mean±SD
Blood Urea	121.7±30.37 * * *	111.4±29.22	19.46±5.661
Serum Creatinine	11.27±17.44 * * *	8.13±2.40	0.98±0.13

* * * Indicate significant, SD: Standard deviation, +ve: Positive, -ve: Negative, the normal value of blood urea(10-50)mg/dl. The normal value of serum creatinine(0.9-1.3) mg/dl in men and(0.6-1.1) mg/dl in women.

The table (7) indicates that the mean serum ALT level in haemodialysis patients with positive toxoplasmosis was 11.05±6.39 u/l, whereas the mean serum ALT levels in haemodialysis patients with negative toxoplasmosis and the healthy group were 10.04±6.28 u/l and 20.82±5.61 u/l, respectively. This study indicates that toxoplasmosis infection results in a significant increase in mean serum ALT levels ($P < 0.05$).

The mean blood AST level in haemodialysis patients with positive toxoplasmosis was elevated at 20.15±23.95 u/l. In contrast, the average blood AST level in both haemodialysis patients negative for toxoplasmosis and the healthy group was 17.99±10.40 u/l and 19.35±4.334 u/l, respectively. The results clearly indicates a positive link between AST levels and toxoplasmosis infection in affected patients ($P < 0.05$), as illustrated in Table 7.

One finding that emerges from this study is that the mean serum ALP level in haemodialysis patients positive for Toxoplasmosis was 288.4±237.5 u/l, whereas the mean serum ALP levels in both haemodialysis patients negative for Toxoplasmosis and the healthy group were 258.6±203.0 u/l and 131.2±22.46 u/l, respectively. The data clearly indicate a relationship between toxoplasmosis infection and elevated serum levels of ALP in haemodialysis patients positive for toxoplasmosis, as detailed in Table 7.

Table 7: The Mean Levels of Serum ALT, Serum AST and Serum ALP in Hemodialysis Patients and Healthy Group according to Presence of Toxoplasmosis Antibodies.

Parameters	Hemodialysis with Toxoplasmosis +ve	Hemodialysis with Toxoplasmosis –ve	Healthy Group
	Mean±SD	Mean±SD	Mean±SD
S. ALT	11.05±6.396 ***	10.04±6.287	20.82±5.616
S. AST	20.15±23.95 ***	17.99±10.40	19.35±4.334
S. ALP	288.4±237.5 ***	258.6±203.0	131.2±22.46

***: Indicate significant, S. ALT: serum alanine aminotransferase, S.AST: serum aspartate aminotransferase , S. ALP : serum alkaline phosphatase, SD : Standard deviation , +ve : Positive , -ve : Negative , the normal value of alanine aminotransferase(<40 U/L) , the normal value of aspartate aminotransferase(< 40 U/L), the normal value of alkaline phosphatase(98-279 U/L).

Discussion

Toxoplasma gondii is the etiological agent of Toxoplasmosis, impacting both humans and animals, and is believed to induce opportunistic infections that lead to mortality in 40% of haemodialysis patients. Consequently, the screening and detection of toxoplasmosis in these patients is seen as an early intervention for their treatment.[4,5]

Limited research has been conducted in Iraq regarding the seroprevalence of toxoplasmosis in individuals with chronic renal failure. The present investigation revealed a seroprevalence of toxoplasmosis in 39.23% of haemodialysis patients and 28% of the healthy cohort. The prevalence rates of anti-toxoplasmosis IgM and IgG antibodies among haemodialysis patients were 4.6% and 34.6%, respectively, while the corresponding values in the healthy group were 0% and 28%, respectively.

The chronic infection with T. gondii is indicated by the presence of IgG antibodies, signifying that the individual has experienced an infection in the past or a reactivation of the infection due to T. gondii's impact on the immune system. An acute infection is indicated by the presence of IgM antibodies, signifying that the individual has

recently acquired the illness.[6] The current findings align with those of Hamad *et al*.[7], who reported seropositivity rates of anti-toxoplasmosis antibodies IgG and IgM in Kirkuk city at 35.21% and 29.16%, respectively, and with Mirahmadi *et al*.[8], who documented seropositivity rates of 44.5% and 23.5% for anti-toxoplasmosis antibodies IgG and IgM, respectively, in haemodialysis patients and healthy individuals.[7,8]

The results indicate that the seropositivity percentage of T. gondii antibodies was higher in males than in females among haemodialysis patients. This disparity may be attributed to the greater susceptibility of men to infections such as toxoplasmosis, likely due to their higher incidence of kidney failure compared to women. T. gondii is influenced by elevated hormone levels, with men displaying greater testosterone levels, which may lead to heightened susceptibility to Toxoplasma infection, either due to an impaired immune response or insufficient personal hygiene that increases exposure to infection sources.[9,10] This conclusion aligns with prior observations from research conducted by Musa *et al*.[10] and Yousif[11], which indicated that T. gondii predominantly infects males more than females.

Concerning the detection of the T. gondii B1 gene in circulating blood, the T. gondii B1 gene was identified in only 27.4% of haemodialysis patients, while it was absent in the healthy group. The rationale for this may be that despite the advantages of serological approaches in diagnosing T. gondii infection, these methods still possess significant drawbacks, including the inability to identify the presence of the parasite in immunocompromised patients. Conversely, the Real-Time PCR assay is an accurate approach for identifying the presence of parasites, since it can detect minimal quantities of T. gondii DNA, reaching as low as 0.05 µl, making it a crucial diagnostic tool for immunocompromised patients, such as those undergoing haemodialysis.[12-14]

The findings of this work endorse the notion that Real-Time PCR utilising the B1 gene exhibits superior sensitivity for diagnosing T. gondii infection, attributable to its elevated sensitivity, specificity, and excellent performance.[15-18]

The discovery of the T. gondii B1 gene aligns with the findings of Mirahmadi *et al*.[8], Sakban and A'aiz[12], and Yarahmadi *et al*.[19], who reported its prevalence in haemodialysis patients at 29.4%, 20%, and 29.55%, respectively.

The renal system filters metabolic waste from the blood and removes excess bodily fluids.[20] Renal function tests are essential biomarkers for assessing the presence of kidney disease and its response to treatment.[21] The primary indicators of normal kidney function are urea and creatinine, whose elevated levels signal renal impairment.[11,22] This study aims to elucidate the relationship between toxoplasmosis infection and renal function testing. Blood urea and serum creatinine levels were assessed in haemodialysis patients, revealing significantly elevated values in those infected with toxoplasmosis.

Certain investigations have identified a correlation between toxoplasmosis and an elevated risk of renal disorders, hence the rise in urea and creatinine levels in haemodialysis patients may be elucidated. The parasite induces urine irregularities and glomerular lesions, leading to compromised kidney function, which can result in the buildup of waste products and excess fluid in the body, culminating in a condition known as uremia and elevated renal biomarkers.[23,24]

The results of the present investigation corroborate prior research linking toxoplasmosis infection to kidney function tests (blood urea and serum creatinine).[23-25]

Furthermore, during Toxoplasmosis infection, numerous alterations occur in the liver that impact liver enzyme activity (S.AST, S.ALT, and S.ALP). Consequently, these enzymes are frequently utilised as indicators to evaluate liver damage induced by Toxoplasmosis.[24] To evaluate the correlation between liver enzyme activities (transaminases) and toxoplasmosis infection, liver enzyme activities were quantified in both haemodialysis patients and healthy individuals. It was revealed that haemodialysis patients without toxoplasmosis exhibited lower levels compared to other groups.[24]

In the present investigation, although there was a notable elevation in S.AST and S.ALT activity in haemodialysis patients infected with T. gondii, their levels remained within the normal range. This may elucidate the subtle effects of toxoplasmosis on S.ALT and S.AST activity in haemodialysis patients. In contrast, S.ALP levels were dramatically elevated beyond the normal range in haemodialysis patients, indicating the adverse effect of infection on this enzyme. The capacity of T. gondii to induce toxoplasmosis depends on the immune system condition of healthy persons, while in immunocompromised patients, the immune system is damaged (e.g., haemodialysis patients). In these patients, infection with toxoplasmosis contributed to heightened oxidative stress and inflammation, resulting in tissue injury and organ failure, particularly in the liver.[20,24,26] These results were comparable with the findings of Abdulwahab and Abdullah[24], Babekir *et al*.[21], and Babekir *et al*.[20], who showed a rise in liver enzyme levels in patients infected with toxoplasmosis.

Conclusions

The findings of the current study demonstrate that serological and molecular detection of T. gondii infection, particularly in immunocompromised individuals such as haemodialysis patients, is essential to prevent the dissemination of infection. Regular screening for toxoplasmosis is necessary, and this study has explored the correlation between toxoplasmosis infection and renal and liver function tests.

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