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Iran J Parasitol

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Iranian Society of Parasitology
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Original Article

Evaluation of *Toxoplasma* and *Toxocara* Prevalence in Chronic Kidney Disease (CKD) Patients Using Serologic and Molecular Technique in Selected Medical Centers of Tehran, Iran

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Received 15 Dec 2024
Accepted 21 Mar 2025

Keywords:
Chronic kidney diseases;
Toxoplasma;
Toxocara

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Abstract

Background: Toxoplasmosis and toxocariasis are two zoonotic diseases with global impact. Chronic kidney disease (CKD) can lead to complications, associated with reduced immune responses that predispose them to frequent parasitic infections. We aimed to determine the prevalence of toxoplasmosis and toxocariasis in CKD patients to propose a new way to control them in dialyses duration in Tehran, Iran.

***Methods*:** Three hundred and sixty-five CKD patients and 72 healthy individuals were tested for anti-*Toxoplasma gondii* (IgG, IgM) and anti-*Toxocara* (IgG) antibodies using conventional ELISA technique. IgM positive samples underwent genetic analysis.

***Results*:** Of the 437 samples studied (365 patients and 72 controls), 182 CKD patients (49.8%) and 20 controls (27.9%) were positive for *Toxoplasma* IgG, and 8 (2.2%) CKD samples were positive for *Toxoplasma* IgM, while none of the control samples were positive for *Toxoplasma* IgM. Molecular analysis of the 8 IgM positive samples with B1gene confirmed the presence of Toxoplasma antigen in all of them. *Toxocara* IgG antibodies showed a lower prevalence in CKD patients (5.7%) compared to controls (9.7%). The study revealed significant differences (P <0.05) in *Toxoplasma* IgG and IgM antibodies, as well as variables such as dialysis and dialysis duration between the two groups of patients and the control group. However, variables such as *Toxocara* IgG, age, gender, lupus, and pets showed no significant difference between the control group and the patients.

***Conclusion*:** The collected data in this study could serve as a reference for future studies and may be useful for examination and evaluation of acute and chronic toxoplasmosis before and after starting dialysis.

DOI: https://doi.org/10.18502/ijpa.v20i2.19028

Introduction

Toxoplasmosis is a foodborne infection caused by *Toxoplasma gondii*. *T. gondii* is an obligate intracellular parasitic protozoan with a worldwide distribution. Toxoplasmosis is mostly asymptomatic, though chronic toxoplasmosis develops when *Toxoplasma* invades the organs and forms tissue cysts (1).

Cats are the main hosts, while mammalians and birds are the intermediate hosts in the *Toxoplasma* life cycle. *Toxoplasma* infectious forms are tachyzoite, bradyzoite, and oocysts (2). Consuming contaminated food sources infects intermediate hosts such as humans and sheep (3). The prevalence of toxoplasmosis in Iran is about 39.3% (4). Serological tests such as ELISA for detecting *Toxoplasma* IgG and IgM antibodies are the most typical diagnosis methods for toxoplasmosis (5).

Toxocara canis and *T. cati* are usually gastrointestinal helminths in dogs and cats. Adult forms of *Toxocara* are observed in the definitive hosts (dogs and cats) (6). Every female *Toxocara* can shed about 200,000 eggs per day. Meanwhile, infected feces can contaminate food and water sources. Paratenic hosts like humans get infected by foodborne transmission and ingestion of eggs (7). *Toxocara* spp. causes a multi-systemic parasitic infection called VLM (Visceral Larva Migrans) or OLM (Ocular Larva Migrans), especially in young individuals. Severe damage may occur in patients with weakened immune systems (8, 9).

Serologic tests such as ELISA or Western blot are useful tools in the diagnosis of *Toxocara*. *Toxocara* IgG levels are the key for diagnosing a previous *Toxocara* infection, though IgM levels do not need to be measured since Toxocariasis symptoms occur at the final stages of disease when IgM is at its lowest (10).

Infectious diseases continue to be a serious problem for patients with immune system defects and chronic diseases. Chronic kidney disease (CKD) is a kidney disorder that causes a gradual decline in the capability of blood filtration in the glomerulus. The loss of kidney function is the major difficulty with CKD. Toxicity of blood, proteinuria, and heart diseases are the severe health complications of CKD (11).

Glomerular filtration rate (GFR) is the key factor in the diagnosis of CKD. GFR is calculated by the blood creatinine level. In healthy individuals, the presence of GFR is higher than 100 mL/min/1.73 m2. In patients with proteinuria, hematuria, and an abnormal kidney imaging result, a decline in GFR level is obvious. CKD patients are categorized into five stages by estimated GFR level. Patients at stage 4 of CKD have severe kidney damage and are referred to dialysis centers (GFR 15–29) and the final stage is kidney failure, with a GFR level lower than 15. Patients with failure conditions receive a suitable kidney transplant (12, 13).

CKD patients, due to previous immunosuppressive treatments, have a weaker immune system compared to healthy individuals (14). This could cause the reactivation of chronic toxoplasmosis. On the other hand, perhaps a former *Toxocara* infection could lead to CKD's gradual progression to the final stages. *Toxocara* antibodies could suppress the immune system or could inhibit autoimmune antibodies' efficiency (15, 16). In patients with lupus or nephrotic syndrome, a previous *Toxocara* infection could be a trigger to cause chronic kidney diseases (17). The correlation between the effect of *Toxoplasma* and *Toxocara* antibodies on CKD is unknown. Therefore, it is recommended that all CKD patients should be consider every six month for *Toxoplasma* IgG titer.

In this study, we aimed to determine the prevalence of toxoplasmosis and toxocariasis in CKD patients.

Materials and Methods

Samples consisted of 365 patients admitted to Urology and Kidney Centers of Tehran,

Iran (Hashemi Nejad Urology and Kidney Hospital and Talaghani Hospital, Urology and Kidney Dept.) in 2022, with the diagnosis of chronic kidney disease based on Glomerular Filtration Rate values and renal failure symptoms such as proteinuria and hematuria, and 72 healthy people from the general population without any history of previous chronic kidney disease with GFR higher than 100 mL/min/1.73 m2. The stages of CKD were defined according to the GFR classification. Variables such as age, gender, history of diabetes or lupus or high blood pressure along with GFR values were collected from all 437 participants via a demographic questionnaire.

The study was approved by the Ethical Committee of the Shahid Beheshti University of Medical Sciences with code No. of IR.SBMU.MSP.REC.1401.041.

Two ml of serum and 3 ml of whole blood in EDTA tubes were collected from patients and the control group under laboratory conditions at each center. All qualified samples were transferred to Parasitology and Mycology Dept., Medicine faculty, Shahid Beheshti University of Medical Sciences, through cold transfer conditions. Samples were kept at -20 °C for performing serological and molecular tests.

The diagnostic tests of *Toxoplasma* and *Toxocara* antibodies were performed using the ELISA method, and all samples were examined based on the presence of *Toxoplasma* IgG and IgM and *Toxocara* IgG. ELISA test for *Toxocara* sp. was performed with NovaLisa® kit (GMBH, Germany). According to the manufacturers' recommendations, IgG absorbance levels < 10 were considered negative, 9 - 11 was considered borderline, and > 11 was Positive. *Toxoplasma* tests were performed with Pishtazteb Diagnostics kit (Pishtazteb, Iran). According to the manufacturers 'recommendations, IgG and IgM absorbance levels < 0.9 were considered negative, 0.9 – 1.1 was considered borderline, and > 1.1 was Positive.

DNA extraction

Extraction of DNA was performed on whole blood samples which were stored at -20 °C. Samples were washed 8 times with 1X PBS buffer and then centrifuged at 8000 rpm to solve Angiotensin Converting Enzyme (ACE) to prevent the presence of any blood clot. Extraction was performed by DNG™-plus (Cinna gen, Iran) commercial DNA extraction kit. Extraction steps were applied following the kit's instructions.

PCR amplification: The PCR technique was performed on the B1 gene used, which amplified the 194pb segment to detect the presence of *Toxoplasma* DNA in extracted samples. The sequence of the forward primer was 5'-GGAACTGCATCCGTTCATGAG-3' and the reverse primer was 5'-TCTTTAAAGCGTTCGTGGTC-3' (18). The PCR reaction contained 3 µl(3µg) of template DNA, 6 µl distilled water, 10 µl Taq DNA Polymerase 2x Master Mix RED (Ampliqon, Denmark) and 0.5 µl of each primer. The PCR cycle consisted of initial denaturation at 94 °C for 5 min followed by 32 cycles at 94 °C for 40 s, 62 °C for 40 s, 72 °C for 40 s and a final extension of 72°C for 5 minutes. Two samples with and without DNA of *Toxoplasma* were utilized as positive and negative controls. 3µL of each PCR product was examined in a 1.5% agarose gel and stained with Ethidium Bromide (EtBr) and visualized under a UV transilluminator.

PCR products were sent to Pishgam Biotech Co. (Iran, Tehran). The sequencing results were edited by Chromas software version 2.5.0 and analyzed with Nucleotide Blast. Then, all sequenced samples were compared with different samples studied in Iran and some other parts of the world registered in the GenBank.

Results

A total of 437 participants were included in this study, and all of them were categorized into CKD patients and healthy individuals. The mean age of the participants was 52 ± 1 years. The minimum age was 18 and

the maximum age was 93 years. Other characteristics such as, sex, history of high blood pressure, lupus, diabetes, and domestic animals were analyzed between the case and the control group (Table 1).

Table 1: Characteristics of the case and control groups

Groups	No.	Female N(%)	Male N(%)	High Blood Pressure N(%)	Diabetes N(%)	Lupus N(%)	Pet N(%)
Patients	365	205 (56.2)	160 (43.8)	4 (1)	0	0	2 (0.54)
Controls	72	46 (63.9)	26 (36.1)	17 (23.9)	16 (22)	2 (2.7)	11 (15.2)
Significance level		> 0.05	> 0.05	> 0.05	< 0.05	> 0.05	> 0.05

The patients consisted of 25 patients with first stage of CKD, 17 patients with second stage of CKD, and 19 patients with CKD 3a and 3b plus 264 patients who were referred to the dialysis department and 40 patients in final stage of CKD who had received transplanted kidney. In the control group, 20 out of 72 samples (27.9%) for *Toxoplasma* IgG antibodies and 7 (9.8%) samples for *Toxocara* IgG were positive in the ELISA assay. None of the control group samples were positive for *Toxoplasma* IgM antibodies. The frequency of all investigated antibodies was analyzed between patients from each group. The results indicated that based on the collected sample size, the patients from the 4th stage of CKD who were referred to the dialysis centers revealed the most seropositive results (Table 2).

Table 2: Distribution of all seropositive investigated antibodies among different stages of CKD patients

CKD stages	Groups	No.	*Toxoplasma* IgM	*Toxoplasma* IgG	*Toxocara* IgG
	1st stage	25	None (%)	22 (88%)	2 (8%)
	2nd stage	17	1 (5.8)	7 (41)	None
	3rd stage	19	None	14 (73.6)	None
	4th stage	264	7 (2.65)	118 (44.6)	17 (6.43)
	5th stage	40	None	21 (52.5)	2 (5)
	Total	365	8(2.19)	182(49.86)	21(5.75)

Only one of the participants in the current study was seropositive for *Toxoplasma* IgG, *Toxoplasma* IgM, and *Toxocara* IgG. Ten out of 365 patients were positive for both *Toxoplasma* IgG and *Toxocara* IgG. In the group of patients who received a kidney transplant at the last stage of CKD, 21 samples for *Toxoplasma* IgG and 2 samples for *Toxocara* IgG were positive. Four out of 365 patients were seropositive for both *Toxoplasma* IgG and IgM antibodies showing a chronic toxoplasmosis profile. The other four patients with positive *Toxoplasma* IgM level were not positive for *Toxoplasma* IgG but one out of these patients was positive for *Toxocara* IgG.

Most of the participants were in the last stages of CKD as well as individuals who referred to dialysis centers (a total of 264 samples). The duration of the dialysis procedure was divided into periods of 6, 12, and 24

months (Table 3). We detected higher levels of *Toxoplasma* IgG in patients with a period of 12 months after starting the dialysis in comparison to patients with 6- and 24-months dialysis periods. There was a correlation between levels of *Toxoplasma* antibody and duration of dialysis with a significant level of <0.001 (Fig. 1). However, there was no significant difference between *Toxoplasma* IgM levels and dialysis duration due to $P > 0.05$.

Table 3: Distribution of dialysis patients per duration length of dialysis process

Dialysis patients (CKD stage4)	Dialysis duration	6 months		12 months		24 months		Total	
	Toxoplasma antibodies	*Toxoplasma* IgM	*Toxoplasma* IgG	*Toxoplasma* IgM	*Toxoplasma* IgG	*Toxoplasma* IgM	*Toxoplasma* IgG	*Toxoplasma* IgM	*Toxoplasma* IgG
	No.	2	98	4	103	1	63	7	264
	Percentage	0.7	37.1	1.5	39.1	0.4	23.8	2.6	100

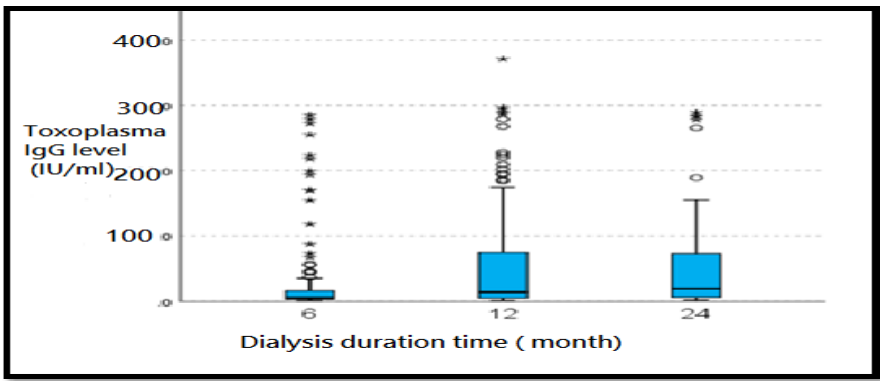

Fig. 1: *Toxoplasma* IgG levels per dialysis time. This diagram shows a significant (PV <0.05) decrease in levels of *Toxoplasma* IgG during the length of using dialysis therapy in CKD patients

According to the PCR results, in all the 8 IgM seropositive patients, *Toxoplasma gondii* DNA was detected by amplifying the 194bp fragment of *Toxoplasma* B1 gene. (Fig. 2) All amplified segments in PCR products were approved by sequencing. The percentage of identities between the isolates from this study with other similar sequences was 98-100% (Fig. 3). The B1 sequences were registered in the GenBank database with accession numbers OP612592 to OP612599.

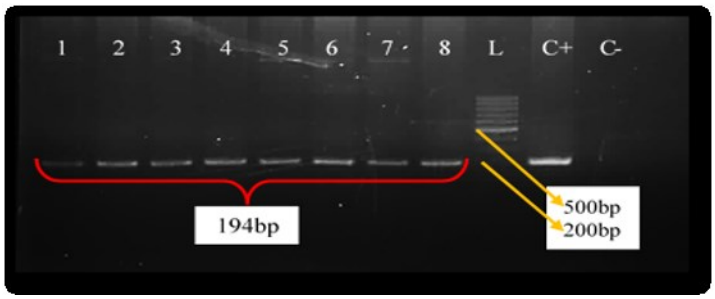

Fig. 2: Gel electrophoresis. Lanes1 -8 (positive samples, L: 100 bp DNA ladder), C+: positive control with amplified 194bp fragment of *Toxoplasma* B1 gene, C-: negative control)

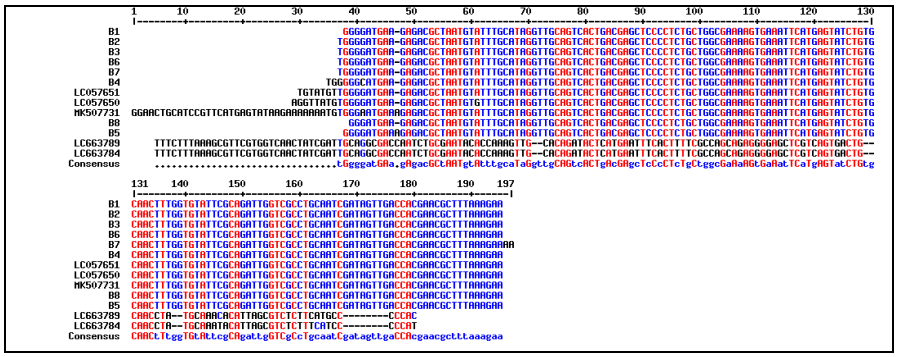

Fig. 3: Alignment results between sequence of current study and other deposited sequences in GenBank

Discussion

Toxoplasma gondii is a parasitic protozoan that can create favorable conditions for the patient to develop opportunistic infections. Examining its presence in patients with renal failure is important and increases the need for care and treatment costs (13).

Toxocara causes visceral larva migrans in accidental hosts. In Iran, the prevalence of toxocariasis in pregnant women is approximately 15.8%. In contrast, the prevalence in healthy individuals in the community has been reported to be around 9.3%. Meanwhile, the frequency of the Toxocara parasite in stray dogs is 24.4% (24). CKD patients, after starting corticosteroid treatment, are more susceptible to secondary infections due to reduced immune levels (24).

In this study, a whole blood sample and serum were collected from 365 patients with chronic kidney failure and 72 healthy individuals with a GFR above 100 who referred to selected kidney and urinary tract centers. In the patient group, 182 samples (49.8%) tested positive for *Toxoplasma* IgG antibodies, and 8 samples (2.2%) tested positive for *Toxoplasma* IgM antibodies. The positive *Toxoplasma* IgM samples were analyzed molecularly for the identification of the B1 gene of the *Toxoplasma* parasite. This aligns with the Saki study. In comparison with the present study, their *Toxoplasma* IgG antibody titer was half that of the IgG titer, and their IgM titer was similar to the IgM titer in the current study, indicating a lower prevalence of this parasite in the mentioned city. However, the similarity in IgM titers suggests a comparable degree of transmission of this infection in patients undergoing dialysis, further increasing the likelihood of transmission of this parasite through dialysis (23).

In another study conducted on 135 dialysis patients and 120 healthy individuals in Qom and Kashan, the titers of *Toxoplasma* IgG and IgM antibodies were positive in 52% and 7.1% of patients, respectively. In our study, these titers were 49.8% and 6.2%, respectively, indicating the similarity of the prevalence of acute and chronic toxoplasmosis between the present study and the two aforementioned cities, indicating the potential for transmission of this parasite during dialysis sessions (25). Another study conducted on dialysis patients in Tehran, reported a prevalence of *Toxoplasma* IgG & IgM antibodies titers 67.3% and 7% in the patient group respectively, which is somewhat similar to the present study, which reported *Toxoplasma* IgG & IgM antibodies titers 49.8% and 6.2% respectively (19). The IgG titer (an indicator of chronic toxoplasmosis)

was higher in their study population, which may be due to the researchers' sampling of hospitalized patients, while in the current study, part of the samples were taken from outpatient subjects. Additionally, the larger number of cases examined in our study provides a more accurate representation, and a more significant point is the percentage of acute cases, where there is considerable similarity between our study and theirs, indicating a higher likelihood of transmission through dialysis. In Arab Mazaar study, in addition to serological tests, molecular assays were conducted to determine the species of the parasite in acute cases on samples that were positive for *Toxoplasma* IgM. The DNA of the parasite was detected in 2 out of 18 serologically positive cases, which were not typed. However, in the current study, using the B1 gene and DNA sequencing molecular methods, Type I *Toxoplasma* was identified in all 8 samples that were serologically positive for *Toxoplasma* IgM, which is the cause of congenital toxoplasmosis (19).

Another study conducted in hospitals of Guilan province on 300 people (150 hemodialysis patients and 150 healthy individuals) in terms of *Toxoplasma* IgG and IgM titers reported positive IgG and IgM titers in 68% and 2.3%, respectively. Their results differed from our study regarding IgG titers, while IgM titers were similar. This discrepancy between the results of our study and above may depend on the geographical location of the sampling, as the parasite's oocysts remain viable in the climatic conditions of the northern regions, with the highest reported prevalence of toxoplasmosis in Iran being from the north of the country (26).

The positive rate of *Toxoplasma* IgG antibodies in the test and control groups was reported at 27.3% and 3.6%, respectively in a study in China, showing a significant difference between them (P=0.005). In the current study, the positive rate of *Toxoplasma* IgG antibodies was reported at 49.8% for the patient group and 27.9% for the control group, which also showed a significant difference with PV<0.001, However, it can be concluded that the rate of *Toxoplasma* infection in dialysis patients with kidney failure is higher than that in healthy individuals, and dialysis may pose a potential risk for the transmission of *T. gondii* infection in CKD patients. This is consistent with the current study, which compared the duration of dialysis with *Toxoplasma* IgG titers and showed that the titer increased over the first year of dialysis, remaining stable and not decreasing for up to two years, with this relationship being significant at *P*<0.001(27).

Toxoplasma IgG antibody titers were reported positive in 39% of cases, and *Toxoplasma* IgM titers were positive in 2.9% in eye patients. None of the samples tested positive for *Toxocara* antibody titers. The molecular analysis in this study focused on identifying the parasite *T. gondii* by examining the B1 gene. DNA parasite was detected in all serologically positive samples for *Toxoplasma* IgM, which is consistent with the current study (10). In 2006, a 41-year-old woman with skin symptoms (skin rash) and proteinuria was admitted to the hospital for measurement of urinary protein and blood creatinine levels. The patient was transferred to the dialysis unit with a diagnosis of renal failure. Given the skin manifestations, serological testing for *Toxocara* was also performed. The patient's *Toxocara* antibody titer was high. In the present study, 6.2% of patients had positive *Toxocara* titers, indicating a history of *Toxocara* infection in patients with renal failure, but it could not be proven whether their infection started before or after dialysis (28). Another case report was of an 8-year-old girl diagnosed with toxocariasis, who was subsequently diagnosed with lupus after autoimmune disease testing in France. In the present study, there were two lupus patients in the population, one of whom had a high *Toxocara* antibody titer, which is consistent with the above results, but it cannot be proven that the *Toxocara* infection is due to the presence of

lupus or vice versa and requires further studies (17).

In another study, *Toxoplasma* IgG antibody titers were positive in 45% of cases, which was similar to our results reported for IgG (49.8%).. In the case of *Toxocara*, antibody titers were positive in 22% of the population. This figure is significantly higher compared to the prevalence of toxocariasis in our study (6.2%), which may indicate contamination of water and food sources in the suburbs of Rome, as well as the keeping of pets, including dogs and cats, and lower hygiene standards and poverty or using update diagnosis methods. It should be noted that another effective factor in the reduction of *Toxocara* antibody titers in the present study is related to the lack of access to accurate and up-to-date kits and the lack of sufficient attention to the presence of this parasite in society, especially today, when keeping pets in homes has become somewhat common (29).

Conclusion

The antibodies against *Toxoplasma* IgG and *Toxoplasma* IgM and the variables like dialysis and dialysis duration had a significant difference in the two groups of patients and control, although variables such as *Toxocara* IgG, had no significant difference between the control group and the patients. It seems that evaluation of acute and chronic toxoplasmosis is necessary before starting dialysis. Infected patients should be treated before dialysis to avoid contamination of the dialysis machines and to prevent the spreading of toxoplasmosis man to man.

Acknowledgements

The authors would like to express their gratitude to Shahid Beheshti University of Medical Sciences (grant numbers: IR.SBMU.MSP.REC.1401.41) and Hasheminejad and Taleghani hospitals and their laboratory supervisors for cooperation in this study.

Conflict of Interests

The authors declared that they have no conflict of interest

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