

Seropositivity of toxoplasmosis among hemodialysis children patients at Zagazig University Pediatrics Hospital, Egypt

Original Article

Howayda SF Moawad¹, Samia E Etewa¹, Samira M Mohammad¹, Mayy A Neemat-Allah², Jad A Degheili³, Mohamed H Sarhan¹

Departments of Medical Parasitology¹, and Pediatrics², Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt, Division of Pediatric Urology, Department of Surgery, Children's Hospital of Eastern Ontario, University of Ottawa, Ontario K1H 8L1, Canada³

ABSTRACT

Background: Toxoplasmosis is a universal opportunistic infection that causes severe complications in immunosuppressed patients. Renal failure is a state of immunodeficiency, especially in children. Therefore, hemodialysis patients may be at high risk for toxoplasmosis.

Objective: To investigate the seropositivity rate of *T. gondii* specific antibodies (anti-*T. gondii* IgM and IgG antibodies) in pediatric hemodialysis patients.

Subjects and Methods: ELISA was used to test serum samples from 67 children on regular hemodialysis and 50 healthy controls for anti-*Toxoplasma* IgG and IgM antibodies in this case-control research. Demographic criteria, duration of hemodialysis, and possible risk factors for toxoplasmosis were recorded. All participants were clinically examined to detect any signs suggestive of toxoplasmosis.

Results: In comparison to total *T. gondii* seropositivity in all participants (23%), it was 16% and 28% in control and hemodialysis groups, respectively. The IgG antibodies were detected in 19 hemodialysis cases and eight control cases. The mean duration of hemodialysis among *T. gondii* seropositive and seronegative patients were 47.6±14.3 and 22.3±6.7 months, respectively. Among *T. gondii* seropositive individuals, the average duration of hemodialysis was substantially longer. In the examined groups, contact with cats and eating semi-cooked meat were the risk factors recorded for *T. gondii* seropositivity.

Conclusion: Toxoplasmosis seropositivity rate was higher in hemodialysis children than in the control group. For early identification and treatment, *T. gondii* screening should be done for all hemodialysis patients before and during hemodialysis.

Keywords: anti-*Toxoplasma* antibodies; ELISA; hemodialysis; renal failure; toxoplasmosis.

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Corresponding Author: Mohamed H. Sarhan, **Tel.:** +20 1020260608, **E-mail:** drsarhan@gmail.com

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INTRODUCTION

Toxoplasmosis is a zoonotic illness caused by *T. gondii*, an obligate intracellular protozoan^[1]. It is a largely undiagnosed, highly neglected disease that can be fatal in humans and animals worldwide^[2]. *T. gondii* infects more than a third of the world's population^[3]. Infection may be contracted by eating *T. gondii* tissue cysts in undercooked or raw meat from infected animals or birds. Swallowing infective oocysts shed by infected cats to contaminate soil, water, and plants, poses a substantial infection source^[2]. Other transmission routes as transplacental, organ transplantation, and blood transfusion can occur^[4]. The disease can be due to an acquired recent infection or reactivation of latent infection^[5].

In immunocompetent individuals, toxoplasmosis is mainly asymptomatic or presents as mild fever and swelling of lymph nodes. In immunocompromised individuals such as hemodialysis patients, organ transplant recipients, AIDS, or malignancy patients, latent forms of the protozoan can be activated and cause generalized lymphadenopathy, encephalitis, cerebral calcifications, epilepsy, myocarditis, pneumonia, and even death^[6,7].

Renal failure is a global public health issue increasing in prevalence with occasional unfavorable outcomes; and requires expensive management^[8]. The most common causes of renal failure in children are chronic glomerulonephritis, congenital anomalies of the urinary system as polycystic kidney disease, renal hypoplasia, dysplasia, obstructive uropathy, steroid-resistant nephrotic syndrome, hemolytic uremic syndrome, autoimmune disorders as systemic lupus erythematosus, diabetic and hypertensive nephropathy^[9,10]. Advanced stages of renal failure need renal replacement therapy as dialysis or renal transplantation that requires long-term immunosuppressive therapy^[11].

One of the most severe and life-threatening problems in dialysis patients is immune system dysfunction. After cardiovascular illnesses, it is the second most common cause of high morbidity and mortality rates among chronic renal failure patients^[12]. This impairment results from disturbed normal renal functions and subsequent uremic toxins accumulation in patients' blood^[10]. These toxins impair humoral and cellular immunity due to high B and T cell apoptosis with consequent lymphopenia, malfunction of the cluster of differentiation 4 (CD4⁺)

T- cells, and decreased phagocytic ability of neutrophils and monocytes^[13].

This immunosuppressive state renders these patients more susceptible to various opportunistic infections, including toxoplasmosis, and are at higher risk of latent infection reactivation^[14]. Hence, a rapid and definitive diagnosis of toxoplasmosis is important in hemodialysis patients^[15]. Specific IgM antibodies are used to diagnose patients for acute toxoplasmosis because these antibodies can be detected at high serum titers during the onset of the disease. Meanwhile, specific IgG antibodies are indicative of chronic toxoplasmosis (previous or latent infection)^[16].

The main goal of this study is to determine the seropositivity rate of *T. gondii* specific antibodies (anti-*T. gondii* IgG and IgM antibodies) using ELISA in hemodialysis patients attending the Hemodialysis Unit at Zagazig University Pediatrics Hospital in Sharqia Governorate, Egypt, compared to healthy controls.

SUBJECTS AND METHODS

This case-control study was conducted at the Hemodialysis Unit of Zagazig University Pediatrics Hospital and Medical Parasitology Department, Faculty of Medicine, Zagazig University, from May 2019 to March 2020.

Study design and population: One hundred seventeen cases aged ≤ 18 years participated in this study, and they were categorized according to their renal status into two groups; the hemodialysis group (n=67) and the control group (n=50). The hemodialysis group included children with chronic renal failure who were on regular hemodialysis (by an arteriovenous fistula or graft) three times per week. These patients did not suffer from other diseases causing immunosuppression such as malignancy, AIDS, diabetes mellitus, bronchial asthma, or received immunosuppressive therapy. At the same time, the control group included healthy participants with no history of renal troubles or any chronic debilitating disease, and their kidney function tests were within the normal range. A comprehensive questionnaire was recorded from all participants, including demographic data (age, gender, residence), duration of hemodialysis, and possible risk factors for toxoplasmosis as the history of contact with cats, eating undercooked meat, or blood transfusion. All participants were clinically examined to detect any signs suggestive of *T. gondii* infection such as fever, skin rash, enlarged lymph nodes, CNS impairment, or organomegaly.

Sampling and serological immunoassays: Sera were obtained from each participant under strict aseptic conditions. Labeled sera were kept at -20°C . Using commercial ELISA kits, serum samples were tested

at a wavelength of 450 nm for anti-*Toxoplasma* IgM and IgG antibodies according to the manufacturer's recommendations (GENESIS diagnostics, California, USA, Product Code: GD081 and GD080, respectively) on an ELISA microplate reader (SLT Lab Instruments, A-5082 Grödig /Salzburg, Austria, serial number. 216692). Results were defined semi-quantitatively by dividing the OD of the test samples by the OD of the calibrator control samples. Antibody index ≥ 1.1 (≥ 32 IU/ml) was considered as a seropositive result of *T. gondii* infection; antibody index 0.9-1.1 was considered borderline positive and re-tested; and antibody index < 0.9 (< 32 IU/ml) was considered as seronegative. The *T. gondii*-specific IgM, IgG, or both reacting samples were confirmed positive.

Statistical analysis: The statistical package for the social sciences (SPSS) software was used to gather, tabulate, and analyze the data. The chi-square test and the t-test were used. Statistical significance was defined as a *P* value of less than 0.05.

Ethical consideration: The study was authorized by Zagazig Faculty of Medicine's Ethical Committee, Egypt. After explaining the purpose of the study, informed consent was obtained from each participant's parents. Results were informed to all participants.

RESULTS

In the present study, the mean age of the hemodialysis cases was 13.6 ± 3.2 years and 10.2 ± 2.7 years for the control cases, with significant statistical differences between both groups. Still, there were insignificant statistical differences between them concerning gender and residence. Male patients represented the highest percentage of both groups, and also, most of them were rural inhabitants. Both groups showed significant statistical differences concerning the possible risk factors for toxoplasmosis except for drinking raw milk (Table 1).

The overall *T. gondii* seropositivity among the hemodialysis and control groups was 23%. Hemodialysis patients had higher total seropositivity (28%) than control participants (16%). Anti-*Toxoplasma* IgM antibodies were not detected in both groups. However, IgG antibodies were detected in 19 hemodialysis cases and eight control cases with insignificant statistical differences between both groups (Table 2).

According to the cause of renal failure in the hemodialysis group, 22 (33%) patients had a history of chronic glomerulonephritis, 14 (21%) patients had a history of congenital urological malformations, 8 (12%) patients had a history of steroid-resistant nephrotic syndrome, 4 (6%) patients had a history of lupus nephritis, 3 (4%) patients had a history

Table 1. Demographic data and the possible risk factors for toxoplasmosis among hemodialysis and control groups.

	Hemodialysis group (n=67)		Control group (n=50)		Total (n=117)		Statistical analysis	
	Mean ± SD		No. (%)		t-test		P value	
Age (Years)	13.6 ± 3.2		10.2 ± 2.7		---		3.76	
							0.04*	
							X²-test	
							P value	
Gender								
Male	41 (61%)		29 (58%)		70 (60%)		0.12	
Female	26 (39%)		21 (42%)		47 (40%)		NS	
Residence								
Rural	43 (64%)		26 (52%)		69 (59%)		1.67	
Urban	24 (36%)		24 (48%)		48 (41%)		NS	
Contact with cats								
Yes	37 (55%)		11 (22%)		48 (41%)		13.06	
No	30 (45%)		39 (78%)		69 (59%)		0.000*	
Contact with soil								
Yes	6 (9%)		11 (22%)		17 (15%)		3.92	
No	61 (91%)		39 (78%)		100 (85%)		0.048*	
Semi-cooked meat								
Yes	21 (31%)		32 (64%)		53 (45%)		12.32	
No	46 (69%)		18 (36%)		64 (55%)		0.000*	
Raw milk								
Yes	13 (19%)		6 (12%)		19 (15%)		1.15	
No	54 (81%)		44 (88%)		98 (85%)		NS	
Blood transfusion								
Yes	36 (54%)		2 (4%)		38 (32%)		32.30	
No	31 (46%)		48 (96%)		79 (68%)		0.000*	

n: Number of cases; SD: Standard deviation; *: Significant difference ($P < 0.05$); NS: Non-significant difference ($P \geq 0.05$).

Table 2. Serological tests for anti-*T. gondii* antibodies among the hemodialysis and control groups.

	Hemodialysis group (n=67)		Control group (n=50)		Total (n=117)		Statistical analysis	
	No. (%)		No. (%)		X ² -test		P value	
IgG positive	19 (28%)		8 (16%)		27 (23%)		2.5	
IgM positive	0 (0%)		0 (0%)		0 (0%)		0.0	
Total seropositivity	19 (28%)		8 (16%)		27 (23%)		2.5	

n: Number of cases; NS: Non-significant difference ($P \geq 0.05$).

of the hemolytic uremic syndrome, and 16 (24%) patients had an unknown cause of renal failure. There were insignificant statistical differences between all groups concerning the total number of IgG and IgM *Toxoplasma* seropositivity. *T. gondii* antibodies were detected in 9 (47%) patients with a history of chronic glomerulonephritis, 3 (16%) patients with a history of congenital urological malformation, 1 (5%) patient with a history of the hemolytic uremic syndrome, 2 (11%) patients with a history of steroid-resistant nephrotic syndrome, and 4 (21%) patients with unknown cause of renal failure (Table 3).

The mean age of the *T. gondii* seropositive patients was 12.4±2.7 years, and 11.8±1.6 years for the *T. gondii* seronegative patients with insignificant

statistical difference between both groups, but there was a significant statistical difference between them concerning the duration of hemodialysis (47.6±2.7 versus 22.3±6.7). Female patients showed an insignificant higher percentage of *T. gondii* seropositivity than male patients (56% versus 44%) who had the higher percentage in the seronegative group; insignificantly most seropositive patients (43:64%) were rural inhabitants. Concerning the possible risk factors for *T. gondii* infection, the statistical differences between both groups were insignificant except for contact with cats (59%) and consuming semi-cooked meat (70%) (Table 4).

Among cases positive for *T. gondii* infection; fever, headache, fatigue, and organomegaly were found in 10

Table 3. The underlying causes of renal failure among the hemodialysis group and associated *T. gondii* total seropositivity.

Renal failure	<i>T. gondii</i> seropositivity		Statistical analysis		
	Cause	No. (%)	No. (%)	X ² -test	P value
Chronic glomerulonephritis		22 (33%)	9 (47%)	2.03	NS
Congenital urological malformations		14 (21%)	3 (16%)		
Hemolytic uremic syndrome		3 (4%)	1 (5%)		
Steroid-resistant nephrotic syndrome		8 (12%)	2 (11%)		
Lupus nephritis		4 (6%)	0 (0%)		
Unknown cause		16 (24%)	4 (21%)		
Total		67 (100%)	19 (100%)		

NS: Non-significant difference ($P \geq 0.05$).

(37%), 12 (44%), 17 (63%), and 8 (30%) respectively, with a statistically significant difference when compared to the *T. gondii* negative cases. While skin rash, blurring of vision, and lymphadenopathy were found in 6 (22%),

7 (26%), and 6 (22%), respectively, with a statistically insignificant difference when compared to the *T. gondii* negative cases (Table 5).

Table 4. Demographic data, duration of hemodialysis and the possible risk factors for toxoplasmosis among hemodialysis and control groups.

	<i>T. gondii</i> seropositive patients (n=27)	<i>T. gondii</i> seronegative patients (n=90)	Total (n=117)	Statistical analysis	
	No. (%)			X ² -test	P value
Gender					
Male	12 (44%)	58 (64%)	70 (60%)	3.64	NS
Female	15 (56%)	32 (36%)	47 (40%)		
Residence				0.86	NS
Rural	18 (67%)	51 (57%)	69 (59%)		
Urbam	9 (33%)	39 (43%)	48 (41%)		
Contact with cats				4.82	0.028*
Yes	16 (59%)	32 (36%)	48 (41%)		
No	11 (41%)	58 (64%)	69 (59%)		
Contact with soil				0.44	NS
Yes	5 (19%)	12 (13%)	17 (15%)		
No	22 (81%)	78 (87%)	100 (85%)		
Semi-cooked meat				8.9	0.003*
Yes	19 (70%)	34 (38%)	53 (45%)		
No	8 (30%)	56 (62%)	64 (55%)		
Raw milk				0.68	NS
Yes	3 (11%)	16 (18%)	19 (15%)		
No	24 (89%)	74 (82%)	98 (85%)		
Blood transfusion				32.30	0.000*
Yes	5 (19%)	33 (37%)	38 (32%)		
No	22 (81%)	57 (63%)	79 (68%)		
	Mean ± SD			X²-test	P value
Age (Years)	12.4 ± 2.7	11.8 ± 1.6		2.83	NS
Duration of hemodialysis (M)	47.6 ± 14.3	22.3 ± 6.7		6.96	0.000*

n: Number of cases; SD: Standard deviation; M: month; *: Significant difference ($P < 0.05$); NS: Non-significant difference ($P \geq 0.05$).

Table 5. The association of clinical manifestations with *T. gondii* seropositivity among the studied hemodialysis patients and the control group.

	Seropositive (n=27)	Seronegative (n=90)	Total (n=117)	Statistical analysis	
	No. (%)			X ² -test	P value
Fever				4.46	0.035*
Yes	10 (37%)	16 (18%)	26 (22%)		
No	17 (63%)	74 (82%)	91 (78%)		
Headache				4.57	0.033*
Yes	12 (44%)	21 (23%)	33 (28%)		
No	15 (56%)	69 (77%)	84 (72%)		
Skin rash				0.92	NS
Yes	6 (22%)	29 (32%)	35 (30%)		
No	21 (78%)	61 (68%)	82 (60%)		
Blurring of visionl				3.00	NS
Yes	7 (26%)	11 (12%)	18 (15%)		
No	20 (74%)	79 (88%)	99 (85%)		
Fatigue				17.1	0.000*
Yes	17 (63%)	19 (21%)	36 (31%)		
No	10 (37%)	71 (79%)	81 (69%)		
Lymphadenopathy				3.50	NS
Yes	6 (22%)	8 (9%)	14 (12%)		
No	21 (78%)	72 (91%)	103 (88%)		
Organomegaly				3.90	0.049*
Yes	8 (30%)	12 (13%)	20 (17%)		
No	19 (70%)	78 (87%)	97 (83%)		
Asymptomatic	4 (15%)	32 (36%)	36 (31%)	4.19	0.041*

n: Number of cases; *: Significant difference ($P < 0.05$); NS: Non-significant difference ($P \geq 0.05$).

DISCUSSION

In fact, parasitic infections play a significant role in developing illnesses, particularly in immunocompromised patients and young children^[17]. Because of the impaired immune system of hemodialysis patients, reactivation of *T. gondii* tissue cysts produces a high and dangerous recurrence of the chronic form of toxoplasmosis^[18]. The goal of the current study was to evaluate the prevalence of toxoplasmosis in pediatric hemodialysis patients compared to healthy cases. The three most common reported etiologies (chronic glomerulonephritis, congenital urological malformations and unknown etiology) of end-stage renal disease in our case-control study, were congruent with those reported by Youssef and Neemat-Allah^[19].

Toxoplasmosis diagnosis can be achieved via serological tests, mouse inoculation, demonstration of the parasite in tissues, Polymerase Chain Reaction (PCR), and tissue culture of clinical specimens. Other less common methods include skin tests and detection of *Toxoplasma* antigen in serum and body fluids. However, quantification of specific antibodies by microscopic detection of neutralized *T. gondii* tachyzoites (Sabin-Feildman dye test) and serological evaluation of *Toxoplasma*-specific IgM and IgG antibodies are considered gold standards^[20]. During acute primary infection with *T. gondii*, anti-*T. gondii* IgM is initially produced. However, IgM titers decline over the next few months, becoming undetectable within a year. The immune system also produces anti-*T. gondii* IgG a few weeks after the initial infection. IgG antibody levels usually peak within one or two months after the infection and persist throughout the lifetime of the infected person^[21].

In our study, the *T. gondii* seropositivity in the hemodialysis patients (28%) as determined by both IgG and IgM evaluation was insignificantly higher than the overall estimate (23%) and the control participants (16%). This rate is higher than that reported by Sharaf *et al.*^[10] who detected 22% *T. gondii* seropositivity in pediatric hemodialysis patients in Cairo Governorate. However, our records were lower than those of Hamza *et al.*^[9], who discovered *T. gondii* seropositivity among adult hemodialysis patients (61.7%) and renal transplant recipients (70.0%) in Alexandria Governorate. Similarly, it is lower than rates in other nations such as Iran (74-76.8%)^[1,7], Turkey (56.0%-76.5%)^[22], and Mexico (56.7%)^[23].

The anti-*Toxoplasma* IgM antibodies were not detected in hemodialysis patients, indicating that those patients were not recently exposed to infection sources or had a reactivation of latent infection. These results agree with Fallahizadeh *et al.*^[6] and Hussein and Molan^[4], who did not detect anti-*Toxoplasma* IgM antibodies in hemodialysis patients. In contrast, Aufy *et*

al.^[24] detected (16.7%) prevalence among hemodialysis patients and (24.1%) among renal transplant recipients; Shehata *et al.*^[25] reported (3.3%), and Sharaf *et al.*^[10] reported (2%) prevalence among hemodialysis adult and children patients, respectively.

In the present study, *T. gondii* IgG seropositivity was insignificantly higher in the hemodialysis group (28%) than in the control group (16%). Similarly, Sharaf *et al.*^[10] detected (20%) among hemodialysis patients and (15%) among the control group. But these results were lower than those of other studies reported by Ocak *et al.*^[22] (76.5%) among hemodialysis patients and (48%) in control subjects; Aufy *et al.*^[24], who detected 56.7% in hemodialysis patients and 23.1% in the control group; Solhjoo *et al.*^[26] who detected 59.10% among hemodialysis patients and 36.40% among healthy individuals; and Bayani *et al.*^[27] whose record reached 80% in hemodialysis patients and 76% in the healthy group. Keeping in mind that IgG seropositivity indicates toxoplasmosis chronicity, the infection may have been acquired before hemodialysis treatment. With the extension of hemodialysis, the disease reached chronicity. Notably, reactivation of latent toxoplasmosis is risky in those immunocompromised patients during dialysis treatment.

There was an insignificant statistical difference between *T. gondii* seropositive and seronegative groups regarding the age in our study. A similar result was obtained in a recent study conducted among the hemodialysis pediatric patients at Ain Shams University Pediatric Hemodialysis Unit^[10]. Besides, exposure to toxoplasmosis was reported to increase with age^[28,29]. Similarly, no statistically significant differences in the percentage of *T. gondii* seropositivity were observed between male and female patients and also between rural and urban inhabitants. This agrees with other studies^[4,27,30] that did not find a significant relationship between *T. gondii* infection rate and gender and residence. In contrast, other studies found a correlation^[31]. The observed diversity could be explained by the differences in sampling methods and lifestyles.

The mean duration of hemodialysis was significantly higher in our series of *T. gondii* seropositive patients. This agrees with other reports^[22,32,33] that also detected a higher mean duration of hemodialysis among seropositive patients than seronegative ones. As such, the more prolonged exposure to hemodialysis, the higher the risk for developing toxoplasmosis.

Among the studied risk factors for toxoplasmosis, our significant recorded 59% revealed that contact with cats is indeed a risk factor for toxoplasmosis. Consumption of semi-cooked meat is another risk factor. These results agreed with those reported by two other studies^[18,34]. On the contrary, concerning animal

exposure, a recent study^[10] detected an insignificant difference between *T. gondii* seropositive and seronegative patients.

There was no significant correlation found between *T. gondii* seropositivity and other risk factors such as raw milk consumption or blood transfusion. This may be attributed to the modesty of the sample size, which presented a limitation for the analysis, thus explaining the statistical insignificance.

Among cases positive for *T. gondii* infection, nonspecific clinical manifestations as fatigue, headache, and fever were reported with a statistically significant difference compared to *T. gondii* seronegative cases. Additionally our study reported organomegaly and lymphadenopathy less frequently, and neurological manifestations of toxoplasmosis were not recorded. Similar results were reported^[9,35]. However, there was no statistically significant increase in the prevalence of organomegaly in their studied patients^[27]. This may be explained in immunocompromised patients by the occurrence of non-specific clinical manifestations of infections in general that may be indistinguishable from the manifestations of the underlying disease *per se*.

In conclusion, hemodialysis patients appeared to have greater *Toxoplasma* seropositivity than healthy controls, implying their higher risk for toxoplasmosis. Toxoplasmosis screening, either serological or molecular, is required before and throughout dialysis treatment. This may aid in the prevention and control of infection in particular patient groupings.

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