ORIGINAL ARTICLE

Prevalence of *Toxoplasma gondii* Infection in Hemodialysis Patients with Chronic Renal Failure and Risk Factors in Diyala Province, Iraq

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ABSTRACT

Introduction: *Toxoplasma gondii* is a protozoan parasite which causes a zoonotic disease called toxoplasmosis. The main purpose of this study was to investigate the seropositivity rate of specific antibodies "anti-*T. gondii* IgG and IgM antibodies using enzyme-linked immunosorbant assay" in hemodialysis patients attending the Teaching Hospital, Baquba City, Diyala Province, Iraq and to determine the potentially preventable risk factors. **Methods: E**ighty five hemodialysis patients with kidney failure and 85 healthy volunteers were selected for this study. **Results:** The percentage of seropositivity for IgG antibodies in patients with hemodialysis was 54.1% while it was 38.2% among the healthy control subjects and the difference was significant between the two groups [Odds Ratio (OR)= 1.8586; 95% Confidence Interval (CI)= 1.0097-3.4212; P= 0.0465]. In contrast, IgM antibodies were not detected in any of the patients or the healthy subjects. Many risk factors were identified, including contact with cats (OR, 2.62; P= 0.0398); eating undercooked meat (OR, 2.6, P= 0.0439); drinking unfiltered water (OR, 2.86, P= 0.0433); and eating outside the home (OR, 5.6, P= 0.0024) as risk factors for toxoplasmosis. However, smoking was not found to be as a risk factor for toxoplasmosis (OR, 2.1, P= 0.1204). **Conclusion:** The results of the present study revealed a high prevalence of toxoplasma infection in hemodialysis patients and therefore, we recommend monitoring these patients for *T. gondii* infection to minimize the spreading of toxoplasmosis via treating the seropositive patients with the available commercial drugs.

Keywords: *Toxoplasma gondii*, Seropositivity, Hemodialysis patients, Healthy subjects

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INTRODUCTION

It has been reported that with age, the incidence of many human chronic diseases including the chronic renal diseases is increasing which in turn disturbs the renal function and consequently, uremia may occur which causes immune –depression that paves the way for various microbial and parasitic infections (1, 2).

Toxoplasma gondii is a cosmopolitan protozoan parasite which causes a worldwide distributed zoonotic disease (toxoplasmosis) which can be transmitted to humans by various ways such as ingestion of oocysts shed by infected cats, ingestion of undercooked or raw infected meat, transplacentally, through organ trans¬plantation, and via the sexual contact (3-5).

In the life cycle, the cats serve as final and intermediate hosts at the same time because both the asexual and sexual reproduction cycles occur in these animals, while humans serve as intermediate hosts because the sexual cycle happens in them (6). The unusual behavior of cats by burying their feces in soil helps the sporulation process of the oocysts and allows them to stay infectious for more than one year (7). After raining, these oocysts come to the surface of the soil and accordingly, any object which comes into contact with soil may become contaminated with the infective oocysts (8, 9).

Bahia-Oliveia et al. (10) stated that "Although toxoplasmosis causes non-symptomatic infections in immunocompetent individuals, it can be a life-threatening risk in immunocompromised individuals". Hemodialysis patients are immunocompromised as a result of uremia and numerous comorbid conditions, disposing them to various bacterial and parasitic infections (11). Recently, Wang et al. (12) conducted a global meta-analysis

to assess the prevalence and odds ratios (ORs) of *T. gondii* infection in immunocompromised individuals and found that the estimated pooled prevalence of *T. gondii* infection in immunocompromised patients was significantly higher than that in the control group and their study was the first to demonstrate that the immunocompromised patients are associated with higher odds of *T. gondii* infection.

Few studies have been carried out in other provinces of Iraq, other than diayala Province, about the sero-epidemiological aspects of toxoplasmosis in hemodialysis patients with chronic kidney diseases. Therefore, the main purpose of the this study, which is the first to be conducted in this province, was to investigate the seropositivity rate of specific antibodies (anti-*T. gondii* IgG and IgM antibodies using enzyme-linked immunosorbant assay) in hemodialysis patients attending the Hemodialysis Unit at the Teaching Hospital in Baquba City, Diyala Province, Iraq in comparison with healthy volunteers. In addition, the aim of the present study was to identify some potentially preventable behavioral risk factors and cultural habits.

MATERIALS AND METHODS

Socio-demographic and behavioral characteristics of patients

A questionnaire has been given to each participant and used to obtain the information regarding the age, sex, residence, and some behavioral habits, such as smoking. All the patients were undergoing hemodialysis at the Hemodialysis Unit at the Baquba Teaching Hospital, Diyala Province, Middle of Iraq during the period from July 2017 and January 2018. In addition, 85 healthy volunteers (control group) were also included.

The inclusion criteria were male and female hemodialysis patients and must willing and able to provide written informed consent and to provide the required information in the questionnaires. The exclusion criteria were: a history or presence of clinically significant cardiovascular, respiratory, gastrointestinal, endocrine diseases or diagnosis of any form of cancer. Patients who received chemotherapy were also excluded. The above mentioned exclusion criteria were also used in selecting the healthy volunteers who were mainly the people attended with the hemodialysis patients and some volunteer blood donors. In addition, the healthy volunteers must not suffer from any kind of renal diseases to be recruited in this study. Each participant was anonymized using a specific ID number which enabled tracking of individuals throughout the study period. Questionnaires were given to all participants regarding the demographic characteristics and some risk factors of toxoplasmosis.

Serological Technique

A blood sample (about 5mL) was collected from each patient and healthy individual and sera were obtained which were stored at -20°C. The Enzyme-Linked Immunosorbent Assay (ELISA; Acon, USA) was adopted to confirm the presence of specific antibodies (IgG and IgM) antibodies. Qualitative ELISA has been used and the microplates have been read at 450nm. Samples were considered positive if the absorbance values were greater than the cut-off absorbance value (0.234). The cut-off value has been calculated using the following equation: Cut-off value= mean absorbance of calibrator 2 – blank absorbtion (0.235-0.001= 0.234). The ELISA assay was conducted according to the manufacturer's instructions.

Ethics approval

The Human Ethical Committee of the College of Sciences, Diyala University, Diyala, Iraq has approved this study (protocol 3/2017). Before the commencement of this study, all participants were informed about the purpose of the study and a written informed consent from each of them was obtained.

Statistical analysis

SPSS v19.0 software was used to analyze the data. Chi-square test was used to perform the bivariate analysis in order to determine the risk factors linked with the seropositivity of toxoplasmosis. In addition, the Odds ratios and the 95% confidence interval were also calculated. P-value ≤ 0.05 was considered statistically significant.

RESULTS

Eighty five hemodialysis patients and 85 apparently healthy volunteers were examined during the period from July 2017 to January 2018. None of the hemodialysis patients exhibits ocular toxopasmosis. The percentage of seropositivity for IgG antibodies in patients with hemodialysis was 54.1% while it was 38.2% among the healthy control subjects and the difference was significant between the two groups [Odds Ratio (OR)= 1.8586; 95% Confidence Interval (CI)= 1.0097-3.4212; P= 0.0465] (Table I). In contrast, IgM antibodies were not detected in any of the patients or the healthy subjects.

Table I: Percentage of seropositivity for anti- *Toxoplasma gondii* IgG and IgM antibodies in patients suffering from kidney failure in comparison with apparently healthy individuals (control group).[a]

Group	Number tested	Number positive		% seropositivity		Patients vs. controls (p-value)
		IgG	IgM	IgG	IgM	
Hemo-dialysis patients	85	46	0	51.1	0	0.0465
Control group	85	33	0	38.2	0	

[a] ELISA was used to detect the anti- *Toxoplasma gondii* IgG and IgM antibodies.

General sociodemographic features (age, gender, and residence area) of kidney patients and healthy control individuals included in the present study and their possible link with the seropositivity for IgG antibodies are shown in Tables II and III. The statistical analysis showed that the seroprevalence of IgG antibodies was not linked with age, sex, and residence area.

Many risk factors were identified in this study (Table IV), including contact with cats and cat feces (OR, 2.62; 95% CI, 1.05-6.6; P= 0.0398); eating undercooked meat (OR, 2.6, 95% CI, 1.02-6.6, P= 0.0439); drinking unfiltered water (OR, 2.86, 95% CI, 1.03-7.91, P= 0.0433); and eating outside the home (mainly in restaurants) (OR, 5.6, 95% CI, 1.84-17.05, P= 0.0024). Although more patients than healthy subjects have been smoking, smoking was not identified as a risk factor because there was no significant difference between the two groups (OR, 2.1, 95% CI, 0.82-5.40, P= 0.1204) (Table IV).

DISCUSSION

Few studies have been carried out in Iraq about the seroprevalence of toxoplasmosis in patients suffering from chronic kidney diseases. The main aim of this study was to investigate the seroprevalence of IgG and IgM antibodies specific for *T. gondii* using ELISA in patients with chronic renal failure (CRF) and doing regular hemodialysis. The percentage of seropositivity for IgG antibodies among CRF patients was 54.1% while it was 38.2% among the healthy volunteers and the difference was significant between the two groups. The positivity rate for IgG antibodies recorded in the present study was higher than the rates reported in the previous studies conducted in Iraq and some other counties on hemodialysis patients. Abdul-Aziz and Zghair (13) studied the seroprevalence of toxoplasmosis in chronic renal failure patients who attended dialysis centers in some of Baghdad hospitals to undergo hemodialysis and

Table II. The impact of age on the seropositivity of anti-*Toxoplasma gondii* IgG among hemodialysis patients and healthy subjects.

Age group (years)	Seroprevalence of T. gondii IgG antibodies						Patients vs. controls (p-value)
	Hemodialysis patients (n= 85)			Control subjects (n= 85)			
	NT	NP (%)	p-value	NT	NP (%)	p-value	
20-39	3	1 (33.0)	reference	8	3 (37.5)	reference	0.8983
40-49	12	8 (66.7)	0.3113	18	8 (44.4)	0.7411	0.2368
50-59	11	8 (72.7)	0.2316	28	10 (35.7)	0.9261	0.0453
60-69	23	10 (43.5)	0.7394	19	9 (47.4)	0.6384	0.8010
>70	33	19 (57.6)	0.4333	12	3 (25.0)	0.5522	0.0626

NT= number tested; NP= number positive

Table III. Gender and residency-related seropositivity of anti-*Toxoplasma gondii* IgG antibodies in chronic renal patients undergoing hemodialysis and apparently healthy subjects.

Variables	Seroprevalence of T. gondii IgG antibodies						Patients vs. controls (p-value)
	Hemodialysis patients (n= 85)			Control subjects (n= 85)			
	NT	NP (%)	p-value	NT	NP (%)	p-value	
Gender							
Males	48	29 (60.4)	reference	44	18 (40.9)	reference	0.0632
Females	37	17 (45.9)	0.1860	41	15 (36.6)	0.6829	0.4021
Total	85	46 (54.1)		85	33 (38.8)		
Residence region							
Rural	42	19 (45.2)	reference	40	12(30.0)	reference	0.1571
Urban	43	27 (62.7)	0.1063	45	21(46.7)	0.1179	0.1307
Total	85	46 (54.1)		85	33(38.8)		

NT= number tested; NP= number positive

Table IV: Univariate analysis of selected risk factors for *Toxoplasma gondii* infection in hemodialysis patients with chronic renal failure and in healthy volunteers (control group)

Risk factors	Seroprevalence of *T. gondii* IgG antibodies				Odds ratio 95%	Confidence interval	P-value
	Hemodialysis patients		Controls				
	MT	NP	NT	NP			
Contact with cats or cat feces	46	29	33	13	2.6244	1.05-6.6	0.0398
Eating of under-cooked meat	46	26	33	11	2.6000	1.02-6.6	0.0439
Drinking unfiltered water	46	20	33	7	2.8571	1.03-7.91	0.0433
Eating in restaurants	46	23	33	5	5.6000	1.84-17.05	0.0024
Smoking	46	22	33	10	2.1083	0.82-5.40	0.1204

NT= number tested; NP= number positive

found that 32.25% of these patients were seropositive for IgG antibodies. Al-Saadawi and Alkhaled (14) investigated the prevalence of anti-*T. gondii* antibodies in hemodialysis patients with CRF attended delivery hospital in Al-Muthanna province (Iraq) and found that 13.04% and 1.09% of the patients were seropositive for IgG and IgM, respectively. In contrast, Al-Dulaimi et al. (15) reported that 80.9% of the patients with CRF who undergoing regular hemodialysis at Al-Kindy Hospital in Baghdad, Iraq were found seropositive for IgG while 44.1% of CRF patients who haven't any hemodialysis session showed seropositivity for the same antibody. The finding of the present study and the other previous studies conducted in Iraq indicate that the hemodialysis patients are at a high risk of getting toxoplasmosis and demonstrate the need for further studies to be done in other parts of Iraq.

The seropositivity recorded in the current study was higher than those recorded in other studies conducted on hemodialysis patients in Iran (7.1%), in Brazil (23%), in Thailand (11%), in Sudan (40.4%) and in Malaysia (51%) (16-20). In contrast, the recorded positivity rate was lower than that reported in similar studies conducted on hemodialysis patients in Egypt (56.7% and 86%) (21, 22), in Turkey(76%) (23), and in Iran (80%) (24) and (80.8%) (25). The observed differencs between the results of the present study and the studies conducted in different countries may be related to various factors such as environmental and cultural differences. Furtado et al. (26) reported that the global differences regarding the prevalence of toxoplasmosis could be attributed to various factors such as country-specific environmental conditions, cultural differences regarding hygienic and feeding habits, climate, and host susceptibility.
In the present study, no significant differences in the percentage of seropositivity were observed between males and females and also between rural and urban patients. Similarly, Jones et al. (27) did not find a significant difference between males and females regarding the prevalence of toxoplasmosis in Brazilian hemodialysis patients. In contrast, some researchers reported a significant link between toxoplsmosis and gender in the Iranian hemodialysis patients (24, 28). The observed diversity could be explained by the differences in sampling methods and life styles. Moreover, Bayani et al. (24) did not observe significant differences in seropositivity among Iranian hemodialysis patients living in rural areas and those living in urban areas.

It has been found that as a parasite, *T. gondii* causes glomerular lesions and urinary abnormalities which lead to renal failure which can be detected by an increase in creatinine levels in the urine (29). Mahboub et al. (30) reported that the increase in the urea concentration in the serum of patients infected with *T. gondii* may be due to the serious kidney damage caused by this parasite (31). On the other hand, Eleftheriadis et al. (32) reported that CRF patients undergoing hemodialysis are prone to acquire various infections. As long as hemodialysis patients are immunocompromised and infection with *T. gondii* can cause serious clinical complications (30), it is possible to propose that toxoplasmosis can pave the way for CRF and vice versa and this depends on which one establishes first.

In the current study, we observed that none of the chronic kidney disease patients was seropositive for the anti-*T. gondii* IgM antibodies. It has been reported that sera that are positive for the anti-*T. gondii* IgG antibodies reveal a chronic pattern of infection and are typically found in patients infected in the most distant past and usually persist for life, while sera that are positive for IgM antibodies reveal acute pattern and are highly suggestive of recently acquired infections and they appear earlier and decline more rapidly than IgG antibodies (33). Similarly, Bayani et al. (24) and Seyyedpour et al. (25) found all the Iranian patients with chronic renal disease and undergoing hemodialysis were negative for the anti-*Toxoplasma gondii* IgM antibody.

Regarding the risk factors, we found that eating undercooked meat (OR, 2.6, P= 0.0439) as a risk factor for toxoplasmosis. Kapperud et al. (9) stated that "individuals who had eaten raw or undercooked mutton, pork, poultry, or minced meat purchased in Norway were at significantly increased risk of infection with *T. gondii*".

Although not so many people own cats in Iraq, many stray cats can be seen in the streets and in the houses of people who do not have cats as they can move freely from house to house. Recently, Retmanasari et al. (34) stated that "a small number of cats do not necessarily indicate there is no risk of infection in that location because a single cat can shed more than 100 million oocysts during the prepatent period (approximately 18 days) and these oocysts can survive outdoors for many months, remain viable for long periods of time in water, and resist freezing and moderately high water temperatures".

The present study identified unfiltered water as a risk factor for the infection with *T. gondii*. Similarly, Retmanasari et al. (35) investigated the seroprevalence of toxoplasmosis in Middle Java, Indonesia, using an EcoHealth approach, combined with geographic information system and reported that consumption of undercooked meat, raw vegetables, and unfiltered water were identified as risk factors for toxoplasmosis. It is well known that water is used for so many different purposes other than human consumption and accordingly, it can be considered as a multipurpose risk factor for the infection with toxoplasmosis.

Eating outside the house (mainly restaurants) was identified as a risk factor for toxoplasmosis in the current study. In contrast, Kapperud et al. (9) did not

identify eating outside of the home as a risk factor for toxoplasmosis in Norway.

In the present study, smoking was not identified as a risk factor for toxoplasmosis. In contrast, Alvarado-Esquivel et al. (34) investigated the seroprevalence and associated risk factors for *T. gondii* infection in healthy blood donors and found a link between toxoplasmosis and smoking in Mexico.

CONCLUSIONS

The results of the present study revealed a high prevalence of toxoplasma infection in hemodialysis patients and therefore, we recommend monitoring these patients for *T. gondii* infection to minimize the spreading of toxoplasmosis by treating the seropositive patients with the available commercial drugs.

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