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Investigation of Anti-*Toxoplasma gondii* Antibodies in the Hemodialysis Patients with ELISA Method

Hemodiyaliz Hastalarında Anti-Toxoplasma gondii Antikorlarının ELISA Yöntemi ile Araştırılması

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ABSTRACT

Objective: The aim of this study was to determine the seroprevalence of *Toxoplasma gondii* in hemodialysis (HD) patients and to reveal the importance of toxoplasmosis as a risk factor in these patients.
Methods: The study was carried out between 26.12.2013 and 01.01.2016 at Van Yüzüncü University Dursun Odabaş the Medical Center on patients with chronic renal failure who entered HD. As the patient group in the study, 150 patients with chronic renal failure who underwent HD; as the control group, 50 people without any known chronic disease and who did not receive any immunosuppressive therapy were included. The ELISA method was used to determine anti-*T. gondii* IgG and IgM antibody levels. A questionnaire including risk factors that may cause the transmission of *T. gondii* was applied to the patient and control groups.
Results: In the study, 89 out of total 150 HD patients (59.3%) were found anti-*T. gondii* IgG antibody seropositive and 4 were (2.7%) anti-*T. gondii* IgM antibody seropositive. Fourteen of 50 healthy individuals in the group (28%) were anti-*T. gondii* IgG antibody positive, while none in this group was anti-*T. gondii* IgM antibody positive. Statistical analysis demonstrated there were separate significant correlations between both anti-*T. gondii* IgG ($p<0.01$) and anti-*T. gondii* IgM antibody ($p<0.05$) frequencies with chronic renal failure. While there were no statistically significant differences in the prevalence of anti-*T. gondii* IgG antibody identification based on gender and age groups, there were significant differences between the prevalence of anti-*T. gondii* IgM antibody based on both gender ($p<0.05$) and age groups ($p<0.05$). Some living conditions and habits of the patient group were evaluated statistically, and a significant correlation ($p<0.05$) was found between eating only raw meatballs and toxoplasmosis seropositivity.
Conclusion: As a result, it was understood that the physicians who monitor of HD patients should assess toxoplasmosis among the risk factors.
Keywords: *Toxoplasma gondii*, hemodialysis patients, seroprevalence

ÖZ

Amaç: Bu çalışmanın amacı hemodiyaziz (HD) hastalarında *Toxoplasma gondii*'nin seroprevalansını saptamak ve bu hastalarda bir risk faktörü olarak toksoplazmozun önemini ortaya koymaktır.
Yöntemler: Çalışma 26.12.2013-01.01.2016 tarihleri arasında Van Yüzüncü Üniversitesi Dursun Odabaş Tıp Merkezi'nde, kronik böbrek yetmezliği olup HD'ye giren hastalar üzerinde yürütüldü. Hasta grubu olarak, kronik böbrek yetmezliği olup HD uygulan 150 hasta; kontrol grubunu olarak ise, bilinen herhangi bir kronik hastalığı olmayan ve herhangi bir immünosüpresif tedavi almayan 50 kişi dahil edildi. Anti-*T. gondii* IgG ve IgM antikor seviyelerini belirlemek için ELISA yöntemi kullanıldı. Hasta ve kontrol grubuna, *T. gondii*'nin bulaşmasına neden olabilecek risk faktörlerini içeren bir anket uygulandı.
Bulgular: Çalışmamızda toplam 150 HD hastasının 89'u (%59,3) anti-*T. gondii* IgG antikoru, 4'ü (%2,7) IgM antikoru yönünden seropozitif bulunmuştur. Kontrol grubundaki 50 sağlıklı bireyin 14'ü (%28) anti-*T. gondii* IgG antikoru yönünden pozitif bulunurken, bu grupta IgM antikoru yönünden pozitiflik saptanmamıştır. Yapılan istatistiksel değerlendirmede, kronik böbrek yetmezliği ile hem anti-*T. gondii* IgG ($p<0,01$) hem de IgM antikoru ($p<0,05$) sıklığı arasında ayrı ayrı anlamlı ilişki saptanmıştır. Çalışmada anti-*T. gondii* IgG antikorunu belirleme sıklığında cinsiyet ve yaş grupları arasında istatistiksel olarak anlamlı fark

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saptanmazken, anti-*T. gondii* IgM antikorunu belirleme sıklığında hem cinsiyet (p<0,05) hem de yaş grupları (p<0,05) arasında fark belirlenmiştir. Hasta grubuna ait bazı yaşam koşulları ve alışkanlıklar, istatistiksel olarak değerlendirilmiş ve sadece çiğ köfte yeme alışkanlığı ile toksoplazmoz seropozitifliği arasında anlamlı bir ilişki (p<0,05) saptanmıştır.
Sonuç: Sonuç olarak HD hastalarının takibini yapan hekimlerin toxoplasmosisi risk faktörleri arasında değerlendirmesi gerektiği düşünülmüştür.
Anahtar Kelimeler: *Toxoplasma gondii,* hemodiyaliz hastaları, seroprevalans

INTRODUCTION

The only species of the *Toxoplasma* genus of Apicomplexa phylum that could settle in humans, other mammals, and birds and become a disease factor is *Toxoplasma gondii* (*T. gondii*). *T. gondii* is the most common zoonotic obligate intracellular protozoan in humans. The definitive host of the parasite is cats and felines, its intermediate host is many warm-blooded animals and including humans. It has three infective forms in its life cycle: Tachyzoite, bradyzoite, and oocyst. Transmission occurs mainly through food and drinks contaminated with oocysts excreted in cat feces, or by eating meat containing tissue cysts undercooked or raw. It can also be transmitted through blood transfusion, organ transplantation and placental transmission from mother to baby (1-4).

It is estimated that approximately 30% of the world population has chronic toxoplasmosis. It has been reported that the *T. gondii* seroprevalence rate is between 7-10% in Norway and the United Kingdom, 44% in France, 50% in Germany, 11% in the USA, and between 17.3% and 78% in Turkey. The socio-economic level of societies, hygiene and sanitation conditions dietary habits and climatic conditions are the factors affecting the incidence of this parasite (5-9).

While Toxoplasmosis is usually asymptomatic and chronic in immunocompetent individuals, it is severe in patients with AIDS and cancer, in immunocompromised or inadequate individuals such as bone marrow and solitary organ transplants and many other patient groups. Moreover, if not controlled, it may result in death. It can cause serious complications such as fatal *Toxoplasma* encephalitis, myocarditis, pneumonia, lymphadenopathy and chorioretinitis in immunosuppressed patients. If *T. gondii* is in placental transmission, the severity of infection in the fetus may increase or fetal deaths may occur depending on the period of pregnancy and the state of the mother's immune system (1,4,10,11).

In patients with end-stage renal disease, in addition to inadequate kidney function, the inability to remove urea, a product of metabolic waste, from the body, causes the formation of a pro-inflammatory environment. The inflammatory environment originating from uremic causes premature aging of the T-cell compartment and disruption of the T-cell-mediated immune system. Due to T-cell-mediated immune dysfunction in hemodialysis (HD) patients, increased susceptibility to infectious diseases, susceptibility to infections, a poor response to vaccination, auto-immune diseases and a high risk of malignancy may be present. Therefore, these patients may be more susceptible to many infections, including infections caused by opportunistic protozoan parasites (12-15). The need for blood transfusion and surgical intervention in HD patients is greater than in the normal population. Like other blood-borne factors, *T. gondii* is also very likely to infect these patients (10,12,16,17).

The objective of the present study is to determine *T. gondii* seroprevalence in HD patients and establish the significance of toxoplasmosis as a risk factor.

METHODS

This study was first approved by the Clinical Research Ethics Committee of the Faculty of Medicine of Van Yüzüncü Yıl University (decision no: 2014/02). The study was conducted between 26.12.2013-01.01.2016 Van Yüzüncü Yıl University Dursun Odabaş Medical Center, on patients with chronic renal failure (CRF) who entered HD.

Patient and Control Group

As a patient group, 150 patients (80 female, 70 male, 26 younger than 35, 124 older than 35 years old) with CRF who underwent HD were included in the study; as a control group, 50 people (28 female, 22 male, 41 younger than 35, 9 older than 35 years old) who did not have any known chronic diseases and did not receive any immunosuppressive therapy were included. Blood samples taken from both groups were centrifuged at 3000 rpm to determine anti-*T. gondii* IgG and IgM antibody levels, and the separated blood sera were stored at -20 °C. A questionnaire including risk factors that may cause transmission of *T. gondii* was applied to the patient group.

ELISA

The reagents prepared in accordance with the ELISA commercial kit (VIRCELL S.L-Granada, Spain) procedure were started to work after they were kept at room temperature for 60 minutes. Anti-*T. gondii* IgM and anti-*T. gondii* IgG antibodies in the plates coated with 2 cut-off, a negative and a positive control sera were determined and Wells for over 100 µL serum (Neolone and Bronidox), the other wells 100 µL sample dilution solution (Neolone, Bronidox phosphate buffer containing protein stabilizers and blue color) was added. 5 µL of each sample was added to the corresponding wells and covered, incubated at 37 °C for 60 minutes. After incubation, the liquid was aspirated from the wells. Five times washing with a washing solution was done so that there were 0.3 mL per well and the remaining liquids were drained. 100 µL of diluted conjugate was added to each well, incubated for 60 minutes and washed again. After the remaining liquids in the wells were drained, 100 µL of substrate solution (trimethylbenzidine) was added and incubated for 20 minutes at room temperature to be protected from light. After adding 50 µL of stop solution (sulfuric acid) to all wells, a reading at 450 nm was performed with a spectrophotometer within one hour.

The samples were classified as negative [antibody level (AL) <9], borderline (AL =9-11) and positive (AL >11; positive AL = [optic density of the samples (OD)/mean OD of cut-off serum) x10]. The study of suspicious sera was repeated with further dilutions until a negative result was obtained.

Statistical Analysis

Descriptive statistics for the categorical variables were presented as count and percent. The chi-square test was calculated to determine relationships between the categorical variables. Z-test was also used for the comparison of proportions. Descriptive

statistics for the studied variables (characteristics) were presented as mean, standard deviation, minimum and maximum values. The statistical significance level was accepted as 5% and calculations were conducted using SPSS (ver. 13) and MINITAB (ver. 14) statistical software.

RESULTS

In the study, 89 out of a total of 150 HD patients (59.3%) were found anti-*T. gondii* IgG antibody seropositive and 4 were (2.7%) anti-*T. gondii* IgM antibody seropositive. Fourteen of 50 healthy individuals in the group (28%) were anti-*T. gondii* IgG antibody positive, while none in this group was anti-*T. gondii* IgM antibody positive. Statistical analysis demonstrated there were separate significant correlations between both anti-*T. gondii* IgG and anti-*T. gondii* IgM antibody frequencies with CRF (Table 1, 2).

While there were no statistically significant differences in the prevalence of anti-*T. gondii* IgG antibody identification based on gender and age groups, there were significant differences between the prevalence of anti-*T. gondii* IgM antibody based on both gender ($p<0.05$) and age groups ($p<0.05$) (Table 1, 2).

In the study, survey data to determine some living conditions and habits of the patient group were evaluated statistically and a significant correlation ($p<0.05$) was found between eating only raw meatballs and toxoplasmosis seropositivity. It was found that 57.1% of 35 people who stated that they had cats at home or in their immediate vicinity were seropositive. But, there was no statistically significant difference between the presence of cats and *T. gondii* seropositivity. There was also no statistically significant difference between surface decontamination and glove use in contact with raw meat and toxoplasmosis seropositivity (Table 3).

DISCUSSION

Although toxoplasmosis is one of the most common infectious diseases in the world, it usually progresses with asymptomatic or non-specific symptoms. However, it causes serious clinical pictures in immunocompromised or inadequate individuals (15).

Globally increasing CRF has been recognized as an important public health problem worldwide. The global prevalence of CRF is estimated at 13.4% (18).

Patients with CRF undergoing HD are considered immunocompromised due to immune response dysfunctions related to phagocytosis, chemotaxis, and the complement system. Therefore, these patients are more vulnerable to opportunistic pathogens such as *T. gondii*. It is very important to know and clinically monitor *T. gondii* seropositivity in these patient groups (14). In the meta-analysis data evaluating *T. gondii* seropositivity in HD patients in Iran; anti-*T. gondii* IgG positivity was observed in 58% of the HD patients and 40% of the healthy control group, anti-*T. gondii* IgM in 2% of HD patients in the study was detected, but these antibodies were not detected in the healthy control group (14). Aufy et al. (19) found that 36.8% of 19 kidney patients who did not undergo HD had anti-*T. gondii* IgG, anti-*T. gondii* IgM in 10.5%; anti-*T. gondii* IgG in 56.7% of 30 patients on regular HD, anti-*T. gondii* IgM in 16.7%; anti-*T. gondii* IgG in 69% of 29 patients who underwent kidney transplantation, anti-*T. gondii*

Table 1. Distribution of anti-*T. gondii* IgG and anti-*T. gondii* IgM antibody prevalence based on gender

Anti-*T. gondii* IgG				**Anti-*T. gondii* IgM**			
Groups	**Gender**	**Positive number %**		**Groups**	**Gender**	**Positive number %**	
HD patients	Famele (n=80)	49	61.3	**HD patients**	Female (n=80)	4	5
	Male (n=70)	40	57.1		Male (n=70)	0	0
	*Z: 0.51; p>0.05				*Z: 2.05; **p<0.05**		
	Total (n=150)	89	59.3		Total (n=150)	4	2.7
	Z: 4.17; **p<0.01				**Z: 2.03; **p<0.05**		
Control	Female (n=28)	7	25	**Control**	Female (n=28)	0	0
	Male (n=22)	7	31.8		Male (n=22)	0	0
	Total (n=50)	14	28		Total (n=50)	0	0

*Result of between-gender comparison, **result of patient-control group comparison, HD: Hemodialysis

Table 2. Distribution of anti-*T. gondii* IgG and anti-*T. gondii* IgM antibody prevalence based on age

Anti-*T. gondii* IgG				**Anti-*T. gondii* IgM**			
Groups	**Age groups**	**Positive number %**		**Groups**	**Age groups**	**Positive number %**	
HD patients	≤35 (n=26)	12	46.2	**HD patients**	≤35 (n=26)	0	0
	≥36 (n=124)	77	62.1		≥36 (n=124)	4	3.2
	*Z: 1.49; p>0.05				*Z: 2.03; **p<0.05**		
	Total (n=150)	89	59.3		Total (n=150)	4	2.7
Control	≤35 (n=41)	10	24.4	**Control**	≤35 (n=41)	0	0
	≥36 (n=9)	4	44.4		≥36 (n=9)	0	0
	Total (n=50)	14	28		Total (n=50)	0	0

*Result of between-age group comparison of HD patient, HD: Hemodialysis

Table 3. Statistical evaluation results for certain life conditions and habits of the patient group

Some of the living conditions and habits	Features	Seronegative number (%)	Seropositive number (%)	Significance value
Cat at home or close proximity	Yes (n=35)	15 (24.6)	20 (22.5)	Z =0.3 p>0.05
	No (n=115)	46 (75.4)	69 (77.5)	
The habit of eating Turkish steak tartare	Yes (n=102)	47 (77)	55 (61.8)	Z =2.05 **p>0.05**
	No (n=48)	14 (23)	34 (38.2)	
Surface cleanliness in contact with raw meat in the kitchen	Yes (n=95)	37 (60.7)	58 (65.2)	Z =0.22 p<0.05
	No (n=55)	24 (39.3)	31 (34.8)	
Glove use in contact with raw meat	Yes (n=122)	51 (83.6)	71 (79.8)	Z =0.6 p>0.05
	No (n=28)	10 (16.4)	18 (20.2)	

IgM in 24.1%; anti-*T. gondii* IgG in 23.1% of the control group of 13 healthy subjects antibody positivity was detected, but in this group of patients, anti-*T. gondii* IgM have not determined antibody positivity. In another study, 31.7% of 120 HD patients had anti-*T. gondii* IgG, anti-*T. gondii* IgM in 3.3% and anti-*T. gondii* IgG in 7% of the control group of 100 people, anti-*T. gondii* IgM in 2% antibodies detected (15).

Various studies have been conducted to determine the seroprevalence of *T. gondii* in patients undergoing HD in Turkey. Yalçın et al. (20) showed anti-*T.gondii* IgG in 76.6% of HD patients and 63.3% of the control group; Şahin et al. (12) reported anti-*T. gondii* IgG in 77.77% of HD patients, anti-*T. gondii* IgM at 1.92% was anti-*T. gondii* IgG in 30.76% of the control group, anti-*T. gondii* IgM at 1.85%; Yazar et al. (21) anti-*T. gondii* IgG in 56.06% of HD patients, anti-*T. gondii* IgM in 1.73%, anti-*T. gondii* IgG in 20% of the control group; Ocak et al. (22) found anti-*T. gondii* IgG seropositivity in 76.5% of HD patients and 48% of the control group. In the study conducted by Hamamcı et al. (23), anti-*T. gondii* was found in 25 of 30 predialysis patients, 28 of 30 HD patients, and 12 of 30 healthy control group but anti-*T. gondii* IgM antibody positivity was not detected. In many studies conducted in Turkey, seropositivity of toxoplasma was also investigated in immunocompromised patient groups and different results were found.

In a study conducted to determine the seroprevalence of *T. gondii* in cancer patients, anti-*T. gondii* IgG antibody positivity was found in 60% of 100 cancer patients receiving chemotherapy and 27% of 100 healthy control group. In addition, anti-*T. gondii* IgM antibody positivity was determined in both cancer patients and one person from the control group (24). In the study conducted on patients with diabetes mellitus (DM); anti-*T. gondii* IgG seropositivity was detected in 40.5% of 74 DM patients and 38.2% of healthy individuals in the control group, but no positivity for anti-*T. gondii* IgM antibodies was detected in either group (25). In a study, anti-*T. gondii* IgG antibody positivity was determined at a rate of 43.5% in HIV-positive patients, and anti-*T. gondii* IgM antibody positivity was not detected in any of the patients (6). In a study conducted on different immunosuppressive patient groups (26), 14.7% of the patients were found to be positive for anti-*T. gondii* IgG and 0.5% for anti-*T. gondii* IgM. In the studies listed above (6,24-26), it is noteworthy that *T. gondii*, known as opportunistic parasitosis, is frequently encountered in individuals with suppressed or impaired immunity.

In one of the studies in which HD patients were evaluated in terms of toxoplasmosis (19), the risk of toxoplasmosis would increase as more dialysis was exposed, in the other (27) it was emphasized that the rate of *T. gondii* infection is higher in HD patients than in healthy people and that HD or blood transfusion carries a potential risk for *T. gondii* infection. In some studies (14,21), it was emphasized that *T. gondii* antibody positivity was high in patients with CRF who underwent HD and that this patient group should be followed up periodically for toxoplasmosis.

In this study, 59.3% of 150 HD patients had anti-*T. gondii* IgG, 2.7% had anti-*T. gondii* IgM, and 28% of the control group, which included 50 people without any known chronic disease, had anti-*T. gondii* IgG positive and no positivity was detected in terms of IgM antibody. In addition, a statistically significant difference was found between the patient and control groups in terms of both anti-*T. gondii* IgG antibody ($p<0.01$) and anti-*T. gondii* IgM antibody ($p<0.05$) positivity. Following the literature, *T. gondii* seroprevalence was found to be higher in HD patients compared to the healthy control group (12,14,20-23). In the statistical comparison between HD patients who are among the immunocompromised patient groups and the control group, the statistical significance of *T. gondii* seroprevalence indicates that toxoplasmosis is a parasitic factor that should be considered in HD patients.

Although toxoplasmosis is seen at any age, as the age increases, the probability of encountering the causative agent of the disease and therefore the positivity rate increases (4). In one of the studies conducted on immunocompromised patients (28), seropositivity of toxoplasmosis was reported to be higher in the 40-50 age group, and in the other (29) in those younger than 30 years of age. Güleşçi and Otkun (30) found the anti-*T. gondii* IgG rate to be 67.5% in cases with a mean age of 53.7%. Şirin et al. (8) detected the lowest anti-*T. gondii* IgG seropositivity in the 0-10 age group (11.1%) and the highest in the >60 age group (55.4%). In a study (31) it was determined that the rate of seropositivity increased with increasing age. In our study, similar to the results of the four studies listed above (8,28,30,31), it was observed that there was an increase in the rate of seropositivity in parallel with the advancement of age.

Different results were found in studies evaluating the factors affecting the frequency of toxoplasmosis. Alvarado-Esquivel et al. (32) determined a statistically significant relationship between *T. gondii* infection and undercooked meat consumption, raw cow and goat milk consumption, and the presence of cats at home. Tekay and Özbek (33) reported that consumption of raw meat carries a great risk for the formation of tissue cyst of *T. gondii* and this is responsible for the high seropositivity rate. In our study, the relationship between the presence of cats in the patients' homes or in their immediate surroundings, surface cleaning in contact with

raw meat, the use of gloves in contact with raw meat, the habit of eating raw meatballs and the frequency of toxoplasmosis were evaluated, and there was a statistically significant relationship between the habit of eating only raw meatballs and the frequency of this parasitosis ($p<0.05$).

CONCLUSION

As a result, developing strategies to prevent toxoplasmosis in immunocompromised patients such as HD revealed the necessity of focusing on risk factors for transmission and activation. It should not be forgotten that there is a risk of transmission of this infection by transfusion of blood or blood products to these patients during HD the procedure of patients with CRF and necessary precautions should be taken. Before it reaches the point of treating *T. gondii* infection with medication, every segment of society, especially patients with impaired immunity, should be informed about the transmission forms of the disease. Considering the results of our study, it was concluded that toxoplasmosis is important in HD patients and that it would be appropriate to evaluate this disease among risk factors by physicians who follow-up HD patients.

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* Information

This article is an abbreviated version of the Master's Thesis of Şehriban Yürektürk.

* Ethics

Ethics Committee Approval: Before the research, Van Yüzüncü Yıl University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee's 27.02.2014, permission was obtained with the decision numbered 2014/02.

Informed Consent: Informed consent was obtained.

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* Authorship Contributions

Concept: Ş.Y., Design: Z.T.C, H.Y., Data Collection or Processing: Ş.Y., Z.T.C., Analysis or Interpretation: Ş.Y., Z.T.C, H.Y., Literature Search: Ş.Y., Z.T.C, H.Y., Writing: Ş.Y, Z.T.C.

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