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Original Article

Serological and Molecular Survey of *Toxoplasma gondii* Infection in Hemodialysis Patients with Chronic Renal Disease in Zahedan, Iran

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ABSTRACT

Background: *Toxoplasma gondii* is one of the most prevalent zoonotic opportunistic parasitic infections in the world. The aim of this study was to examine chronic and acute toxoplasmosis molecularly and serologically in Hemodialysis patients.

Methods: A total of 238 serum samples of hemodialysis patients with chronic renal disease (119 samples) as case group and healthy individuals (119 samples) as control group were enrolled in Zahedan City, southeastern Iran. The molecular and serological detection of toxoplasmosis were conducted on all samples using nested-PCR and ELISA.

Results: The prevalence rates of anti-T. gondii IgM and IgG and *T. gondii* DNA in the case group were respectively 0%, 44.5% and 29.4% and the corresponding values in the control group were 0.8%,23.5% and 2.52 % respectively (p≥0.05).

Conclusion: In conclusion, preliminary *Toxoplasma gondii* infection screening is required using serological techniques, particularly in hemodialysis patients who are frequently exposed to hemodialysis so as to stop infection dissemination.

Keywords: *Toxoplasma*, Hemodialysis patients, Nested-PCR, Zahedan

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Introduction

Toxoplasmosis is one of the most prevalent zoonotic opportunistic parasitic infections throughout the world, induced by an intracellular obligate protozoan, named *Toxoplasma gondii* (*T. gondii*) (1). Nearly a third of the world's population are infected with this type of parasite, by consuming vegetables, soil, and water contaminated with oocysts excreted by cats as the specific hosts, and/or by eating uncooked or raw meats with cysts in the tissues of warm-blooded vertebrate animals as the indirect hosts (2, 3). Besides, other routes, such as blood transfusion and organ transplantation are effective in transmitting infection from donors to recipients (4-6). The nature of this infection varies from self-limiting asymptomatic to highly fatal or morbid, where primary conditions and immunity status may affect the final outcome of this infection to a great extent (7). In recipients, where a seropositive donor transmits infection to an immunocompromised seronegative recipient, it will lead to an increase in the reactivation of chronic toxoplasmosis in the former donor and causes a fatal or severe disease in the next recipient (5, 8, 9). There are varied occurrence levels of *Toxoplasma* throughout the world and in various regions of Iran, depending on various factors, including culture, age, nutrition, geographical situation, contact with soil, health status, pets, and the like, turning it into a major public health concern (10-13). In addition, the type of the underlying condition in some individuals is effective in intensifying toxoplasmosis. In general, the seroprevalence rates of *Toxoplasma* in healthy people and immunocompromised patients in Iran are 33-45% and 43-56%, respectively (9, 14). In some patients, including renal hemodialysis patients (HP) and kidney patients, toxoplasmosis cannot be diagnosed using only clinical manifestations; hence, the accurate and certain diagnosis of the disease could be possible by serological and parasitological assessments (15). Hemodialysis patients are not generally categorized as immunosuppressed patients, for a decrease in cell-induced immunity in kidney disorders leads to the high risk of the revitalization of inactive toxoplasmosis (7, 16). Hence, this study was conducted to examine chronic and acute toxoplasmosis molecularly and serologically in Hemodialysis patients in Zahedan City, southeastern Iran, in 2018.

Material and Methods

Study site

This case-control study was carried out using the nonprobability sampling method on 238 individuals who divided into the case (119 cases) and control (119 cases) groups at the hemodialysis departments of Khatemolanbia and Imam Ali hospitals of Zahedan University of Medical Sciences in Zahedan City, from April to September 2018. The case group consisted of hemodialysis patients with chronic renal disease and the control group consisted of healthy individuals without renal complications. Zahedan, being the capital of the province of Sistan and Baluchistan, is in the Balochistan region, bordering Afghanistan and Pakistan in southeastern Iran (Figure 1). The climate of this city is cool in winter and hot in summer, with a population of approximately 550,000. Questionnaires, including demographic data about age and sex, were filled out by the participants in the course of sample collection.

Figure 1. Geographic Map of Sistan and Baluchistan Province in Iran, Study location (zahedan) is displayed with arrow

Serological test

Serum samples (5 ml) of 238 individuals including 119 hemodialysis patients (case group) and 119 healthy individuals (control group) were obtained. In addition, to extract DNA for subsequently use, the buffy coat was taken from two groups. Next, the obtained samples were delivered to the serology laboratory of the department of mycology and parasitology of Zahedan University of Medical Sciences, Zahedan, Iran. The serum samples were stored at -20°C prior to use and measured concerning anti-*Toxoplasma* IgM and IgG, making use of a commercial scale enzyme immunoassay kit (PishtazTeb Diagnostics Co., Tehran, Iran), based on the manufacturer's protocol. The positive cut-off value of IgG and IgM antibodies was defined as the upper limit of the 10 and 1. 1 U/mL, respectively.

DNA extraction and Nested-PCR

DNA extraction was applied to the entire buffy coat of the samples. Making use of the DynaBio™ Tissue/Blood DNA Extraction Mini Kit (Takapozist, Iran), DNA was extracted. DNA extraction was conducted according to the Company's protocol. Next, purified DNA was kept at -20 ºC. The extraction of positive DNA was applied to RH strain of *T. gondii* (which was provided from the Department of Parasitology, Tarbiat Modares University, Iran). Nested-PCR was conducted to amplify the 194 bp fragment of B1 gene of *T. gondii*. Making use of the external primers of R1: 5′-CCGCAGCGACTTCTATCTCT -3′ and F1: 5′-TCAAGCAGCGTATTGTCGAG-3′, the first stage of PCR was passed through. Likewise, Nested-PCR was conducted using the internal primers of R2: 5′-TCTTTAAAGCGTTCGTGGTC-3′ as well as F2: 5′-5′-GGAACTGCATCCGTTCATGAG-3′ (18). Amplification was applied to the ultimate volume of 15µl of reaction mixes, including 1µl of reverse and forward primers and 7.5µl of the altered reaction of Taq DNA polymerase Master Mix RED (Bioneer Co., Korea), including 250 Um of dNTPs, 1U of Top DNA polymerase, 30 Mm of KCL, 10 Mm of Tris-HCL, 2 µl of template DNA, 1.5 Mm of Mgcl2, as well as 4.5µl of distilled water. PCR conditions included 5 minutes at 95°C succeeded by thirty 20-second cycles at 94°C, 20 seconds at 72°C, 20 seconds at 53°C and the ultimate elongation of 10 minutes at 72°C. Next, the primary product of PCR was diluted 1:25, using distilled water; it was subsequently used as a template at Nested-PCR's 2nd stage.

The 2nd amplification task was done using the comparable volume of 15µl, including 1µl of the second reverse and forward primers, 7 µl of the altered reaction of Taq DNA polymerase Master Mix RED (Bioneer Co., Korea), 2µl of the product of PCR at the diluted ratio of 1:25 and 5µl of distilled water. Nested-PCR was conducted under comparable reaction conditions of the 1st round, excluding annealing which was used in 35 cycles. To confirm Nested-PCR findings, the analysis of the products was carried out utilizing the 1% agarose gel for separation

purposes, stained with an ethidium bromide solution and examined under the UV light. At the final stage, the amplified length of the 194 bp fragment of B1 gene of *T. gondii* was showen (18, 19).

Ethical consideration

This study was performed as part of M.Sc. student thesis of Masoumeh Sani Haidari, and it was financially supported by the grant No.171 and the Ethics Committee (Permit Number: IR. Zaums.Rec.1396.173) provided from Zahedan University of Medical Sciences, Zahedan, Iran.

Results

Of the 119 Hemodialysis patients, 58 ones (48.7%) were male and 61patients (51.3%) were female. Furthermore, the age range of the subjects was between 22 and 68 years, and the mean age of participants was 39.1. The antibodies of IgG seropositivity were identified in the sera of 28 individuals (23.5%) of the HG group and 53 individuals (44.5%) of the HP group. As it is seen in Table 1, no individual in the HP group and 0.8% in the HG group were IgM seropositive ($p \geq 0.05$). The results of the statistical analysis showed that IgG antibodies were of a higher level in the HP group than in the HG group ($p \geq 0.05$), yet no significant difference was found between them in terms of the IgM antibodies of *T. gondii*. In addition, there was no significant relationship between sex and the prevalence of *Toxoplasma* antibodies.

Table1. The relationship between seroprevalence of *T. gondii* and sex in hemodialysis and control groups

Toxoplasmosis	**Samples N (%)**		**IgG Positivity N (%)**		**Sex/ IgG Positivity** **Male N(%)**	**Female N(%)**	**P value**
Hemodialysis Patients (HP)	119	(100%)	53	(44.5%)	29 (24.33%)	24 (20.1%)	P≥0.05
Healthy Group (HG)	119	(100%)	28	(23.5%)	15 (12.6)	13 (10.9)	
Toxoplasmosis	Samples N (%)		IgM Positivity N (%)		Sex/ IgM Positivity Male N(%)	Female N(%)	P value
Hemodialysis patients (HP)	119	(100%)	0	(00)	0 (00%)	0 (00%)	P≥0.05
Healthy individuals (HG)	119	(100%)	1	(0.85%)	0 (00%)	1 (0.85%)	

Toxoplasma DNA in 35 samples of HP group (29.4%) and 3 samples of HG group (2.52%) were identified, respectively. Nearly, all samples of positive PCR were belonged to the groups with negative and positive IgG antibodies of *T. gondii*, and none of them were belonged to the control group. Furthermore, molecular positive and Toxoplasma-positive samples were prevalent in 19 individuals (15.97%) of HP patients (Table 2, Figure. 2).

Table2. The frequency of *Toxoplasma* IgG antibody results with ELISA method based on Nested-PCR in HP group

ELISA / **Nested- PCR**	**Samples N (%)**	**IgG Positivity N (%)**	**IgG Negative N (%)**	**P value**
Nested- PCR Positive	35 (29.4%)	19 (15.97%)	16 (13.43%)	p≥0.05
Nested-PCR Negative	84 (70.6%)	50 (42%)	34 (28.6%)	
Total	119 (100%)	69 (57.97%)	50 (42.03%)	

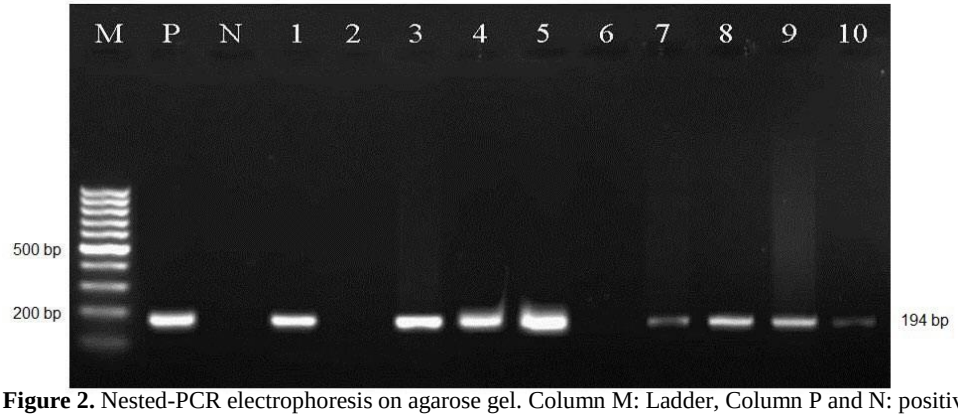

Figure 2. Nested-PCR electrophoresis on agarose gel. Column M: Ladder, Column P and N: positive and negative control, Column 1, 3-5 and 7-10: positive sample, and column 2 and 6: negative samples

Discussion

Infection with *T. gondii* in immune-competent people is frequently associated with a high level of prognosis, yet in immune-compromised individuals, like HP patients, it could be deadly as a result of infection reactivation (9). Therefore, the examination of Toxoplasma DNA using Nested-PCR in IgG positive patients helps the timely and definite diagnosis of the infection. In this study, the *T. gondii* parasite was identified to be circulating DNA of gene B1 in 35 (29.4%) samples of the HP group. As many as 19 samples with positive PCR were related to patients who were positive for IgG anti-Toxoplasma gondii. The values in the results of the present study were higher than those of other studies conducted in other parts of the world. In the study by Saki *et al.*, (2013) it was demonstrated that PCR yielded positive results in 1.4% (4/280) of HP patients, with no positive result in healthy individuals (20). In two previous studies conducted in this regard, three positive DNA samples of *T. gondii* were detected in HP patients (20, 21). In addition, in the study conducted by Rezavand *et al*., (2016) five patients (6%) were diagnosed with *Toxoplasma* DNA positive using PCR (22). Furthermore, the sreological results of the present study indicated that the number of individuals who were positive for IgM (0%) and IgG (44.5%) antibodies among hemodialysis patients differed significantly from that of the healthy individuals (0.8% and 23.5%, respectively). In the same manner, in Solhjoo *et al.* (2010) study in Jahrom Town, Fars province, Toxoplasma IgG antibodies were found in 59.10 percent of HP patients and 36.40 percent of healthy individuals (23). In another study, *T. gondii* IgG seropositivity has been reported in 76% and 80% of healthy volunteers and HP patients (P>0.05), respectively (20). Two studies in Khuzestan (Southwest of Iran) have reported significantly higher levels of *T. gondii* IgM and IgG antibodies in HP patients than in the HG group (24, 25). In continuation of this investigation, in a study by Ebrahim Zadeh *et al.* (2014) in Sistan and Baluchistan province, the seroprevalence levels of the IgG antibody for *Toxoplasma* among HG and HP groups were respectively 29.7% and 56.7% (p<0.036). While, the antibody for *T. gondii* IgM was identified in only 13.5% of the HP group (18). In the two studies done in East Azerbaijan province and Chaharmahal and Bakhtiari province, in 2015, higher levels of the *Toxoplasma* IgG antibody in HP patients (70% and 45%) than in the HG group (68% and 33%) have been reported (26, 27). In a study conducted by Rasti *et al*. (2016) in Iran's central cities of Kashan and Qom, the seroprevalence levels of IgG of *T. gondii* in HP patients and the HG group were shown to be 33.3% and 63% respectively; in addition, only one case of IgM seropositivity was identified in the HP group. Furthermore, the prevalence rates of IgM and IgG anti-T.gondii in HP patients were respectively 3.3% and 60%; however, the corresponding values were respectively 0% and 37.8% in the healthy group (28). In a study conducted by Seyyedpour *et al.* (2016), the antibodies of *T.* gondii IgG were found in 80.8% of the HP group and 91% of the healthy group; while, no individual was positive for IgM antibody of *T. gondii* in the HP group, 2.76% of the healthy group were diagnosed to be positive (29). Dorri *et al*. (2017) have reported 43.4% and 73.7% rates of IgG anti-*Toxoplasma* in HG and HP groups in Sistan and Baluchistan province, respectively (30). Nevertheless, in another study, the IgM and IgG of *T. gondii* were respectively 6.3% and 43.8% in HP patients and the

percentages were from 5% to 27.5% in the healthy group (31). In another study done in Turkey, the prevalence rates of IgM and IgG anti-*T. gondii* in HP patients were respectively 1.7% and 56.06% and *Toxoplasma* IgG antibodies were reported in 76.5% of HP and 48% of HG groups (32). In addition, in two other studies, high levels of toxoplasmosis have been discovered in chronic HP patients (33, 34). However, in a study in China, the positive levels of the IgG antibodies of *T. gondii* in HG and HP groups were respectively 3.6% and 27.3% (35). The results of all mentioned studies imply that the seroposivity level of toxoplasmosis has been higher in HP patients than in healthy individuals, depending on the duration of dialysis. In addition, in diagnosing active toxoplasmosis, molecular techniques are more sensitive than serological ones (20, 32, 35).

Conclusions

The results of the present study indicated that the timely monitoring of the *T. gondii* infection using serological techniques and PCR, particularly in HP patients who are more exposed to the dialysis procedure, is required for preventing infection dissemination.

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Ethical consideration

The present study was verified by the Ethics Committee of Zahedan University of Medical Sciences, Iran (IR.Zaums.Rec.1396.173).

Competing interest

The authors declare that they have no conflict of interest.

References

1. Karami MF, Rafiei A, Raiesi O, Getso M, Akhlaghi E, Jalali P, et al. The Relation between toxocariasis and toxoplasmosis co-infection and the presence of Rheumatoid Factor (RF) in people with hydatidosis in Southwestern Iran, from 2013 to 2018. J Parasit Dis 2019; 43(3):379-84. doi: 10.1007/s12639-019-01101-x
2. Eskandarian A, Jahani S, Hejazi H, Yousefi H, Raissi V. Investigation of *Toxoplasma gondii* infection in cutaneous leishmaniasis patients of the Isfahan province. Int J Infect 2017; 4(2):e40260. doi: 10.5812/iji.40260.
3. Sekandarpour S, Jafari Modrek M, Shafiei R, Mohammadiha A, Etemadi S, Mirahmadi H. Determination of parasitic burden in the brain tissue of infected mice in acute toxoplasmosis after treatment by fluconazole combined with sulfadiazine and pyrimethamine. Eur J Med Res 2021; 26:65.
4. Soltani S, Khademvatan S, Saki J, Shahbazian H. Detection of toxoplasmosis in renal transplant recipients by ELISA and PCR methods in Ahvaz, south-west of Iran. Jundishapur J Microbiol 2013; 6(9):e7642. doi : 10.5812/jjm.7642.
5. Zarean M, Shafiei R, Gholami M, Fata A, Balaghaleh MR, Karimi A, et al. Seroprevalence of anti–Toxoplasma gondii antibodies in healthy voluntary blood donors from Mashhad City, Iran. Arch Iran Med 2017; 20(7): 441-445.
6. Zarean M, Shafiei R, Gholami M, Fata A, Rahmati Balaghaleh M, Kariminik A, et al. Seroprevalence of anti-Toxoplasma gondii antibodies in healthy voluntary blood donors from Mashhad city, Iran. Arch Iran Med 2017; 20(7):441-5.
7. Mirahmadi H, Nozari B, Raissi V, Alijani E, Etemadi S. Frequency and parasite load of *Toxoplasma gondii* in hemodialysis patients based on RE gene by real-time PCR. Gene Reports 2021; 23:101145. doi: 10.1016/j.genrep.2021.101145.
8. Dalimi A, Abdoli A. Latent toxoplasmosis and human. Iran J Parasitol 2012; 7(1):1-17.
9. Mirahmadi H, Hasanzadeh R, Raeesi HM, Fallahi S, Shahraki MK, Badirzadeh A. Loop-mediated isothermal amplification (Lamp) assay to detect toxoplasmosis in schizophrenia patients. Iran J Parasitol 2020;15(3):299-306.
10. Jafari Modrek M, Hasanzadeh R, Foroutan M, Mirahmadi H, Rahmati-Balaghaleh M, Zarean M. Seroprevalence and molecular evaluation of *Toxoplasma gondii* in Schizophrenic patients hospitalized in Sistan and Baluchestan province, Southeast of Iran. Trop Biomed 2019; 36(2):422-9.

11. Keshavarz H, Mamishi S, Daneshvar H. The prevalence of *Toxoplasma* infection in hospitalized patients in selected hospitals of Kerman. J Kerman Univ Medical Sci 2000; 7(3):129-36. [In Persian].
12. Rezazadeh Varaghchi J, Ahmadi Shadmehri A, Ahmadi Shadmehri A. Seroepidemiology of toxoplasmosis in women referred to Birjand Milad genetic counseling center during 2011-2013. J Kerman Univ Medical Sci 2015; 22(5):524-32. [In Persian].
13. Kamyabi Z, Atapour M. Investigation of the prevalence of *Toxoplasma* antibodies in women during marriage consultation in Kerman city. J Kerman Univ Medical Sci 1999; 6(3):127-33. [In Persian].
14. Shafiei R, Riazi Z, Sarvghad M, Galian Sharifdini M, Mahmoodzadeh A, Hajia M. Prevalence of IgG and IgM anti-toxoplasma gondii Antibodies in HIV positive patients in Northeast of Iran. Iran J Pathol 2011; 6(2):68-72.
15. Arab-Mazar Z, Fallahi S, Koochaki A, Mirahmadi H, Seyyed Tabaei SJ. Cloning, expression and immunoreactivity of recombinant *Toxoplasma gondii* GRA5 protein. Iran J Microbiol 2016; 8(5):331-37.
16. Foroutan M, Rostami A, Majidiani H, Riahi SM, Khazaei S, Badri M, et al. A systematic review and meta-analysis of the prevalence of toxoplasmosis in hemodialysis patients in Iran. Epidemiol Health 2018; 40:e2018016. doi: 10.4178/epih.e2018016.
17. Raissi V, Bayat F, Taghipour A, Raiesi O, Ibrahim A, Getso M, et al. Seroepidemiology and risk factors of toxoplasmosis among children age ranged from 1 to 14 years referred to medical diagnostic laboratories in Southeast Iran. Clin Epidemiol Glob Health 2020; 8(2):595-9. doi: 10.1016/j.cegh.2019.12.009.
18. Ebrahim Zadeh A, Bamedi T, Etemadi S, Shahrakipour M, Saryazdipour K. Toxoplasmosis as a complication of transfusion in hemodialysis patients. Iran J Ped Hematol Oncol 2014; 4(1):22-5.
19. Mousavi M, Saravani R, Jafari Modrek M, Shahrakipour M, Sekandarpour S. Detection of *Toxoplasma gondii* in diabetic patients using the Nested PCR assay via RE and B1 genes. Jundishapur J Microbiol 2016; 9(2):e29493. doi: 10.5812/jjm.29493.
20. Saki J, Khademvatan S, Soltani S, Shahbazian H. Detection of toxoplasmosis in patients with end-stage renal disease by enzyme-linked immunosorbent assay and polymerase chain reaction methods. Parasitol Res 2013; 112(1):163-8. doi: 10.1007/s00436-012-3120-6.
21. Bayani M, Mostafazadeh A, Oliaee F, Kalantari N. The prevalence of *Toxoplasma gondii* in hemodialysis patients. Iran Red Crescent Med J 2013; 15(10):e5225. doi: 10.5812/ircmj.5225.
22. Rezavand B, Mahmoodzadeh Poornaki A, Rahim Mokhtari K, Mohammad A, Andalibian A, Abdi J. Identification and determination of the prevalence of *Toxoplasma gondii* in patients with chronic renal failure by ELISA and PCR. Asian Pac J Trop Dis 2016; 6(5):347-9. Doi: 10.1016/S2222-1808(15)61044-1.
23. Solhjoo K, Sotoodeh Jahromi A, Parnian-Rad A. Anti-Toxoplasma gondii antibodies in haemodialysis patients. Am J Infect Dis 2010; 6(1):13-7. doi: 10.3844/ajidsp.2010.13.17.
24. Maraghi S, Yadyad MJ, Sheikhi M, Shamakhteh F, Latifi SM. Study the anti-toxoplasma antibodies (IgG and IgM) in hemodialysis patients of Abadan and Khoramshahr cities southwest Iran in 2011 using ELISA. Jundishapur J Microbiol 2013; 6(7):e7113. doi:10.5812/jjm.7113.
25. Kazemi F, Fallahizadeh S, Feizhaddad MH. Seroepidemiological study of toxoplasmosis in hemodialysis patients of Ahvaz, Southwest of Iran. Int J BioMed Public Health 2018; 1(1):12-6. doi: 10.22631/ijbmph.2018.55340.
26. Hamidi F, Etemadi J, Ghabouli Mehrabani N, Mahami Oskouei M, Motavalli R, Ardalan MR. Comparison of *Toxoplasma gondii* seropositivity in hemodialysis and peritoneal dialysis patients. Journal of Coastal Life Medicine 2015; 3(8):621-2. doi: https://doi.org/10.12980/JCLM.3.2015J5-76.
27. Khalili B, Mortezaei S, Fazeli M. The comparison of anti-Toxoplasma antibody (IgM, IgG) in hemodialysed patients and those undergoing chemoterapy with healthy blood donor, shahr-E-kord, 1392. Iran J Parasitol 2015; 10(Suppl 1):311-311.
28. Rasti S, Hassanzadeh M, Soliemani A, Hooshyar H, Mousavi SG, Nikoueinejad H, et al. Serological and molecular survey of toxoplasmosis in renal transplant recipients and hemodialysis patients in Kashan and Qom regions, central Iran. Ren Fail 2016; 38(9):970-3. doi: 10.3109/0886022X.2016.1172940.
29. Seyyedpour SH, Afshar P, Barzegarnejad A, Kalhori S, Agah R. Evaluation of anti-Toxoplasma gondii antibodies in hemodialysis patients with chronic kidney disease in Sari,

Iran. Nephrourol Mon 2016; 8(6):e40182. doi: 10.5812/numonthly.40182.

30. Dorri M, Dabirzadeh M, Maroufi Y, Afshari M, Badri Chokamy M. Prevalence of anti-Toxoplasma IgG and IgM in hemodialysis patients comparing to healthy individuals in Sistan area, Iran. J Nephropharmacol 2017; 6(2):106-9. doi: 10.15171/npj.2017.13.

31. Aufy SM, Mahgoub AM, Saadi MG, Adel Elmallawany M. Serological detection of *Toxoplasma gondii* in chronic renal failure patients and renal transplant recipients. J Egypt Soc Parasitol 2009; 36(4):943-50.

32. Ocak S, Duran N, Eskiocak AF, Aytac H. Anti-Toxoplasma gondii antibodies in hemodialysis patients receiving long-term hemodialysis therapy in Turkey. Saudi Med J 2005; 26(9):1378-82.

33. Abbas MM, Zaki M, Afify NA. Prevalence of *Toxoplasma gondii* and *Cytomegalovirus* antibodies in patients with chronic renal failure. J Egypt Soc Parasitol 1996; 26(3):671-6**.**

34. Esposito R, Orlando G, Inzoli C, Calello G, Moroni M, Rivolta E, et al. Asyptomatic parasitic infections in renal transplant recipients and in haemodialysis patients. Boll Ist Sieroter Milan 1987; 66(3):210-3.

35. Tong DS, Yang J, Xu GX, Shen GQ. Serological investigation on *Toxoplasma gondii* infection in dialysis patients with renal insufficiency. Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi 2011; 23(2):144-53. [In Chinese].