HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Parasitological research doi: 10.1016/S2222-1808(15)61044-1

Identification and determination of the prevalence of *Toxoplasma gondii* in patients with chronic renal failure by ELISA and PCR

Babak Rezavand[1], Abbas Mahmoodzadeh Poornaki[2], Khojasteh Rahim Mokhtari[2], Alireza Mohammad[3], Ammar Andalibian[4], Jahangir Abdi[1*]

[1]*Department of Parasitology, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran*

[2]*Department of Parasitology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran*

[3]*Department of Medical, Parasitology and Mycology, School of Public Heath, Tehran University of Medical Science, Tehran, Iran*

[4]*Department of Parasitology, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran*

ARTICLE INFO

Article history:
Received 24 Dec 2015
Received in revised form 15 Mar, 2nd revised form 1 Apr, 3rd revised form 6 Apr 2016
Accepted 25 Apr 2016
Available online 4 May 2016

Keywords:
Toxoplasma gondii
Diagnosis
Opportunistic infections
ELISA
PCR

ABSTRACT

Objective: To detect *Toxoplasma gondii* (*T. gondii*) among end-stage renal disease (ESRD) patients.
Methods: This case-control study was conducted on 180 blood samples. In compliance with all ethical principles, 90 blood samples were taken from hemodialysis patients with ESRD and 90 samples from healthy volunteers. *T. gondii* screening was done using ELISA to search for immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies and by PCR for amplification of the *T. gondii* genome using specific primers.
Results: The results were analyzed using SPSS software. Out of 90 patients on hemodialysis, 54 (60.0%) were positive for anti-toxoplasma IgG antibody, 3 (3.3%) for anti-toxoplasma IgM antibody and 5 patients (6%) were positive by PCR. From 90 healthy volunteers, 34 (37.8%) were positive for anti-toxoplasma IgG antibody. All the healthy volunteers were negative for anti-toxoplasma IgM antibody and in PCR. Compared with the gold standard method of ELISA, PCR had 100% sensitivity and 98.9% specificity in detection of *T. gondii*.
Conclusions: PCR alongside serologic methods can be valuable for *T. gondii* screening. Given the high prevalence of *T. gondii* among hemodialysis patients with ESRD, *T. gondii* screening together with sanitary control of biological agents was recommended in dialysis units.

1. Introduction

Chronic renal failure is a debilitating disease with many systemic complications for the patient. The disease is manifested with progressive, irreversible loss of functional renal tissue, such that the remaining kidney bulk is no longer able to do its role[1]. This disease develops over several years after the acute renal failure attacks, eventually leading to hemodialysis, peritoneal dialysis or kidney transplantation[2-4]. End-stage renal disease (ESRD) is one of the major problems of health organizations, and is the most common cause of death in these patients worldwide[5]. ESRD presents clinically in the form of uremic syndrome, in which glomerular filtration rate is decreased to less than 10 mL/min estimated. There are about 1 million ESRD patients under hemodialysis in the world[6,7]. According to the Iranian Dialysis Center, there are about 12 500 patients diagnosed with this disease in the country in 2006[8]. Studies have supported the high incidence of opportunistic infectious agents among those undergoing dialysis, especially in uremic patients[4]. *Toxoplasma gondii* (*T. gondii*) is an intracellular opportunistic parasite, which could endanger the patient's life, particularly in those with compromised immune systems like AIDS patients[9]. The cat is the reservoir and spread agent of the disease, and the disease transmission occurs through ingestion of cysts[10]. *T. gondii* forms cysts in the surrounding tissue cells to protect itself against immune activity in healthy people[11]. The disease is usually asymptomatic in those with healthy immune systems, and only a small percentage of them show disease symptoms. If the immune system of the body is weakened, the cysts can be reactivated, and acute, disseminated and systemic forms of the diseases can be manifested[12]. Patients undergoing hemodialysis are not generally classified as immunosuppressed patients, but there is evidence of immune system disorders among uremic patients[13]. Considering this fact and the high number of hemodialysis patients, the goal of

*Corresponding author: Jahangir Abdi, Department of Parasitology, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran.
Tel/Fax: +9808432227109
E-mail: abdi-j@medilam.ac.ir, jahangirabdi@yahoo.com
This work was approved by ethical committee of Ilam University of Medical Sciences (number of 0748).
The journal implements double-blind peer review practiced by specially invited international editorial board members.

present study aimed to detect *T. gondii* using serological methods as a gold standard and molecular diagnosis in ESRD patients and healthy control people.

2. Materials and methods

This case-control study conducted in Tehran on 90 patients with chronic renal failure and 90 healthy volunteers. Informed consent was obtained for sampling from the subjects. To test the titer of anti-*T. gondii* antibody, 5 mL blood was drawn from the subjects, and the samples were sent to the laboratory. Serum samples were used for ELISA immunoglobulin G (IgG) and ELISA immunoglobulin M (IgM), and whole blood was used for the detection of *T. gondii* by PCR.

2.1. Serological tests

Toxoplasma IgG and IgM antibodies were quantitated using VIRO-IMMUN kit made in Germany.

2.2. Genome extraction

DNA was extracted from whole blood samples using phenol-chloroform test. The DNA extracted from whole blood samples was transferred to –80 ℃ after determining the purity of DNA until PCR.

2.3. PCR

T. gondii genome amplification was done using PCR. Specific primers of TR1: ACGAACACTCGCAGAGATGA and TR2: GATCCTTTTGCACGGTTGTT were used for *B1* gene[2]. Deionized water was used as negative control, and positive control was the RH strain ready in the Department of Parasitology of Teerthanker Mahaveer University.

PCR was done in a final volume of 25 μL by adding 0.8 μL of magnesium chloride, dNTP, *Taq* polymerase enzyme, 2.5 μL PCR buffer, DNA template and 1 μL of primers at a concentration of 1 pmol/L.

PCR thermal schedule consisted of 35 cycles, 3 min for first denaturation and 30 s for denaturation of DNA strands, annealing at 45 ℃ for 30 s, extension at 72 ℃ for 30 s and final extension at 72 ℃ for 5 min.

A total of 10 μL of amplified PCR product was electrophoresed on 1.5% agarose gel. The amplified DNA was visualized under transilluminator instrument after staining with ethidium bromide.

2.4. Statistical analysis

For statistical analysis, SPSS 20 software was used. The significance test between parameters involved in the study was done using independent *t*-test and Fisher exact test.

3. Results

The mean age of hemodialysis patients and healthy volunteers was (43.8 ± 17.13) and (41.8 ± 18.9) years, respectively. Using *t*-test, no significant difference was observed between the two groups in terms of age ($P = 0.64$).

From 90 hemodialysis patients, 34 (37.8%) were female and 56 (62.2%) were male. Out of 90 healthy controls, 39 (43.3%) were female and 51 (56.7%) were male. *t*-test showed no significant difference between the two groups in terms of gender ($P = 0.64$).

The results showed that from a total of 90 hemodialysis patients in the control group, 54 (60%) were positive for IgG and 3 (3.3%) were positive for IgM. Out of 90 healthy volunteer subjects in the control group, 34 (37.8%) were positive for IgG and 0 (0%) was positive for IgM.

T. gondii-specific DNA amplification by PCR in blood samples from patients and healthy volunteers showed that 5 samples (6%) of hemodialysis patients and no sample (0%) of healthy volunteers became positive.

Sensitivity and specificity of PCR were calculated based on the gold standard for detection of *T. gondii* (ELISA). The results showed that the sensitivity and specificity of PCR in toxoplasma diagnosis were 100% and 98.9%, respectively. The positive and negative predictive values for PCR were 100% and 60%, respectively (Table 1). To find the relationship between the results obtained using ELISA IgG, ELISA IgM and PCR with kidney transplant duration in the control group, Fisher exact test, χ^2 and Fisher exact tests were used, respectively. The results of these tests showed no significant relationship with kidney transplant duration in patients.

Table 1
Positive and negative predictive values for PCR.

PCR		ELISA IgG		Total	ELISA IgM		Total
		Negative	Positive		Negative	Positive	
Negative	Count	92	83	175	175	0	175
	% Within ELISA	52.6%	47.4%	100%	98.9%	0%	97.2%
Positive	Count	0	5	5	2	3	5
	% Within ELISA	0%	5.7%	2.8%	60%	100.0%	2.8%
Total	Count	92	88	180	177	3	180
	% Within ELISA	100%	100%	100%	100%	100%	100%

To find the correlation between positive results of samples using the PCR between patients and healthy volunteers, the Fisher exact test was used. Results indicated no significant correlation between PCR results in patients and healthy subjects. To find the correlation between positive sample results using the ELISA IgM between patients and healthy volunteers, Fisher exact test was used. The results obtained showed no significant correlation between ELASA IgM of patients and healthy subjects (Table 2).

Table 2
Correlation between ELASA IgM of patients and healthy subjects.

Correlation	Sex		Duration of transplantation	
	Female	Male	Upper 6 months	Under 6 months
IgG positive	8 (27.6%)	19 (90.5%)	30 (57.1%)	24 (51.7%)
Statistical significance	0.001		0.13	
IgM positive	1 (4.8)	1 (3.4%)	2 (0.0%)	1 (6.9%)
Statistical difference	0.99		0.61	
PCR positive	2 (9.5%)	1 (3.4%)	3 (4.8%)	2 (6.9%)
Statistical difference	0.57		0.67	
Total	21 (42.0%)	29 (58.0%)	21 (42.0%)	29 (58.0%)

To control the correlation between positive samples using the ELISA IgG between patients and healthy volunteers, Fisher exact test was used. The results revealed a significant correlation between the results of ELASA IgG in patients and healthy controls. From 90 patients who received a kidney transplant, 54 (60%) and from 90 healthy volunteers 34 (37.8%) were positive about *T. gondii* antibodies.

4. Discussion

After infection by *T. gondii*, cellular immunity plays a major role to protect the body against this organism[14]. After stimulation of the immune system, activation of macrophages and production of type 1 cytokine increase the level of interferon gamma (IFNγ). IFNγ induces

nitric oxide production, resulting in destruction of toxoplasma tachyzoites inside the cell. Finally, IFNγ performs its anti-toxoplasma activity by production of nitric oxide[15]. Uremia can lead to immune system compromise in patients with chronic kidney disease[16]. Intensification of uremia causes domination of Th2 responses in dialysis patients, which is associated with a sharp decline in IFNγ with increased serum levels of interleukin-13[17]. Domination of Th2 response on Th1 in uremic conditions in patients with chronic kidney disease leads to chronic inflammation, which increases the main Th2 lymphocytes[18].

Solhjoo *et al*.[19] in a study on patients with chronic renal failure in Jahrom reported the levels of IgG, IgM and IgA anti-toxoplasma antibodies to be 59.10%, 6.80% and 6.80%, respectively. They also addressed the direct relationship between increased levels of anti-toxoplasma antibodies with increasing duration of hemodialysis[19]. In our study, there was no significant correlation between dialysis duration and prevalence of *T. gondii* among hemodialysis patients. This difference seems to result from different climatic and geographical conditions of subjects and control of biological agents in dialysis units in large cities. The study of Ocak in Turkey was conducted on 255 hemodialysis patients and 50 healthy subjects[20]. The level of anti-toxoplasma antibody in patients and controls was 75.5% and 48% in patients and control subjects, respectively, which was consistent with our results[20]. Despite the direct relationship between the prevalence of antibodies to *T. gondii* with geographical differences, studies in different parts of the world have supported the high prevalence of *T. gondii* in ESRD patients. Mohammadi Manesh *et al*. in Egypt performed a serological study on 60 patients with a history of chronic renal failure, and reported 38.3% prevalence of *T. gondii* compared to normal subjects[21]. In our study, there was a significant correlation between the prevalence of anti-*T. gondii* IgG antibodies in ESRD patients and healthy volunteers. This can show the role of hemodialysis in the transmission process of *T. gondii*. In this study, we used PCR besides the gold standard ELISA assay for *T. gondii* screening.

Results showed that PCR has a higher sensitivity and specificity than ELISA in *T. gondii* screening. It seems that uremia as a reason suppressing Th1 can give the right conditions for incidence of intracellular pathogens[18]. Therefore, further studies in uremic patients to detect intracellular infectious agents, especially *T. gondii* seem to be necessary. Finally, we recommended *T. gondii* screening in those subjects to hemodialysis to prevent likely transmission of the infectious agent during the dialysis process.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Project support was provided by the Ilam University of Medical Sciences. Hereby the Acknowledgment is made deputy of research and technology.

References

[1] Tonelli M, Wiebe N, Culleton B, House A, Rabbat C, Fok M, et al. Chronic kidney disease and mortality risk: a systematic review. *J Am Soc Nephrol* 2006; **17**(7): 2034-47.

[2] Izadi M, Jonaidi Jafari N, Mahmoodzadeh Poornaki A, Sadraei J, Rezavand B, Mirzaei HR, et al. Detection of *Toxoplasma gondii* from clinical specimens of patients receiving renal transplant using ELISA and PCR. *Nephrourol Mon* 2013; **5**(5): 983-7.

[3] Tarapore D, Lima PU, Carneiro J, Christensen AL. To err is robotic, to tolerate immunological: fault detection in multirobot systems. *Bioinspir Biomim* 2015; **10**(1): 016014.

[4] Alvarado-Esquivel C, Sánchez-Anguiano LF, Mendoza-Larios A, Hernández-Tinoco J, Pérez-Ochoa JF, Antuna-Salcido EI, et al. Prevalence of *Toxoplasma gondii* infection in brain and heart by immunohistochemistry in a hospital-based autopsy series in Durango, Mexico. *Eur J Microbiol Immunol (Bp)* 2015; **5**(2): 143-9.

[5] Kalamas AG, Niemann CU. Patients with chronic kidney disease. *Med Clin North Am* 2013; **97**(6): 1109-22.

[6] Moeller S, Gioberge S, Brown G. ESRD patients in 2001: global overview of patients, treatment modalities and development trends. *Nephrol Dial Transplant* 2002; **17**(12): 2071-6.

[7] Schmaderer C, Heemann U, Moog P. [Update nephrology 2013]. *MMW Fortschr Med* 2013; **155**(9): 56-9; quiz 60-1. German.

[8] Aghighi M, Heidary RA, Zamyadi M, Mahdavi-Mazdeh M, Rajolani H, Ahrabi S, et al. Dialysis in Iran. *Iran J Kidney Dis* 2008; **2**(1): 11-5.

[9] Rostami MN, Eskandari E, Garoosi Z, Mohajeri N, Rezaian M, Keshavarz H. Serological study of *Toxoplasma gondii* infection using IFA method in renal transplant recipients. *Iran J Parasitol* 2006; **1**(1): 31-8.

[10] Esmaeilzadeh M, Shamsfard M, Kazemi A, Khalafi SA, Altome SA. Prevalence of protozoa and gastrointestinal helminthes in stray cats in Zanjan Province, north-west of Iran. *Iran J Parasitol* 2009; **4**(3): 71-5.

[11] Dubey JP. The history of *Toxoplasma gondii*-the first 100 years. *J Eukaryot Microbiol* 2008; **55**(6): 467-75.

[12] Dalimi A, Abdoli A. Latent toxoplasmosis and human. *Iran J Parasitol* 2012; **7**(1): 1-17.

[13] Kimmel M, Alscher DM, Dunst R, Braun N, Machleidt C, Kiefer T, et al. The role of micro-inflammation in the pathogenesis of uraemic pruritus in haemodialysis patients. *Nephrol Dial Transplant* 2006; **21**(3): 749-55.

[14] Salinas N, Olguín JE, Castellanos C, Saavedra R. T cell suppression in vitro during *Toxoplasma gondii* infection is the result of IL-2 competition between Tregs and T cells leading to death of proliferating T cells. *Scand J Immunol* 2014; **79**(1): 1-11.

[15] Swierzy IJ, Muhammad M, Kroll J, Abelmann A, Tenter AM, Lüder CG. *Toxoplasma gondii* within skeletal muscle cells: a critical interplay for food-borne parasite transmission. *Int J Parasitol* 2014; **44**(2): 91-8.

[16] Tsigou E, Aloizos S, Myrianthefs P, Gourgiotis S, Tsakris A, Baltopoulos G. The immune response after stimulation with wall components of Gram-positive bacteria and fungi. *Immunol Lett* 2014; **159**: 23-9.

[17] Wang IK, Wu YY, Yang YF, Ting IW, Lin CC, Yen TH, et al. The effect of probiotics on serum levels of cytokine and endotoxin in peritoneal dialysis patients: a randomised, double-blind, placebo-controlled trial. *Benef Microbes* 2015; **6**(4): 423-30.

[18] Alvarez-Lara MA, Carracedo J, Ramírez R, Martín-Malo A, Rodríguez M, Madueño JA, et al. The imbalance in the ratio of Th1 and Th2 helper lymphocytes in uraemia is mediated by an increased apoptosis of Th1 subset. *Nephrol Dial Transplant* 2004; **19**(12): 3084-90.

[19] Solhjoo K, Jahromi AS, Parnian-Rad A. Anti-*Toxoplasma gondii* antibodies in haemodialysis patients. *Am J Infect Dis* 2010; **6**(1): 13-7.

[20] 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.

[21] Mohammadi Manesh R, Hosseini Safa A, Sharafi SM, Jafari R, Bahadoran M, Yousefi M, et al. Parasites and chronic renal failure. *J Renal Inj Prev* 2014; **3**(4): 87-90.