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Detection of toxoplasmosis in hemodialysis Egyptian patients using serological and molecular techniques

[1]Reham K. Nahnoush, [2]Elham M. Youssif and [3]Omayma M. Hassanin

[1]*Department of Medical Parasitology, Faculty of Medicine, Cairo University*
[2]*Department of Internal Medicine, Faculty of Medicine, Cairo University*
[3]*Department of Molecular Biology, Faculty of Medicine Ain-Shams Research Institute, Ain-Shams University*

Address For Correspondence:
Reham K. Nahnoush, Department of Medical Parasitology, Faculty of Medicine, Cairo University
E-mail: dr_reham_kamal@hotmail.com

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ABSTRACT

Toxoplasma gondii is considered one of the most prevalent opportunistic protozoan parasite, which can be life threating in vulnerable subjects. The aim of this study was to detect toxoplasmosis among 150 hemodialysis patients (HD) and 90 healthy individuals. Screening of *T. gondii* was done serologically by using ELISA to search for IgM and IgG antibodies and molecularly by quantitative real time PCR for amplification of *T. gondii* genome using specific primers. Toxoplasma IgG seropositivity was detected in 60% (90/150) among HD and 31.11% (28/90) of the control group. Rate of anti-*T. gondii* IgG antibody was significantly higher in HD patients than the control group. IgM seropositivity was detected in 14.6% (22/150) among HD group while nothing detected (0%) in healthy volunteers. *Toxoplasma* DNA was detected in 3 positive samples (2%) among HD patients, two of them were belonged to patients with IgG positive antibodies while only one belonged to patient with IgM positive antibodies. The results of this study showed that patients on hemodialysis were positive for *Toxoplasma* antibodies by serological tests and PCR confirmed the quantitative parasitic load in the peripheral blood in three cases, which indicates that patients on hemodialysis are at great risk of active infection with *Toxoplasma*.

INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii* (*T. gondii*) which is an obligate intracellular protozoan parasite (Dubey and Beattie, 1988). It was reported to infect about one-third of human population worldwide (Weiss and Dubey, 2009). Wide range of hosts could be infected also, as birds and domesticated mammals. Cats are the final hosts while humans and most vertebrates are intermediate hosts (Robert-Gangneux and Darde, 2012). This parasite exists in varying forms as tachyzoites, tissue cysts and oocysts which transmit the infection via different ways as oral route, organ transplantation, placental route, circulating leukocytes in addition to blood transfusion (Atmaca *et al*. 2004). Toxoplasmosis is usually asymptomatic in immunocompetent individuals, and only a small percentage may express self-limited symptoms. While in immunocompromised individuals it could be life threatening due to high risk of activation and relapse of the disease in these defenseless patients (Weiss and Dubey, 2009). Patients on hemodialysis are not generally classified as immunosuppressed patients but impairment of cell mediated immunity was reported among patients with renal disorders, so they are at risk of several infections (Cohen and Stosor, 2013). *Toxoplasma gondii* is considered one of the most prevalent opportunistic protozoan, as it may invade central nervous system and cause encephalitis and encephalopathy in vulnerable subjects (Nissapatorn, 2009 and Ho and Marra, 2014). Thus the aim of this study was to detect acute and chronic toxoplasmosis, serologically by

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detecting IgM and IgG using Enzyme Linked Immunosorbent Assay (ELISA) and molecularly by quantitative real time Polymerase Chain Reaction (qPCR) among hemodialysis patients in comparison to healthy control individuals which can indicate their risk of acquiring infection.

MATERIALS AND METHODS

Study design and Population:

A cross-sectional study conducted at Internal medicine department (Nephrology unit), Kasr Alainy Teaching Hospital in Egypt, during the period from March 2014 to November 2015. The study included 240 participants; all of them were informed about the purpose and signed on written consent prior to collection of samples. They were categorized into two groups according to their renal status, group of hemodialysis patients (HD) (n=150) and control group (n=90). The former group included chronic renal failure patients who were on regular haemodialysis while the other group included apparently healthy participants with no history of renal troubles and their kidney functions were within normal range. A structured comprehensive questionnaire was administered to all individuals that included some enquiries including demographic data and duration of dialysis for hemodialysis patients. Clinical examination was done to detect any signs suggestive of *Toxoplasma* infection such as fever, enlarged lymph nodes, hepatosplenomegaly and skin rash.

Serological Tests:

Venous samples were collected into a sterile tube, then for serological work the blood sample was left for 10 minutes to clot and then centrifuged at 3000 rpm for 5 minutes. The serum was then separated and stored at -20°C. Enzyme-linked immunosorbent assay was used for detection of *Toxoplasma gondii* IgM antibodies in human sera, with a commercial ELISA kit DRG® Toxoplasma IgM (EIA-1799) (DRG International, Inc., USA) following the manufacturers´ kits instructions. Sample was considered negative < 0.9 AU/ml, equivocal 0.9 to 1.0 AU/ml, while positive ≥ 1.0 AU/ml. Also, each serum sample was subjected for measurement of *T. gondii* IgG antibodies by using the commercial available kit NovaLisa™ *Toxoplasma* IgG-ELISA (NovaTecImmundiagnostica GmbH (D-63128 Dietzenbach, Germany). Absorbance at 450 nm is read using an ELISA microwell plate reader. The sample was considered negative < 30 IU/ml, equivocal 30-35 IU/ml, while positive > 35 IU/ml. Equivocal cases were considered negative as it was not accessible to obtain a second serum sample later to be tested.

DNA extraction and Molecular technique:

Genomic DNA was extracted from whole blood samples using the DNeasy® Blood and Tissue Kit (Qiagen, CA, USA). Genomic amplification was done by qPCR using the Light Cycler® instrument (Roche Diagnostics, Hoffmann-La Roche Ltd, USA) with the Light Cycler® fast Start DNA Master SYBR Green dye. Primers from bases (5´-CCG TTGGTT CCG CCT CCT TC-3´) and (5´-GCAAAA CAG CGG CAG CGT CT-3´) were used to amplify *Toxoplasma* B1 gene of 35-fold repeats. The reaction mixture (20µl Master SYBR Green kit; Roche Diagnostic) contained 0.5 µM of each primer, 5mM MgCl2 and 5µl template DNA. Amplification was performed for 50 cycles: 5s denaturation at 95°C, 10s annealing at 61°C and 15 s extensions at 72°C, with an overallramp rate of 20°C s. The resulting PCR fragment of *T. gondii* was analyzed using the Light Cycler® Red 640 (detected in channel 640). This PCR technique was performed according to **Contini *et al.* 2005.** Serial dilutions of cloned purified *Toxoplasma* genomic materials from 10^1 to 10^6 genomic equivalents were used as positive control samples. Standard curve was created using the previously mentioned positive samples for the absolute quantification of the unknown samples. Negative control was applied with ultrapure water.

Statistical analysis:

All data, information and test results were recorded and analyzed by using SPSS version 18 software and chisquare test. A $P \leq 0.05$ was regarded as statistically significant.

Results:

According to the results of the present study, anti-*Toxoplasma* IgG antibodies were detected in 60% (90/150) and 31.11% (28/90) in HD patients and control group, respectively. While anti *T. gondii* IgM antibodies were detected in 14.6% (22/150) among HD patients but not detected in the control group with statistically significant difference between the two groups ($p<0.05$) (Table 1). The mean duration of hemodialysis in *Toxoplasma* seropositive and seronegative patients was investigated in this study. It was significantly higher in Toxoplasma seropositive patients (Table 2). Regarding molecular detection of *Toxoplasma* DNA in blood samples of HD patients and healthy volunteers. Three positive samples (2%) were detected among hemodialysis patients. Quantitative genomic estimation of these 3 positive samples in qPCR was from 1.2×10^2, 2.3×10^3 and 6×10^7 genomic equivalents respectively. The first 2 samples were IgG positive,

while the third sample was IgM positive reflecting the highest genomic level of circulating *Toxoplasma* parasitic stages in peripheral circulation (Table 3).

Table1: Anti-*Toxoplasma* IgG and IgM antibodies among the studied groups.

	Renal failure with haemodialysis No. (%)	Control group No.(%)
IgG positive	90 (60 %)	28 (31.11%)
IgG negative	60 (40 %)	62 (68.8%)
IgM positive	22 (14.6%)	0 (0%)
IgM negative	128 (85.3%)	90 (100%)

Statistically significant (P≤0.05)

Table 2: Mean duration of hemodialysis among Toxoplasma seropositive and seronegative hemodialysis patients

Toxoplasma seropositive haemodialysis patients	52.92 ±33.8 months
Toxoplasma seronegative haemodialysis patients	29.4 ± 21.93 months

Table 3: Anti Toxoplasma IgG and IgM antibodies among PCR positive samples.

PCR positive samples (Numerical calculation)	Anti *Toxoplasma* IgG	Anti *Toxoplasma* IgM
1- 1. 2 x 10^2genomic equivalents	+	-
2- 2. 3x 10^3 genomic equivalents	+	-
6x 10^7 genomic equivalents	-	+

Discussion:

Toxoplasmosis is an intracellular infection which necessitates activated cellular immunity to control it (Girndt, 2002). Many studies reported impairment of cellular immunity in patients on dialysis and uremic ones. This was reported to be due to reduction in number of T cells in patients suffering from renal disorders and the increase in suppressor cells. Reduction in the number of natural killer cells and phagocytic activity was also reported. Unfortunately, dialysis cannot fix up this impairment (Ebrahim *et al*. 2014). These factors probably are responsible for suppression of immunity in uremia and high incidence of infection in patients on dialysis (Schollmayer and Bozkurt, 1988). ELISA technique is used in this study for detection of anti *Toxoplasma gondii* IgG and IgM antibodies in hemodialysis patients and healthy individuals. It is the commonest method used for detection of toxoplasmosis and diagnosis of primary infection (Remington *et al*. 2004). Moreover, Quantitative Real time PCR was used in this study for detection of *Toxoplasma gondii* circulating genomic substances at low concentration in blood samples, qPCR is also used because serological tests alone are usually not useful for distinguishing recent from past infections, anti-*Toxoplasma* IgM antibodies may stay detectable for more than 1year after primary infection (Liesenfeld *et al*. 1997). In the present study, *Toxoplasma gondii* IgG seropositivity was significantly higher in hemodialyzed group 60% than in control group 31.11% (p value <0.05), while anti *Toxoplasma* IgM antibodies were detected in 14.6 % in HD group whereas nothing detected in the control group with statistically significant difference between the two groups. Similarly, Solhjoo *et al*. 2010 in Jahrom, stated that anti *Toxoplasma gondii* IgG antibodies were detected among 59.10% HD patients and 36.40% healthy people. In another study which was done in Egypt by Aufy *et al*. 2009, seropositivity of *Toxoplasma* IgG antibodies was recorded in 56.7% of HD patients and 23.1% in the control group, while IgM was reported in 16.7% compared to none in control group with statistically significant difference. On the other hand, in a study conducted by Maraghi *et al*. 2013 in Abadan and Khoramshahr cities, it was stated that *Toxoplasma* IgG seropositivity was 40.67% among HD patients and 26% in healthy people, which showed lower IgG seroprevalence than the current study. Other studies reported higher rate of seropositivity for anti-*Toxoplasma* IgG antibodies in hemodialysis patients, as Ocak *et al*. 2005 who reported anti *Toxoplasma* IgG antibodies in 76.5% among dialysis patients, and 48% in control subjects. In addition, Bayani *et al*. 2013 determined anti-*Toxoplasma* IgG antibodies among 80% of hemodialysis patients and 76% in healthy volunteers. Regardless the various rates of anti-*Toxoplasma* antibodies reported by the previously mentioned work, y*et al*l studies found higher seropositivity among the on-dialysis patients than healthy individuals. In the present study, clinical presentations suggestive of toxoplasmosis were not observed. This was in accordance with Remington *et al*. 2001 who stated that acute *Toxoplasma* infection is often clinically silent. Concerning the mean duration of hemodialysis for *Toxoplasma* seropositive and seronegative patients in this study, it was found to be 52.92 ±33.8 and 29.4 ±21.93 months respectively, which indicated that the mean duration of hemodialysis was significantly higher in *Toxoplasma* seropositive patients. Similarly other studies by Yazar *et al*. 2003 and Ocak *et al*. 2005 reported higher mean duration of dialysis for seropositive patients than in seronegative ones. Thus, the more exposure to dialysis, the more the risk of toxoplasmosis. On the other hand, qPCR was reported to be highly sensitive and specific to detect low level of circulating DNA related to various organisms including *Toxoplasma* (Romand *et al*. 2004). Therefore, the technique was applied in the current study to confirm the presence of *Toxoplasma* infection at earlier phases and succeeded to detect genomic concentrations from 10^2 to

10^6. Detection of *Toxoplasma* genomic materials by molecular methods is reported to be more sensitive than serological testing in immunodeficient patients where interpretation of antibody titers is difficult and so serology is of limited value in case of reactivation (Holliman *et al*. 1992 and Iqbal *et al*. 2003). In this study *Toxoplasma gondii* genomic substances were detected in 3 cases (2%) among 150 hemodialyzed patients, two of them were belonged to patients with IgG positive antibodies while only one belonged to patient with IgM positive antibodies. Nearly similar, Saki *et al*, 2013 recorded positive results related to *Toxoplasma* DNA in 4 cases (1.4%) among 280 blood samples of hemodialyzed patients, they were IgM positive cases. In conclusion, the results of this study showed that patients on hemodialysis were positive for *Toxoplasma* antibodies by serological tests and PCR confirmed the quantitative parasitic load in the peripheral blood in three cases, which indicates that patients on hemodialysis are at great risk of active infection with *Toxoplasma*, likely due to reactivation of a chronic infection. Thus prevention, screening and treatment programs should be carried out to prevent dissemination of this infection through haemodialysis procedure.

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