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

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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 12500 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

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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 VIROIMMUN kit made in Germany.

2.2. Genome extraction

DNA was extracted from whole blood samples using phenolchloroform test. The DNA extracted from whole blood samples was transferred to \(-80^\circ C\) 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 Tehran Mahaveer University.

PCR was done in a final volume of 25 \( \mu L \) by adding 0.8 \( \mu L \) of magnesium chloride, dNTP, Taq polymerase enzyme, 2.5 \( \mu L \) PCR buffer, DNA template and 1 \( \mu L \) of primers at a concentration of 1 \( \text{pmol/L} \).

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

A total of 10 \( \mu L \) 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 patients, Fisher exact test, \( \chi^2 \) and Fisher exact tests were used, respectively. The results of these tests showed no significant relationship with kidney transplant duration in patients.

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).

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 (IFN\( \gamma \)). IFN\( \gamma \) induces...
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