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Anti-*Toxoplasma gondii* Antibodies in Haemodialysis Patients with Chronic Renal Failure

Süleyman Yazar[1], Funda Demirtaş[1], Şaban Yalçın[1], Ozan Yaman[1], Bulent Tokgöz[2], Cengiz Utaş[2], and İzzet Şahin[1]

Departments of [1]Parasitology and [2]Nephrology, Erciyes University Medical Faculty, Kayseri, Turkey.

This study aimed to determine the prevalence of anti-Toxoplasma gondii antibodies in haemodialysis patients with chronic renal failure (CRF). Methods: One hundred and seventy three haemodialysis patients, and 40 healthy controls, were studied for the prevalence of anti-Toxoplasma gondii antibodies by a micro enzyme-linked immunosorbent assay (ELISA). Anti-*T. gondii* IgG antibodies were detected in 97 (56.06%) haemodialysis patients and 8 (20%) controls with a statistical significance. In addition, anti-*T. gondii* IgM antibodies were detected in 1.73% of patients, but none of the controls. In conclusion, a high percentage of positivity for Toxoplasma antibodies in patients with CRF undergoing haemodialysis was noticed, thus parasitological surveys of CRF patients should be periodically performed to prevent the possible dissemination of toxoplasmosis through the dialysis procedure.

Key Words: *Toxoplasma gondii*, haemodialysis, chronic renal failure, ELISA

INTRODUCTION

Toxoplasma gondii, a worldwide distributed parasite, is closely related to other coccidia, and also has certain similarities to malarial parasites. The parasites were first discovered in the North African rodent, Ctenodactylus gundii, hence the species was given the name "gondii". Although serological evidence indicates a high rate of human exposure to this organism, the disease itself is relatively rare. *T. gondii* can infect many vertebrates, including humans, but the definitive host is the house cat and other members of the Felidae family.[1]

This organism is an obligate intracellular parasite found in two forms in humans. The actively proliferating trophozoites, or tachyzoites, are usually seen in the early, more acute phases of the infection. The resting forms, or tissue cysts, are primarily found in muscle and brain tissues, probably as a result of the host immune response.[1]

Toxoplasma infections can be acquired postnatally, and are categorized into four groups:

a) Lymphadenitis, fever, headache, and myalgia, with a possibility of splenomegaly and a brief erythematous rash
b) Typhus-like exanthemaous form with myocarditis, meningoencephalitis atypical pneumonia, and possible death
c) Retinochoroiditis, which may be severe and require enucleation
d) CNS involvement, which is usually fatal.[2]

T. gondii are transmitted parenterally, flourish in states of immunosuppression, and most *Toxoplasma* infections are asymptomatic.[3] The large numbers of people who are serologically positive for *T. gondii* suggests that the majority of infections are benign, with most people exhibiting few (e.g., cold or light case of the flu) or no symptoms.

About 20% of the U.S. population is seropositive for immunoglobulin G (IgG) for *T. gondii*, making this one of the most prevalent, and probably the only, chronic parasitic infection

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Reprint address: requests to Dr. Süleyman Yazar, Department of Parasitology, Erciyes University Medical Faculty, Kayseri-Turkey. Tel: 90-352-4374937/23401, Fax: 90-352-4375285, E-mail: syazar@erciyes.edu.tr

lasting a human lifetime with no known consequences.[4] The rates of seropositivity have been reported as about 29% in Egypt,[5] and 23.1% at İzmir[6] and 36% at Kayseri,[7] both in Turkey.

T. gondii in its dormant bradycyst form is generally not associated with symptoms; however, with immunosuppression of the host, reactivation of the bradizoites causes the potentially lethal disease, toxoplasmosis.[8]

Many studies have been carried out on the immune response in patients with CRF, and proved there was impairment of cell-mediated immunity.[9] CRF patients are under risk from a variety of infections,[10] either due to their depressed immune status,[11] or through haemodialysis.[12]

In this study the subjects were selected from haemodialysis patients with CRF, and were tested with the micro enzyme-linked immunosorbent assay (ELISA) for *T.gondii* antibodies. This study aimed to determine the seroprevalence of anti-*T.gondii* antibodies in patients with CRF undergoing haemodialysis.

MATERIALS AND METHODS

Patients and sera

In this study, 173 patients with CRF, undergoing haemodialysis, aged between 15 and 90 (mean: 43.54 ± 15.21) were selected from patients under treatment at the Erciyes University Medical Faculty Nephrology department, between 2000 and 2002. In addition, we selected 40 healthy volunteers as a control group, who were aged between 18 and 56 (mean: 42.24 ± 16.28). Blood samples were taken from the brachial vein of all the patients and healthy volunteers under sterile conditions. The sera were separated after centrifugation at 1000 rpm for 10 min., and stored at -20 ℃ until required.

Serologic technique

We used the micro ELISA technique for *T. gondii* employing anti-*T. gondii* IgG and IgM antibodies ELISA kits purchased from the commercial manufacturer EUROIMMUN, which were performed following the manufacturer's instructions.

Statistical analyses

Chi-square tests were used in the statistical analyses, and were performed using SPSS V.10.0 for Windows.

RESULTS

In the presented study, 97 of the 173 (56.06%) patients and 8 of the 40 (20%) healthy volunteers were positive for the IgG antibodies (Table 1).

Table 1. The Percentage of Anti-*T. gondii* IgG Antibodies in CRF Patients Undergoing Haemodialysis, and in the Control Group

Cases	IgG (+)	IgG (-)
Patients	97 (56.06%)	76 (43.94%)
Controls	8 (20%)	32 (80%)

$p<0.001$.

The percentage of haemodialysis patients found positive for the anti-*T. gondii* IgG antibody was 56.06%, which was significantly greater than the 20.0% in the healthy volunteers ($p<0.001$).

Three patients were positive for IgM antibodies (1.73%) in the patient group, although all of the subjects in the control group were determined as seronegative by the ELISA. The percentage of haemodialysis patients positive for the anti-*T. gondii* IgM antibodies (1.73%) was found to be greater than in the healthy volunteers (0 %), but the difference between the groups was not statistically significant ($p>0.05$).

In this study, we also investigated the relationship between length of time on haemodialysis treatment and anti-*T. gondii* IgG antibodies seropositivity (Table 2).

We observed an increase in the seropositivity rate with increasing length of time on dialysis treatment, indicating a statistically significant difference between these two parameters ($p<0.01$).

Table 2. Anti-*T. gondii* IgG Antibodies Seropositivity of Haemodialysis Patients, and the Length of Time on Dialysis Treatment

	IgG (+) patients	IgG (-) patients
Number of patients and percentage	97 (56.06%)	76 (43.94%)
Length of time on dialysis treatment in months	57.11 ± 34.05	25.92 ± 23.62

DISCUSSION

T. gondii is found in two different stages in humans. The actively proliferating intracellular forms are called trophosoites, or tachyzoites, and are crescent shaped. Many different tissues may be parasitized by these organisms, particularly lung, heart, lymphoid organ and CNS tissues. The resting forms, or cyst stages, are found in the tissues, and contain the more slowly developing bradizoites.[13]

Toxoplasmosis can vary from an asymptomatic, self-limiting infection, to a fatal disease, as seen in patients with congenital infections, or in debilitated patients, where the underlying conditions may influence the final outcome of the infection. In immunocompromized patients, the infection most often involves the nervous system, with diffuse encephalopathy, meningoencephalitis, or cerebral mass lesions.[13]

The most frequent protozoan causing opportunistic infections in immunocompromised individuals is *Toxoplasma gondii*. Its association with severe manifestations of immuno suppression has been known for several decades, and the occurrence of encephalitis, and the disseminated disease, has subsequently been observed in different clinical conditions, such as lymphoreticular neoplasies, solid organ transplantation, and currently in patients with AIDS.[14] Following the occurrence of AIDS, toxoplasmosis has become the most common cause of encephalitis in the United States.[15]

The diagnosis of toxoplasmosis is mainly based on a combination of clinical and laboratory data. In clinical practice, serological tests are routinely employed to detect IgM and IgG-specific antibodies, including indirect immunofluorescence and immunoenzymatic tests (ELISA), with the latter showing higher sensitivity and specificity.[16] The use of more sensitive and specific methods, such as the polymerase chain reaction (PCR), has been shown to be effective for diagnosis of congenital and ocular toxoplasmosis,[17] but PCR detection of parasitemia in patients with AIDS and toxoplasmic encephalitis is only useful in cases of the disseminated infection.[18]

The present results revealed a higher percentage of positivity for the *T. gondii* IgG antibodies in CRF patients undergoing haemodialysis (56.06%) than in the controls (20%), with statistical significance (Table I). These findings may be due to the patients with CRF being immunocompromised, increasing their susceptibility to this infection.[19]

Infection is a frequent complication, and the major cause of death, of end-stage renal disease.[20] Cellular and humoral immune responses are suppressed in uremic subjects,[21] and impaired cell functions have been reported.[22] Schollmayer and Bozkurt[23] suggested the absolute number of circulating T-cells was reduced and suppressor cells increased, and that haemodialysis did not restore the impairment of the immune status in CRF. The depression in the cell-mediated immunity in CRF has been attributed to vitamin B_6 deficiency,[24] antimicrobial therapy[25] and activation of cellular immune suppressor mechanisms.[26] These events probably contribute to the acquired immune suppression in uremia, and the high incidences of infection among dialysis patients.[20] In addition, patients receiving haemodialysis spent prolonged periods of time together in health care facilities, thus their risk of infection increases.

Esposito et al.[27] reported a significantly higher prevalence of *T. gondii* in renal transplant recipients compared to patients on chronic haemodialysis. Abbas et al.[28] found a positive correlation between the frequency of dialysis sessions and the positivity for *Toxoplasma*. In our study, the percentage of haemodialysis patients positive for the anti-*T. gondii* IgG antibody was found to be

(56.06%) significantly greater than in the healthy volunteers (20%). In addition, a positive correlation was found between the length of time on dialysis treatment and the sero-positivity for *Toxoplasma* ($p < 0.001$). This stresses the role of the haemodialysis procedure itself in the process of transmission of toxoplasmosis. The risk of infection increases with the increasing length of time on dialysis treatment.

Individuals undergoing immunosuppressive therapy, such as organ transplant recipients, who had previously been infected with *T. gondii,* might show an altered serological profile of this protozoan compatible with reactivation, such as increased IgG antibody titres, or less frequently, increased titres of acute phase antibodies. Thus, the kidney transplant patients with the latent state *T. gondii* may undergo acute reactivation, making the results of our study more meaningful in this respect.

In conclusion, patients with CRF undergoing haemodialysis should be screened for *Toxoplasma* before dialysis, and parasitological surveys of CRF patients should be periodically performed to prevent the possible dissemination of toxoplasmosis through the dialysis procedure.

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