Anti-*Toxoplasma gondii* antibodies in patients with chronic heart failure

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Chronic heart failure (CHF) involves interactions between the cardiovascular, neuroendocrine and immune systems. This study investigated the seropositivity rate for anti-*Toxoplasma* IgG and IgM antibodies by ELISA in patients with CHF. Ninety-seven patients with CHF and 50 healthy volunteers were selected for this investigation. The seropositivity rate for anti-*Toxoplasma* IgG antibodies among CHF patients (68%) was significantly higher than in healthy volunteers (36%). Thus, parasitological screening of this group of patients should be periodically performed to prevent the possible dissemination of toxoplasmosis.

INTRODUCTION

Toxoplasma gondii is worldwide in distribution, is closely related to other coccidia and also has certain similarities to malarial parasites. The parasites were first recovered in the North African rodent called *Ctenodactylus gundi*, hence the species was named *gondii*. Although serological evidence indicates a high rate of human exposure to the organism, toxoplasmosis is relatively rare. *T. gondii* can infect many vertebrates as well as humans, but the definitive host is the house cat and other members of the Felidae (Garcia & Bruckner, 1997a).

This organism is an obligate intracellular parasite and is 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 the brain, probably as a result of the host immune response (Garcia & Bruckner, 1997b).

In humans, infection occurs via ingestion of contaminated undercooked food (especially meat) or transplacentally during acute infection in pregnancy, leading to congenital toxoplasmosis. In the immunocompetent host, *Toxoplasma* infection is usually asymptomatic but can produce a mild self-limiting illness with lymphadenopathy similar to infectious mononucleosis. Congenital infection, however, is a very serious condition with a lethal prognosis in about 10% of cases and high rates of disabling sequelae. Toxoplasmosis

is a lifelong condition, but the foetus is only at risk of congenital disease when acute infection occurs in pregnancy. Placental contamination is a prerequisite to congenital infection and occurs almost exclusively following primary infection when there is maternal parasitaemia. The infected placenta then acts as a reservoir from which the parasite can spread to the foetus, leading to multisystemic disease. When the mother is chronically infected by *T. gondii*, the parasite is dormant in the maternal tissues and there is no parasitaemic phase. Only rarely has congenital infection been reported from a chronically infected immunocompromised mother with a reactivation of toxoplasmosis. Foetal transmission can occur immediately after maternal infection or be delayed by several weeks (Daffos *et al.*, 1988).

Fortunately, most postnatal *Toxoplasma* infections are asymptomatic, with clinical toxoplasmosis affecting only a limited number of immunocompetent individuals. Symptomatic infection can be categorized into four groups: (1) lymphadenitis, fever, headache and myalgia, with a possibility of splenomegaly and a brief erythematous rash; (2) typhus-like exanthematous form with myocarditis, meningo-encephalitis, atypical pneumonia and possible death; (3) retinochoroiditis, which may be severe, requiring enucleation; (4) CNS involvement (Beaver *et al.*, 1984).

There are large numbers of *T. gondii*-seropositive humans, which suggests that the majority of infections are mild, with most people exhibiting few (e.g. cold or light case of the flu) or no symptoms. Statistics reveal that approximately 2.5% of AIDS patients have been diagnosed with cerebral toxoplasmosis.

The diagnosis of toxoplasmosis is mainly based on a combination of clinical and laboratory data. In clinical practice,

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Abbreviations: CHF, chronic heart failure; IL, interleukin; IFN, interferon; NK, natural killer.

serological tests are routinely employed to detect IgM and IgG specific antibodies, for example ELISA, which shows a high sensitivity and specificity (Fuccillo *et al.*, 1987). Serological tests can be helpful because the absence of anti-*T. gondii* IgM virtually excludes recent infection in immunocompetent patients. Although IgM antibodies typically disappear a few weeks or months after infection, they can remain elevated for more than 1 year. Thus, the presence of anti-*T. gondii* IgM antibodies does not necessarily indicate that the infection was acquired recently. This issue is important in the evaluation of pregnant women because congenital transmission of *T. gondii* in immunocompetent women occurs almost exclusively when infection is acquired during gestation (Montoya & Remington, 2000).

The ELISA used in this study is an enzyme immunoassay for the detection of *T. gondii*-specific IgG and IgM antibodies in human serum. The *T. gondii* antigen used to coat the microplate comes from tachyzoite ultrasonate enriched with membrane proteins. The conjugate consists of a peroxidase-labelled mAb specific to human gamma chains. The IgG antibody titre rises shortly after the stimulation of IgM antibody. The IgG titre may continue to rise for as long as 2 months and remain elevated for years, even though the clinical symptoms have disappeared (Garcia & Bruckner, 1997b). Anti-*T. gondii* IgG results are interpreted as follows: negative, indicates absence of prior exposure to *T. gondii*; positive, indicates prior exposure to the *T. gondii* and the patient has acquired toxoplasmosis.

Chronic heart failure (CHF) involves interactions between the cardiovascular, neuroendocrine and immune systems. Natural killer (NK) cells, as their name implies, are an important cytolytic component of the innate immune system. These cells contribute the first line of non-antigen-specific defence against infections, and there is evidence that they play a role in tumour surveillance (Kagi *et al.*, 1996). It has been reported that a subgroup of patients with heart failure exhibited NK cell anergy to activation by interleukin (IL)-2 and gamma interferon (IFN- γ) (Vredevoe *et al.*, 1995). Thus, it can be claimed that CHF patients are at risk of a variety of infections, especially opportunistic infections, due to their depressed immune status.

Toxoplasmosis in patients who are immunocompromised by virtue of underlying CHF has received relatively little attention. In the present study, we evaluated the anti-*T. gondii* seropositivity rate of patients with CHF using micro-ELISA. This patient group was immunocompromised because of the underlying diseases so they were at risk of opportunistic infections. We tried to underline the risk of severe toxoplasmosis with this study, which summarizes the results of the monitoring of IgG and IgM antibodies to *T. gondii* in patients with CHF.

METHODS

Patients and their sera. In this study, 97 patients with CHF aged between 23 and 87 years (mean $\pm$ SD, 63.64 $\pm$ 11.46) were randomly

selected from patients who applied to Erciyes University Medical Faculty Cardiology department. In addition, we selected 50 healthy volunteers as a control group aged between 21 and 85 years (mean $\pm$ SD, 62.91 $\pm$ 12.14). Blood samples were taken from the brachial vein of all patients and healthy volunteers under sterile conditions. The sera were separated by centrifugation at 1000 r.p.m. for 10 min and stored at -20°C until the analysis.

Serological technique. The micro-ELISA technique for *T. gondii* was used. Anti-*T. gondii* IgG and IgM antibody ELISA kits were purchased from Bio-Rad. The technique was performed following the manufacturer's instructions.

Statistical analyses. The chi-square test was used for the statistical analyses and was performed by SPSS v.10.0 for Windows.

RESULTS AND DISCUSSION

In the present study, 66 of 97 (68%) cases in the patient group and 18 of 50 (36%) healthy volunteers (control group) were found to be positive for IgG antibodies. The percentage of people who were anti-*T. gondii* IgG positive in the CHF patient group was found to be significantly greater than in healthy volunteers ($P < 0.05$). Only one patient was positive for IgM antibodies in the CHF patient group, although all of the subjects in the control group were seronegative by ELISA. The percentage of people who were anti-*T. gondii* IgM positive in the CHF patient group (1.03%) was greater than in the healthy volunteers (0%), but the difference between groups was not statistically significant ($P > 0.05$).

In this study, we also investigated the relationship between duration of CHF illness and anti-*T. gondii* IgG antibody seropositivity. We observed that the seropositivity rate increased with the increasing duration of CHF illness. This result indicates a correlation between these two parameters ($P < 0.05$).

Infection with the opportunistic protozoan parasite *T. gondii* is widespread in humans and animals, and toxoplasmosis emerges as a life-threatening risk in situations of immunodeficiency (Navia *et al.*, 1986). In immunocompetent hosts, the parasite induces strong T-cell-mediated type 1 immunity (Denkers & Gazzinelli, 1998), with production of proinflammatory cytokines and IFN- γ . *T. gondii* infection is lethal in the absence of these cytokines (Gazzinelli *et al.*, 1994; Scharton-Kersten *et al.*, 1996). However, the strong Th1 response generated during *T. gondii* infection must be tightly regulated by anti-inflammatory factors, without which the immune response triggered by the parasite leads to immunopathology (Neyer *et al.*, 1997). Thus, while IFN- γ is required for resistance to *Toxoplasma*, excessive levels of the cytokine are lethal (Mordue *et al.*, 2001).

On the other hand, NK cells make up ~10–15% of peripheral blood lymphocytes in humans (Campbell & Colonna, 2001) and represent the first line of defence against many pathogens. Upon activation, NK cells begin to proliferate and secrete cytokines as a means of communication with

other components of the immune system, in particular T cells. NK cells also participate in humoral immunity; NK-cell-deficient mice are unable to generate some immunoglobulin isotype subclasses (Satoskar *et al.*, 1999). Impairment of NK cell function could ultimately weaken adaptive cellular and humoral immune responses to multiple pathogens (Moretta *et al.*, 2002; Kos, 1998). In vulnerable populations (e.g. patients with advanced heart failure), even modest impairment of immune function can have deleterious consequences, resulting in morbidities from infectious exposures that would not be life-threatening in healthy subjects.

Interestingly, some proinflammatory cytokines, especially IL-6, mediate biological functions in the cardiovascular, neuroendocrine and immune systems. Levels of IL-6 have been shown to be elevated in serum and plasma of patients with CHF (Deswal *et al.*, 2001; Baumgarten *et al.*, 2000). It has also been reported that a subgroup of patients with heart failure exhibited NK cell anergy to activation by IL-2 and IFN- γ (Vredevoe *et al.*, 1995).

About 20 % of the population of the USA is seropositive for IgG for *T. gondii*, making this one of the most prevalent infections, and probably the only chronic parasitic infection, lasting a human lifetime without any known consequences (Dubey & Beattie, 1988). In France, the rate of seropositivity has been reported to reach 80 %, which may be partly due to eating habits, for example preferring undercooked or raw meat (Feldman, 1982). In Turkey, the rate of seropositivity was reported to be 23.1 % in Izmir (Altintas *et al.*, 1998) and 36 % in Kayseri (Yazar *et al.*, 2000).

Toxoplasmosis can vary from an asymptomatic, self-limiting infection to a fatal disease, as seen in patients with congenital infections or in debilitated patients in whom underlying conditions may influence the final outcome of the infection. In immunocompromised patients, the infection most often involves the nervous system, with diffuse encephalopathy, meningoencephalitis or cerebral mass lesions (Garcia & Bruckner, 1997b).

The most frequent protozoan causing opportunistic infections in immunocompromised individuals is *T. gondii*. Its association with severe manifestations of immunosuppression has been known for several decades, and the occurrence of encephalitis and disseminated disease has since been observed in different clinical conditions such as lymphoreticular neoplasias, solid organ transplantation and, at present, mainly in patients with AIDS (Ferreira & Borges, 2002). Following the emergence of AIDS, toxoplasmosis became the most common cause of encephalitis in the USA (Luft & Remington, 1998).

In conclusion, due to the probability of immunodeficiency and predisposition to opportunistic infections including toxoplasmosis, CHF patients should be periodically screened for *Toxoplasma* to prevent possible dissemination.

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