A Trojan Horse in Intensive Care Unit: *Toxoplasma gondii*

Yoğun Bakım Ünitesinde Bir Truva Atı: *Toxoplasma gondii*

Özlem Selvi CAN, MD, Msc,[a]
Şaban YALÇIN,MD, Assis.Prof.,[b]
Osman MEMİKOĞLU, MD Assoc.Prof.,[c]
Enver ÖZGENCİL, MD, Msc,[a]
Şirali OBA, MD, Msc,[d]
Melek TULUNAY, MD, Prof.,[a]
Necmettin ÜNAL, MD, Prof..,[a]
Mehmet ORAL, MD, Prof.,[a]
Hacer SERDAROĞLU, MD, Msc[e]

Departments of
[a]Anesthesiology and Reanimation,
[c]Clinical Bacteriology and Infectious Diseases,
Ankara University Faculty of Medicine,
[d]Clinic of Anesthesiology and Reanimation,
Medicana Hospital,
[e]Clinic of Anesthesiology and Reanimation,
Güven Hospital, Ankara
[b]Department of
Anesthesiology and Reanimation,
Harran University Faculty of Medicine,
Şanlıurfa

Geliş Tarihi/*Received:* 01.02.2011
Kabul Tarihi/*Accepted:* 18.06.2011

This article was presented as a poster in the 27th International Symposium on Intensive Care and Emergency Medicine, 2007, Brussels, Belgium.

Yazışma Adresi/*Correspondence:*
Şaban YALÇIN, MD, Assis.Prof.
Harran University Faculty of Medicine,
Department of Anesthesiology and
Reanimation, Şanlıutfa,
TÜRKİYE/TURKEY
sabanyalcin@yahoo.com

doi:10.5336/medsci.2011-23054

ABSTRACT Objective: Toxoplasmosis attains clinical significance as an opportunistic infection with high mortality in immunosuppressed individuals. The aim of this study was to assess the anti-*Toxoplasma gondii* antibody seropositivity rate in intensive care unit patients (ICU) who are immunosuppressed due to underlying critical illness. **Material and Methods:** One hundred and three (51 males, 52 females) patients in intensive care units and 78 healthy volunteers (39 males, 39 females) were enrolled in the study. Blood samples were collected from all patients within the first 24 hours of hospitalization in the ICU and anti-*T. gondii* IgG and IgM antibodies were tested by enzyme-linked immunosorbent assay. **Results:** IgG antibodies to *T. gondii* were positive in 58 (56.3%) patients in the ICU and in 19 (24.3%) healthy volunteers. IgM antibodies were positive in 15 patients (13.8%) in the ICU and in five healthy volunteers (6.4%) in the control group. The difference in the IgG seropositivity rate between the patient and the control group was statistically significant ($p< 0.05$). The seropositivity rate of anti-*T.gondii* IgM was higher in the patient group with no significance ($p> 0.05$). **Conclusion:** Toxoplasma seropositivity is common in ICU patients. Toxoplasmosis should be considered in immunosuppressed patients in the ICU and serological screening may be performed to prevent severe toxoplasmosis.

Key Words: Toxoplasma; intensive care; enzyme-linked immunosorbent assay

ÖZET Amaç: Toksoplazmoz, bağışıklığı baskılanmış bireylerde yüksek mortalite ile seyreden fırsatçı bir enfeksiyon olarak klinik öneme sahiptir. Bu çalışmada, zemindeki kritik hastalıkları nedeniyle bağışıklığı baskılanmış olan yoğun bakım hastalarında anti-*Toxoplasma gondii* antikorlarının prevalansının belirlenmesi amaçlandı. **Gereç ve Yöntemler:** Yoğun bakım birimlerinde yatan 103 hasta (51 erkek, 52 kadın) ve 78 sağlıklı gönüllü (39 erkek, 39 kadın) çalışmaya dahil edildi. Kan örnekleri yoğun bakımda yattıkları ilk 24 saat içinde alındı ve enzim aracılı immünosorbent deney yöntemi ile anti-*T. gondii* IgG ve IgM antikorları araştırıldı. **Bulgular:** Yoğun bakımda yatan 58 hastada (%56,3) ve 19 gönüllüde (%24,3) anti-*T. gondii* IgG antikorları pozitif bulundu. IgM antikorları ise yoğun bakımda yatan 15 hastada (%13,8) ve 5 gönüllüde (%6,4) saptandı. Gruplar arasında IgG seropozitiflik oranı istatistiksel olarak anlamlı bulundu ($p< 0,05$). Buna karşılık, anti-*T. gondii* IgM oranı yoğun bakım hastalarında fazla olmasına rağmen, bu fark istatistiksel açıdan anlamlı değildi ($p> 0,05$). **Sonuç:** Yoğun bakım hastalarında toksoplazma seropozitifliği nadir değildir. Bağışıklığı baskılanmış yoğun bakım hastalarında toksoplazmoz olabileceği akılda tutulmalı, ciddi toksoplazmoz gelişme riskini önlemek için serolojik araştırmalar yapılmalıdır.

Anahtar Kelimeler: Toksoplazma; yoğun bakım; immünoenzimatik yöntem

Turkiye Klinikleri J Med Sci 2012;32(1):120-3

Toxoplasma gondii, a worldwide-distributed parasite, may infect many vertebrates, including humans, but the natural host is house cat and other members of the Felidae family.[1] 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 brain tissues, probably due to the host immune response.[1]

Toxoplasmosis attains clinical significance in two major situations, as a cause of congenital infection and as an opportunistic infection with high mortality in immunosuppressed individuals.[2-7] Twenty percent of the U.S. population is seropositive for *T. gondii* immunoglobulin G (IgG), making this one of the most prevalent and probably the only chronic parasitic infection lasting a human lifetime.[8] Seropositivity rates for *T. gondii* in two large cities in Turkey were reported as 23.1% for İzmir and 36% for Kayseri.[9,10] The high seropositivity rates suggest that the majority of infections are mild, with most people exhibiting few (ie, cold or mild case of flu) or no symptoms. On the other hand, studies have shown higher rates of symptomatic infection in immunecompromised individuals such as those with HIV infection.[11] The prevalence of *Toxoplasma gondii*-induced encephalitis may increase up to 40% in patients with AIDS.[12-14] Toxoplasmosis in intensive care unit (ICU) patients who are immunocompromised due to underlying critical illness has received relatively little attention. The aim of this study was to assess the anti-*Toxoplasma gondii* antibody seropositivity rate in ICU patients by testing for IgG and IgM antibodies to *T. gondii*.

MATERIAL AND METHODS

SUBJECTS

Written informed consent was obtained from all individuals or their family members and the institutional review board approved the study. The study was run in accordance with the ethical principles for human investigations, as outlined by the Second Declaration of Helsinki. The study group consisted of 103 (51 males, 52 females) patients hospitalized in the ICU of the Department of Anesthesiology and Reanimation Ankara University Medical Faculty between 2005 and 2006 and 78 healthy volunteers (39 males, 39 females). Blood samples were collected under sterile conditions from all patients within the first 24 hours of hospitalization in the ICU. Samples were centrifuged at 1000 r.p.m. and the sera were separated. The sera were stored at -20 C° until tested.

Non-medical staff members of the Ankara University Medical Faculty and their relatives comprised the control group. All volunteers were healthy individuals without any known medical problems. Blood samples were collected from all healthy volunteers under sterile conditions and were centrifuged at 1000 r.p.m. to separate the sera. The sera were stored at -0C° until tested.

SEROLOGICAL TECHNIQUE

Enzyme-linked immunosorbent assay (ELISA) was used for determination of anti-T. gondii IgG and IgM antibodies. The ELISA kit was provided by a commercial manufacturer (EUROIMMUN, Germany). The test procedure was run according to the instructions of the manufacturer, which used peroxidase-labelled anti-human IgG (rabbit) to determine IgG antibodies and peroxidase-labelled anti-human IgM (goat) to determine IgM antibodies. The cut-off values recommended by the manufacturer were used for IgG (10 IU ml^{-1}) and IgM antibodies. The absorbance of serum samples was divided by the absorbance of the calibrator; if the ratio was >1 the sample was considered positive for IgM antibody.

STATISTICAL ANALYSIS

SPSS version 15.0 for Windows pocket program was used. Chi-squared test was adopted for categorical variables. The difference was considered statistically significant when the $p< 0.05$.

RESULTS

The age of the patient group ranged between 18 and 81 (mean age 53.9 ± 13.9) and the control group between 24 and 66 (mean age 51.4 ± 9.2). The diagnoses of patients on the first day of admission were as follows; respiratory failure (34 patients), multi-trauma (25 patients), malignancy (14

patients), renal failure (13 patients), neurological disorders (12 patients), and liver failure (5 patients). Mean APACHE II score on admission was 20.1±3.2. Fifty-eight patients (56.3%) in the ICU and in 19 (24.3%) individuals in the control group were positive for IgG antibodies to *T. gondii*. IgM antibodies were positive in 15 patients (13.8%) in the ICU and in 5 healthy volunteers (6.4%) in the control group. The sero-prevalence distributions of the two groups were shown in Table 1. The difference in the IgG seropositivity rate between the patient and the control group was statistically significant ($p< 0.05$). The seropositivity rate of anti-*T. gondii* IgM was higher in the patient group with no significance ($p> 0.05$) (Table 1).

DISCUSSION

T. gondii is the most common protozoan causing opportunistic infections in immunocompromised individuals and has been associated with severe manifestations of immunosuppression in different clinical conditions such as lymphoreticular neoplasias, solid organ transplants and AIDS.[15] We investigated the seropositivity rate of toxoplasmosis in ICU patients, using ELISA to determine levels of anti-*T. gondii* IgG and IgM antibodies on the first day of ICU stay. The present results revealed higher positivity rates for *T. gondii* IgG and IgM antibodies in ICU patients (56.3%) compared with the controls (24.3%) with a statistically significant difference (Table 1). These findings may be attributed to the altered immunity of ICU patients.

In immunocompetent individuals acute acquired Toxoplasma infection is usually self-limiting and rarely requires specific treatment.[16] Following acute infection, *T. gondii* remains viable in the form of tissue cysts which reproduce slowly throughout the life span of the host, thus characterizing the chronic phase of infection. During this phase, the tissue cysts are controlled by the humoral and cellular immune system, involving Tlymphocytes and macrophages, which are continuously stimulated by parasite antigens. As a result, parasite multiplication is more active and persists for longer periods in less immunologically active tissues such as the central nervous system.[17,18] Immunocompromised hosts, especially those with deficient cellular immunity, are at risk of recrudes- cence of chronic infection and dissemination, with the occurrence of fulminating disease. Patients with neoplasia, collagen tissue disease, and transplant recipients under immunosuppressive therapy or haemodialysis patients with chronic renal failure have deficient cellular immunity, and this makes them susceptible to the infection.[5,6] In immunocompromised patients, the infection most often involves the nervous system, with diffuse encepha- lopathy, meningoencephalitis or cerebral mass lesions.[1]

Critically ill patients frequently demonstrate profound immunity abnormalities due to their illness or to treatment regimes used.[19] In the ICU, systemic inflammatory response syndrome or shock develops in a high number of admitted patients, which induces the release of catecholamines and glucocorticoids into the systemic circulation.[20] During systemic inflammatory response syndrome and the compensatory anti-inflammatory response syndrome, the immune function may be impaired.[21-24] Individuals with immunity abnormalities such as ICU patients, who had been previously infected with *T. gondii*, may show increased IgG antibody titres or less frequently, increased titres of acute-phase antibodies, which may be interpreted as reactivation. *T. gondii* infection produces IgM, IgA, IgE and IgG antibodies, with the first three being detected early during the course of infection. IgM antibodies that indicate acute infection are the first to occur and can be detected 7-15 days after infection followed by a progressive decline and disappearance of these antibodies within a few months. IgG antibodies indicate chronic in-

TABLE 1: Anti-*T. gondii* IgG and IgM antibodies in intensive care unit patients and controls.

	ICU patients (n= 103)		Control group (n= 78)	
	n	%	n	%
Anti-*T.gondii* IgG*	58	56.3	19	24.3
Anti-*T.gondii* IgM	15	13.8	5	6.4

ICU, intensive care unit; IgG, immunoglobuline G; IgM, immungolobuline M, *T. gondii, Toxoplasma gondii.*

* $p< 0.05$.

fection and an increased titre of IgG antibodies might show reactivation.[15] Results compatible with reactivation or acute infection may influence the treatment protocol of the ICU patient.

The diagnosis of acute toxoplasmosis is primarily centered on a combination of clinical and laboratory data. The serologic diagnosis of toxoplasmosis is very complex and has been discussed extensively in the literature; a number of additional procedures, some of which are automated, have been developed. These methods include enzyme immunoassays, some of which are automated, ELISA tests, direct agglutination, immunofluorescence assay (IFA), immunocapture and immunoblot. The ELISA is the most common method because of its efficacy, ease of use, and low cost and availability. More sensitive and specific procedures such as polymerase chain reaction has been shown to be effective.[15]

In conclusion, *Toxoplasma* seropositivity is common in ICU patients; thus toxoplasmosis should be considered in immunocompromised patients in the ICU and serological screening may aid to prevent severe toxoplasmosis.

REFERENCES

1. Garcia SL, Bruckner AD. Tissue protozoa (Toxoplasma gondii). Diagnostic Medical Parasitology. 3rd ed. Washington, DC: American Society for Microbiology Press; 1997. p.111-21.
2. Remington JS, Desmonts G. Toxoplasmosis. In: Remington JS, Klein JO, eds. Infectious Diseases of the Fetus and Newborn Infant. 3rd ed. Philadelphia, PA: Saunders; 1990. p.89-195.
3. Carey RM, Kimball AC, Armstrong D, Lieberman PH. Toxoplasmosis. Clinical experiences in a cancer hospital. Am J Med 1973;54(1): 30-8.
4. Ambroise-Thomas P, Pelloux H. Toxoplasmosis - congenital and in immunocompromised patients: a parallel. Parasitol Today 1993;9(2): 61-3.
5. Yazar S, Yaman O, Eser B, Altuntas F, Kurnaz F, Sahin I. Investigation of anti-Toxoplasma gondii antibodies in patients with neoplasia. J Med Microbiol 2004;53(Pt 12): 1183-6.
6. Yazar S, Demirtaş F, Yalçin S, Yaman O, Tokgöz B, Utaş C, et al. Anti-Toxoplasma gondii antibodies in haemodialysis patients with chronic renal failure. Yonsei Med J 2003;44(2): 288-92.
7. Tansel O, Ekuklu G, Kunduracılar H, Eker A, Yuluğkural Z, Yüksel P. [Seroepidemiology of toxoplasmosis and the theoretical incidence of congenital toxoplasmosis in women of reproductive age in Edirne, Turkey. A community based study]. Turkiye Klinikleri J Med Sci 2009;29(1):84-90.
8. Dubey JP, Beattie CP. Toxoplasmosis of Animals and Man. 1st ed. Boca Raton, Fla: CRC Press; 1988. p.220.
9. Altintas N, Yolasigmaz A, Yazar S, Sakru N, Kitapcioglu G. [Investigation of Toxoplasma antibodies in residence of central Izmir and surrounding rural area]. Acta Parasitol Turc 1998;22(3):229-32.
10. Yazar S, Karagoz S, Altunoluk B, Kilic H. [Investigation of anti-Toxoplasma gondii antibodies in patients suspected of toxoplasmosis]. Acta Parasitol Turc 2000;24(1):14-6.
11. Cetinkaya Z, Yazar S, Gecici O, Namli MN. Anti-Toxoplasma gondii antibodies in patients with schizophrenia--preliminary findings in a Turkish sample. Schizophr Bull 2007;33(3): 789-91.
12. Pereira-Chioccola VL, Vidal JE, Su C. Toxoplasma gondii infection and cerebral toxoplasmosis in HIV-infected patients. Future Microbiol 2009;4(10):1363-79.
13. Krick JA, Remington JS. Toxoplasmosis in the adult--an overview. N Engl J Med 1978;298 (10):550-3.
14. Luft BJ, Remington JS. AIDS commentary. Toxoplasmic encephalitis. J Infect Dis 1988;157(1):1-6.
15. Ferreira MS, Borges AS. Some aspects of protozoan infections in immunocompromised patients- a review. Mem Inst Oswaldo Cruz 2002;97(4):443-57.
16. Israelski DM, Remington JS. Toxoplasmosis in the non-AIDS immunocompromised host. Curr Clin Top Infect Dis 1993;13:322-56.
17. Darcy F, Santoro F. Toxoplasmosis. In: Kierszenbaum F, ed. Parasitic Infections and the Immune System. 1st ed. New York: Academic Press; 1994. p.163–201.
18. Sims TA, Hay J, Talbot IC. An electron microscope and immunohistochemical study of the intracellular location of Toxoplasma tissue cysts within the brains of mice with congenital toxoplasmosis. Br J Exp Pathol 1989;70 (3):317-25.
19. Tanaka S, Toh Y, Minagawa H, Mori R, Sugimachi K, Minamishima Y. Reactivation of cytomegalovirus in patients with cirrhosis: analysis of 122 cases. Hepatology 1992;16(6): 1409-14.
20. Kutza AS, Muhl E, Hackstein H, Kirchner H, Bein G. High incidence of active cytomegalovirus infection among septic patients. Clin Infect Dis 1998;26(5):1076-82.
21. Tamion F, Richard V, Lyoumi S, Daveau M, Bonmarchand G, Leroy J, et al. Gut ischemia and mesenteric synthesis of inflammatory cytokines after hemorrhagic or endotoxic shock. Am J Physiol 1997;273(2 Pt 1):G314-21.
22. Bone RC. Immunologic dissonance: a continuing evolution in our understanding of the systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS) Ann Intern Med 1996;125(8): 680-7.
23. Webster NR, Galley HF. Immunomodulation in the critically ill. Br J Anaesth 2009;103(1): 70-81.
24. Marshall JC, Charbonney E, Gonzalez PD. The immune system in critical illness. Clin Chest Med 2008;29(4):605-16, vii.