Annals of Tropical Medicine & Parasitology, Vol. 105, No. 5, 367–371 (2011)

Detection of IgM and IgG anti-Toxoplasma antibodies in renal transplant recipients using ELFA, ELISA and ISAGA methods: comparison of pre- and post-transplantation status

M. J. GHARAVI[*], S. JALALI[†], S. KHADEMVATAN[‡] and S. HEYDARI[§]

[*]*Department of Parasitology, Tehran University of Medical Sciences, Tehran, Iran*
[†]*Tehran University of Medical Sciences, Tehran, Iran*
[‡]*Department of Parasitology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
[§]*Department of Parasitology, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

Received 20 December 2010, Revised 6 June 2011,
Accepted 13 June 2011

In the transplant recipient patients receive immunosuppressive therapy, the possibility of reactivation of the old infection or acquisition of infection from a donor's tissue increases. In this study, IgM and IgG anti-Toxoplasma immunoglobulins seroconversion in renal transplant recipients (RTRs) have been evaluated before and after transplantation.

This is a prospective cohort study on a total of 102 RTRs. Two serum samples were obtained from each patient. The first was taken before administration of any immunosuppressive drugs such as corticosteroids and the second was taken 3 months after transplantation. The IgM and IgG anti-Toxoplasma antibodies were assayed by enzyme-linked flourescence assay (ELFA) and enzyme-linked immunosorbent assay (ELISA) techniques. IgM/immunosorbent agglutination assay (ISAGA) method has also been used.

All RTRs were tested for toxoplasmosis before and after transplantation. ELFA identified 65 (63.7%) pre-transplantation samples as IgG+ and did not detect any positive IgM samples. However, IgM was detected in three (2.9%) post-transplantation samples by this method. Forty-nine (48%) pre-transplantation samples were reported IgG+ by ELISA and no IgM positive sample was identified by this method. ELISA has detected two (1.9%) IgM-positive reactions in post-transplantation samples. By IgM/ISAGA method, we have detected no IgM positive reactions in pre-transplantation samples, whereas 3 months later (second sampling) IgM antibody was detected in 3 (2.9%) cases.

Secondary toxoplasmosis infection was observed in 30 cases per 1000 RTRs, which indicates that screening for toxoplasmosis infection should be performed in developed countries for these patients. On the other hand, as the risk of re-active toxoplasmosis infection exists in developing nations, they should consider the necessary preventive measures to control this condition.

INTRODUCTION

Toxoplasmosis is a widespread infection reported throughout the world and affects human, mammals and birds. The protozoan is an obligatory parasite with two sexual and asexual life cycles. Three forms of tachyzoite, pseudocyste and tissue cyst exist in asexual hosts and oocyst is produced during the sexual cycle (David and William, 2006). Outbreak of infection is different based on age, geographical situations, food habits as well as many epidemiological agents. In Iran, seroprevalence has been reported to be between 40 and 70% at different regions (Hazrati Tappeh *et al.*, 2006).

Soil, water and food — especially vegetables — that have been contaminated by oocysts, and consumption of the meat contaminated by tissue cysts, such as kebab, are sources of transmission. Toxoplasmosis

Reprint requests to: Mohammad Javad Gharavi, Department of Parasitology, Tehran University of Medical Sciences, Tehran, Iran.
E-mail: gharavi_m_j@yahoo.com.

DOI: 10.1179/1364859411Y.0000000022

occurs in acquired and congenital types and the latter is more important (Gharavi *et al.*, 2005; Hazrati Tappeh *et al.*, 2006).

Toxoplasmosis is usually asymptomatic in adults (70–80% cases) and also in patients with normal immune system. Symptoms include: mild fever, lymphadenopathy and exanthematic rashes (20–30% cases). In addition, in immunocompromised patients such as HIV positive and immunosuppressed individuals and in congenital toxoplasmosis it is likely to observe serious manifestations such as encephalopathy, CNS disorders, convulsion, pneumonia, myocarditis, signs of brain calcification, etc. (Trikha and Wig, 2001). These manifestations are signs of severe toxoplasmosis (<10% cases) (Montoya and Liesenfeld, 2004).

Several studies have shown that immunosuppressed individuals including renal transplant recipients (RTRs) are susceptible to secondary acquired toxoplasmosis (Meroni *et al.*, 1997). The acute complications including encephalopathy, meningoencephalitis and meningitis could be similar to the conditions caused by other diseases which make differential diagnosis a complicated process. Diagnosis of toxoplasmosis cannot be made by clinical findings alone; it needs confirmatory parasitological and serological evaluations (Petersen *et al.*, 2005). The serological methods are based on sensitivity and specificity (based on immune complex mechanism) including affinity and avidity (Petersen *et al.*, 2005). However, it should be noted that parasitological methods are more valid (Gharavi *et al.*, 2008). According to the level of outbreak of infection in Iran, some regions have reported a level of more than 70%. This percentage could be higher in patients after renal transplantation, warranting appropriate measures for proper diagnosis, treatment and prevention, and especially control of relapse in renal transplantation patients. Pre- and post-transplantation screening for anti-toxoplasma antibodies could be considered as an appropriate assessment. This study aims to compare the value of the new diagnostic method enzyme-linked flourescence assay (ELFA) with immunosorbent agglutination assay (ISAGA) and enzyme-linked immunosorbent assay (ELISA) in diagnosis of endogenous infection in RTRs. The other issues of interests are sensitivity and specificity of these methods and the economical and scientific justification for screening the toxoplasmosis in RTRs.

MATERIALS AND METHODS

Sampling

1. The samples were collected from 102 RTRs during 12 months in Hasheminejad and Labafinejad hospitals. The first sampling was performed before transplantation and before any immunosuppressive drugs were given the patients.
2. Sera were kept at -20°C until examination.
3. The second sampling was performed 3 to 4 months later.
4. Demographic data such as gender, age, occupation, and any contacts with cat, seropositivity of Toxoplasma, and the dose of immunosuppressive drugs were collected by a questionnaire.
5. After each test, the samples were kept at $-70^{\circ}C$ until the end of the study, and the samples with equivocal results were tested again.

Methods

1. ELFA method: it is an enzymatic sandwich that results in generation of a florescent product. The measurements were performed by IgG/ELFA and IgM/ELFA kits (VIDAS, BioMerieux Mercy l'Etoile Co., France). According to the manufacturer's instructions the IgG level <4 UI/ml was reported as negative and >8 UI/ml was reported as positive. For IgM, the level <0.55 UI/ml was

negative and >0.65 UI/ml was positive. This method was able to assay and detect IgG and IgM.

2. ELISA method: It tracks any complex including antigen and antibody couples. The measurements were performed by IgG/ELISA kit (Genesis Co., UK) and IgM/ELISA kit (Anthose, Austria). According to the manufacturer's protocol, the IgG anti-Toxoplasma <15 UI/ml was reported negative and the level >15 UI/ml was reported positive. In regard to IgM levels lower than 1 UI/ml was reported negative and levels equal or higher than 1 UI/ml was reported positive. This method was able to assay and detect IgG and IgM.
3. ISAGA method: The principle of the test is the agglutination of antigen of Toxoplasma antigen by specific IgM antibodies in patient's sera. It is a mixture of two methods of Direct Agglutination and ELISA. We employed the commercial kit Toxo-ISAGA (BioMerieux, Mercy I'Etoile, France) in which IgM monoclonal antibody was linked to the solid phase. In the absence of specific antibodies formulated Toxoplasma precipitated in wells but for positive reaction agglutination occurred. This method was only suitable for evaluating the IgM antibody. It is a qualitative and ocular method which is done as duplicate. The results for each serum is read by two dilutions and are reported as 0, 1^+, 2^+, 3^+, 4^+. Finally, the aggregate of both dilutions from 0 to 5^+ is negative. However, if it is 6^+ or higher, it is positive.

FINDINGS

In this study, the youngest RTR was 15 and the oldest 65 years (Table 1) and 70% of patients were male and 30% female.

Among 102 sera with ELFA method before transplantation, 65 cases (63.7%) were positive for IgG, with serotiters ranging from 15 UI/ml to more than 300 UI/ml. In this method, all of the RTRs were negative for IgM. In the second referral in 3 months later, after transplantation, the frequency of IgG^+ samples was the same as the first sampling and also three cases (2.9%) were positive for IgM that indicated secondary toxoplasmosis.

From a total of 102 sera that were tested with ELISA method, the number of IgG^+ patients before transplantation was 65 (63.7%), in which the lowest level was 20 and the highest level was 150. All of the RTRs were negative for IgM. For the second referral, two cases (1.9%) of patients had positive IgM that indicated secondary toxoplasmosis.

Among the total sera tested by IgM/ISAGA method before transplantation, all of the cases had negative IgM and in the re-sampling, three cases (2.9%) were positive for IgM that indicated secondary toxoplasmosis (Table 2).

DISCUSSION

For many years, toxoplasmosis has been considered as an opportunistic infection in immunocompromised patients (Hwa and Kim, 2004; Yazar *et al.*, 2004; Yuan *et al.*, 2007). Wulf *et al.* (2005) have explained the problems of toxoplasmosis in RTRs such as lack of clinical awareness and difficulties in

TABLE 1. Patients' age distribution

Age groups	Frequency	
(years)	%	Number
15–20	7.8	8
21–30	23.5	24
31–40	23.5	24
41–50	13.7	14
51–60	21.5	22
61–70	9.8	10

TABLE 2. The presence of IgM/IgG Toxoplasma antibodies in RTRs before and after transplantation by ELFA, ELAISA and ISAGA methods

Positive cases	First referral		Second referral	
Methods	Number	%	Number	%
IgG^+/ELFA	65	63.7	65	63.7
IgM^+/ELFA	0	0	3	2.9
IgG^+/ELISA	49	48.1	49	48.1
IgM^+/ELISA	0	0	2	1.9
IgM^+/ISAGA	0	0	3	2.9

establishing a diagnosis. This disease contributes to the high mortality up to 65%, and 15 of 35 cases (43%) were diagnosed at autopsy. Moreover, the clinical presentation of toxoplasmosis in RTRs varies. Fever is the most frequent clinical sign (80%), followed by pneumonia and generalized neurological signs such as headaches, drowsiness and lethargy (Walker and Zunt, 2005). Toxoplasmosis should be considered in the differential diagnosis of pneumonia, culture-negative sepsis, and encephalitis in RTRs. However, screening will help in identifying patients at risk, especially seronegative recipients with seropositive donors, and can help in establishing the diagnosis by showing seroconversion. If no standard screening is performed, specimens should be stored for later testing. Increased awareness and early diagnosis may improve, otherwise poor outcome of toxoplasmosis in RTRs can occur (Wulf *et al.*, 2005).

Toxoplasmosis does not represent a major risk in kidney transplanted patients. However, life-threatening toxoplasmosis with pulmonary and cerebral manifestations has been previously reported by Iqbal *et al.* (2003). He suggested that despite the low risk, it must always be considered in seropositive patients, especially when the donor is seropositive. In any case, the presence of anti-Toxoplasma antibodies should be routinely assessed in donors and recipients prior to transplantation to obtain baseline serology and evaluate the potential risk of Toxoplasma infection after transplantation (Iqbal *et al.*, 2003). Although the results of the comparative methods are close to each other, the automatic methods (CLIA, ELFA) are preferred because of a high reproducibility, lower personnel budget, etc. Therefore, Gharavi *et al.* (2008) suggested using these methods to diagnosis of toxoplasmosis. He motioned that IgM/ISAGA is the most suitable diagnostic method for IgM detection (Gharavi *et al.*, 2008). In addition, confirmatory diagnostic method in Toxoplasma Serological Profile with a positive or equal IgM titer was emphasized by Montoya *et al.* (2002). They described the avidity test result, and in accordance with our study, showed the superiority of ELFA method with VIDAS system over other methods (Montoya *et al.*, 2002).

According to our finding, secondary toxoplasmosis in RTR as a main risk factor is not considered, meanwhile in some patients due to possibility of presence of secondary infection that lead to severe toxoplasmosis. Consequently, monitoring transplant patients as a group of patients being treated with immunosuppressed drugs should not be neglected. Despite the previous studies (that we mentioned before), we claim that for immunocompromised individuals such as patient who take immunosuppressive drugs, and considering the rate of secondary toxoplasmosis risk (3%), we must notice the secondary toxoplasmosis in RTRs.

Also, in accordance with our results, we suggest that ELFA method is higher sensitive and more specific than others, thus has more trustful results. In fact, acute infections especially congenital toxoplasmosis, in order to identify IgM, the IgM/ISAGA method has the same validity of ELFA.

ACKNOWLEDGEMENTS. This project was granted by student's budget project of Iran University of Medical Science (IUMS) and Central Fardis Laboratory. Therefore, we are very grateful to them and we would like to thank and honor Dr Nejad Gashti, Professor of IUMS; Mr Fatemi and Ms Zare, from

Labafinejad Hospital; Ms Nasirdivani and Ms Miraei, from Hasheminejad Hospital as well as all respected personnel of renal transplant section and laboratories of both hospitals.

REFERENCES

David, T. J. & William, A. P. (2006). *Toxoplasma gondii*. In *Medical Parasitology*, 9th edn, eds Markell, E. K. & Voge, E. M. pp. 140–149. St. Louis, MO: Saunders Elsevier.

Gharavi, M. J., Oormazdi, H. & Roointan, E. S. (2008). A comparative study on sensitivity and specificity of conventional and unconventional IgG and IgM assays for diagnosis of Toxoplasmosis. *Iranian Journal of Public Health*, **37**, 42–45.

Gharavi, M. J., Rahnama, N. & Jahani, M. R. (2005). Seroepidemiological survey of Toxoplasma infection of mentally retarded children. *Iranian Journal of Public Health*, **34**, 19.

Hazrati Tappeh, K. H., Gharavi, M. J., Makhdoumi, K., Rahbar, M. & Taghizadeh, A. (2006). Prevalence of cryptosporidium spp. infection in renal transplant and hemodialysis patients. *Iranian Journal of Public Health*, **35**, 54–57.

Hwa, E. O. & Kim, I. (2004). Toxoplasmic lymphadenitis with toxoplasma bradyzoites. *The Korean Journal of Pathology*, **38**, 330–332.

Iqbal, J., Nampoory, M. R. N., Johnv, K. V., Khalid, N. & Al-Mousawi, M. (2003). Determination of antibodies to Toxoplasma gondii and CMV in renal transplant recipients. *Transplantation Proceeding*, **35**, 2703–2705.

Meroni, V., Zerrilli, E., Genco, F., Nocita, B., Poletti, F. & Minoli, L. (1997). Incidence of acquired toxoplasmosis in seronegative recipients of solid organ transplant. *Clinical Microbiology Reviews*, **10**, 107.

Montoya, J. G. & Liesenfeld, O. (2004). Toxoplasmosis. *Lancet*, **363**, 1965–1967.

Montoya, J. G., Liesenfeld, O., Kinney, S., Press, C. & Remington, J. S. (2002). VIDAS test for avidity of toxoplasma-specific immunoglobulin G for confirmatory testing of pregnant women. *Journal of Clinical Microbiology*, **40**, 2504–2508.

Petersen, E., Victoria, B. M., Guy, E., Liesenfeld, O., Meroni, V., Naessens, A., Spranzi, E. & Thulliez, Ph. (2005). European multicenter study of the LIAISON automated diagnostic system for determination of toxoplasma gondii-specific immunoglobulin G (IgG) and IgM and the IgG avidity index. *Journal of Clinical Microbiology*, **43**, 1570–1574.

Trikha, I. & Wig, N. I. (2001). Management of toxoplasmosis in AIDS. *Indian Journal of Medical Sciences*, **55**, 87–98.

Walker, M. & Zunt, J. R. (2005). Parasitic central nervous system infections in immonocompromised hosts. *Clinical Infectious Disease*, **40**, 1005–1015.

Wulf, M. W. H., Crevel, R., Portier, R., Meulen, C. G., Melchers, W. J. G. & Galama, J. M. D. (2005). Toxoplasmosis after renal transplantation: implications of a missed diagnosis. *Journal of Clinical Microbiology*, **43**, 3544–3547.

Yazar, S., Yaman, O., Eser, B., Altuntas, F., Kurnaz, F. & Sahin, I. (2004). Investigation of anti-Toxoplasma gondii antibodies in patients with neoplasia. *Journal of Medical Microbiology*, **53**, 1183–1186.

Yuan, Z., Gao, S., Liu, Q., Xia, X., Liu, X., Liu, B. & Hu, R. (2007). Toxoplasma gondii antibodies in cancer patients. *Cancer Letters*, **254**, 71–74.