Iranian J Parasitol: Vol.2, No.4, 2007, pp. 1-6

Original Article

Determination of Antibodies (IgG, IgM) against *Toxoplasma gondii* in Patients with Cancer

**M Ghasemian [1], Sh Maraghi [2], J Saki [1], M Pedram [2]*

[1]Mycoparasitology Dept. School of Medicine, Jundi-Shapour University of Medical Sciences, Ahwaz, Iran
[2]Shafa Hospital and Research Center of Thalasemia and Haemoglobinopathy, Ahwaz, Iran

(Received 9 May 2007; accepted 10 Nov 2007)

Abstract

Background: The aim of this study was determination of antibodies (IgG, IgM) against *Toxoplasma* in malignant patients in order to refer the patients on time to the physician for treatment.

Methods: This study was carried out on 252 malignant patients and 252 healthy normal subjects (as control) obtained from Shafa Hospital and Medical Diagnostic Laboratory (Iran-Zamin), in Ahwaz city. Patient's information was recorded in a questionnaire before sampling. Serum samples of patients were examined for IgG and IgM antibodies by ELISA technique using Trinity kits.

Results: The results of this study revealed the presence of *Toxoplasma* antibodies in 114 (45.2%) cases of patients who were positive for *Toxoplasma* IgG antibodies, and 26 (10.3%) cases were confirmed to be positive for *Toxoplasma* IgM antibodies and also 17 (6.7%) of cases had both IgG and IgM antibodies against *Toxoplasma gondii*. In control group 92 (36.5%) cases and 15 (6%) cases revealed seropositive for IgG and IgM antibodies, respectively. There were no significant differences between sex, close contact with cat, living region, chemotherapy, and seropositivity rate of toxoplasmosis in patients. Comparing the age groups, the highest seropositive rate showed in the age of 51 years or higher, and their rates had tendency to increase with age in both groups. No seropositivity significant relationship was found between patients and control group.

Conclusion: According to the prevalence of positive cases in these patients, it is necessary to examine the patients for toxoplasmosis before, during and after chemotherapy.

Keywords: *Toxoplasmosis, Cancer, ELISA, Iran*

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite occurring with a global distribution amongst human and animals. Transmission to human occurs either through ingestion of *T. gondii* oocysts shed into the environment via cat faeces, or by eating raw or uncooked meat of infected animals. Under normal immune conditions, *Toxoplasma* infection is largely asymptomatic, but in those individuals who are immunocompromised, such as individuals with AIDS, malignant patient under chemotherapy or organ transplant recipients, the parasite can become widely disseminated, causing severe toxoplasmosis and/or encephalitis (1-3). As an effective vaccine has not yet been developed, continuous and detailed epidemiological surveillance is required to estimate the risk of infection, especially in pregnant women, and the likelihood of reactivation in immunocompromised individuals.

The diagnosis of toxoplasmosis is most commonly made by detecting the immunoglobulin (IgG and IgM) antibodies in the serum samples of patients using variety methods (ELISA, IFA, IgM-ISAGA, etc) (4). So the aim of this work was to determine the antibodies (IgG, IgM) against *Toxoplasma* in malignant patients to encourage awareness of this opportunistic parasite and in

***Corresponding author**: Tel: 0152-5362179 Fax: 0611-3360082 E-mail: mehr-977@ yahoo.com

order to referring the patients on time to the physician for treatment.

Materials and Methods

Study population

A cross-sectional sero-survey of *Toxoplasma* IgG and IgM antibodies in 252 malignant patients and 252 healthy normal subjects as control, which they nearly were similar to the patient group for age and sex, were conducted. This study was undertaken in 2004-2005. The study population, consisting of malignant patients confined to bed in Shafa Hospital, Ahwaz City, south of Iran as well as those referred to Medical Diagnostic Laboratory (Iran-Zamin) in this city. Questionnaire forms including baseline information were filled by caretakers before sampling.

Serological method

All collected serum samples were separated and preserved at -20 ^{o}C until being examined. The antibodies (IgG/IgM) in patients were measured by ELISA technique (Torch-IgG, IgM-Trinity Biotech Company) according to the manufacturer's instructions.

Statistical analysis

SPSS 11.5 software was used for analyzing the data. In order to check for statistic difference, chi-square test was adopted. A p-value of <0.05 was considered to be significant.

Results

The frequency of IgG and IgM anti-*Toxoplasma* antibodies in malignant patients and control group are shown in Table 1 and 2. In all patients and control group, seropositivity rose gradually with age. A significantly higher rate (59.4 %) in patients and 71% in control group were found in the groups aged ≥51 years and 41-50 years, respectively (Table 3 & 4) (P <0.001). The seropositive rate of males and females were 42.6% and 47.7%, in patient group and 31.5% plus 40.3% in control group, respectively (Table 3 & 4). Among seropositive cases of patients, 44.2% had close contact with cats. There was no significant difference between seropositivity subjects and close contact with cat (Table 3). A relationship between *T. gondii* seroprevalence, the effect of chemotherapy and living region (urban or rural) of the population has also been studied. No seropositivity significant relationship was found with these factors (Table 3). Otherwise there was no seropositivity significant difference between patients and control groups.

Table 1: Status of antibodies to *Toxoplasma gondii* in 252 malignant Patients

	Results of IgM			
Results of IgG	**Positive**	**Borderline**	**Negative**	**Total**
	N0. (%)	No. (%)	No. (%)	No. (%)
Positive	17 (6.7)	10 (4)	87 (34.5)	114 (45.2)
Borderline	1 (0.4)	0 (0)	11 (4.4)	12 (4.8)
Negative	8 (3.2)	9 (3.6)	109 (43.3)	126 (50)
Total	26 (10.3)	19 (7.6)	207 (82.1)	252 (100)

Table 2: Status of antibodies to *Toxoplasma gondii* in 252 control group

	Results of IgM			
Results of IgG	**Positive**	**Borderline**	**Negative**	**Total**
	No. (%)	No. (%)	No. (%)	No. (%)
Positive	10 (4)	1 (0.4)	81 (32.1)	92 (36.5)
Borderline	1 (0.4)	0 (0)	10 (4)	11 (0.4)
Negative	4 (1.6)	2 (0.8)	143 (56.7)	149 (59.1)
Total	15 (6)	3 (1.2)	234 (92.9)	252 (100)

Table 3: Risk factors of *Toxoplasma gondii* seropositivity in malignant patients

Factors	**Positive** No. (%)	**Borderline** No. (%)	**Negative** No. (%)	**Total** No. (%)	***P* value†**
Age (years)					
<10	8 (17.8)	4 (8.9)	33 (73.3)	45 (100)	0.001
11-20	20 (40.8)	3 (6.1)	26 (53.1)	49 (100)	
21-30	8 (34.8)	0 (0)	15 (65.2)	23 (100)	
31-40	14 (51.9)	0 (0)	13 (48.1)	27 (100)	
41-50	26 (59.1)	1 (2.3)	17 (38.6)	44 (100)	
>51	38 (59.4)	4 (6.3)	22 (34.4)	64 (100)	
Total	114 (45.2)	12 (4.8)	126 (50)	252 (100)	
Gender					
Male	52 (42.6)	3 (2.5)	67 (54.9)	122 (100)	0.12
Female	62 (47.7)	9 (6.9)	59 (45.4)	130 (100)	
Total	114 (45.2)	12 (4.8)	126 (50)	252 (100)	
Living region					
Urban	86 (47.5)	9 (5)	86 (47.5)	181 (100)	0.45
Rural	28 (39.4)	3 (4.2)	40 (56.3)	71 (100)	
Total	114 (45.2)	12 (4.8)	126 (50)	252 (100)	
Contact with cat					
Yes	53 (44.2)	4 (3.3)	63 (52.5)	120 (100)	0.51
No	61 (46.2)	8 (6.1)	63 (47.7)	132 (100)	
Total	114 (45.2)	12 (4.8)	126 (50)	252 (100)	
Chemotherapy					
Yes	107 (45.1)	12 (5.1)	118 (49.8)	237 (100)	0.67
No	7 (46.7)	0 (0)	8 (53.3)	15 (100)	
Total	114 (45.2)	12 (4.8)	126 (50)	252 (100)	

† Results of Chi-square tests by *P* value of <0.05 as significant difference

Table 4: Risk factors of *Toxoplasma gondii* seropositivity in control group

Factors	Positive No. (%)	Borderline No. (%)	Negative No. (%)	Total No. (%)	*P* value†
Age (years)					
<10	3 (9.4)	1 (3.1)	28 (87.5)	32 (100)	0.001
11-20	5 (14.7)	1 (2.9)	28 (82.4)	34 (100)	
21-30	25 (34.2)	4 (5.5)	44 (60.3)	73 (100)	
31-40	12 (27.9)	1 (2.3)	30 (69.8)	43 (100)	
41-50	22 (71)	2 (6.5)	7 (22.6)	31 (100)	
>51	25 (64.1)	2 (5.1)	12 (30.8)	39 (100)	
Total	92 (36.5)	11 (4.4)	149 (59.1)	252 (100)	
Gender					
Male	34 (31.5)	4 (3.7)	70 (64.8)	108 (100)	0.2
Female	58 (40.3)	7 (4.9)	79 (54.9)	144(100)	
Total	92 (36.5)	11 (4.4)	149 (59.1)	252 (100)	

† Results of Chi-square tests by *P* value of <0.05 as significant difference

Discussion

Although *Toxoplasma* infection is asymptomatic almost in most cases, but can cause acute infection in immunosuppressed patients and congenital toxoplasmosis infants. It has been known that 15-58% of humans are infected with *T. gondii*, but the rate of infection varies widely by location, age and other factors (5).

Disease in immunocompromised individuals (i.e. persons with AIDS, transplant recipients, persons receiving immunosuppressive drugs) usually is due to reactivation of latent infection but can result from acute infection. Toxoplasmosis in these persons leads to lethal meningoencephalitis, focal lesions of the CNS, and less commonly, myocarditis or pneumonitis. The clinical pictures may include headache, seizures, mental status changes, focal neurologic signs, and aseptic meningitis (6). 30-40% of AIDS patients with IgG antibodies to *T. gondii* (indicating chronic latent infection) develop active toxoplasmosis unless they take preventive medication (7, 8).

Diagnosis of toxoplasmosis is primarily made by the use of serological tests. IgG antibodies to *Toxoplasma* are usually present 1-2 weeks after acquisition of the infection and usually persist for life. For immunocompetent persons, seroconversion with high concentrations of *Toxoplasma*-specific IgM and a 4-fold increase in specific IgG titer is indicative of recent infection (9). It is generally accepted that prevalence of antibody in human population depends on geographic, climatic, hygienic, and socioeconomic conditions, as well as on the lifestyle of population.

Several seroepidemiological surveys focused on prevalence of *Toxoplasma* antibody titers in different parts of Iran that indicate the high prevalence rate (10-12). In the work of Asmar et al. the highest and lowest infection rates have been reported from Mazandaran Province (20.5%) and Hormozgan province (2.9%), respectively (10).

In this study approximately 45.2% of patients and 36.5% of control group were confirmed to be positive for *Toxoplasma* IgG antibodies. High titers of IgG antibodies in the absence of IgM antibodies are consistent with chronic latent infection acquired in the past. 10.3% of patients and 6% of control were confirmed to be positive for *Toxoplasma* IgM antibodies that indicate recent infection. In the other hand,

6.7% of patients and 4% of control had both of IgG and IgM antibodies that indicate acute infection. Similar finding for *Toxoplasma* IgG antibodies determination have been reported by some studies (10-12). High seropositive rate in this study may be due to, high chance of contact with contaminated resources (infection by oocysts through contact with infected cats, exposure to contaminated water and food, and through ingestion of undercook meat) and receiving immunosuppressive therapy that leads to reactivation of latent infection.

Seroprevalence of toxoplasmosis is known to increase with age (13-15). In this study, in both groups patients and control, the lowest positive rates were seen in the age of <10 years and the positive rates were slowly increased with age, along with the peak level revealed in >51 years in patients group and 41-50 years in control group. The reason for the rise in quantitative titers with age is not clear. A hypothesis would be that the increase is a reflection of increasing exposure years as the humans get older. Multiple minor infections might at first produce low antibody levels and later higher levels (15).

In the present study, the prevalence of antibody in females was higher than in males, while in some studies showed higher antibody prevalence in males than in females (16, 17), as well as in females was almost the same as that in males (11,18). In accordance of the results of this study, there was not statistically significant difference between seropositivity rate and sex in both patient and control groups.

Various studies have also reported a statistical correlation between toxoplasmosis prevalence and close contact with cats (19, 20). In our study, 46.2% of patients which showed *Toxoplasma* antibody had not close contact with cat. This is consistent with the study of Nateghi Rostami et al (21). However no significant association was found between *T. gondii* seroprevalence and close contact with cat, although different types of serological testes were used for each of these studies.

This study showed higher *Toxoplasma* seropositivity among patients who grew up in urban areas. Nevertheless, there was no significant difference in infection rate in urban and rural areas.

Immunosuppressive patients are exposed to various possible risk factors, which might expose them to *Toxoplasma* primary infection or reactivation. These data showed 55.5% of patients were susceptible to acute *Toxoplasma* infection. For this reason, it is important that patients with toxoplasmosis infection are diagnosed and identified in order to refer them for early therapy or other interventions. In parrarel, it is also important to inform and educate these patients on how to avoid possible risk-factors and prevent *Toxoplasma* infection. Hence, it would be desirable that the antibody status of patients be known before, during and after chemotherapy.

Acknowledgements

The financial support of Research Deputy, Tropical and Infectious Diseases Research of Jondi –Shapour University of Medical Sciences and critical help of Shafa Hospital staff, Mr Absalan, Miss A. Sarkaki M. Esfahani and Miss F. Dalimi are highly appreciated.

The authors declare that they have no Conflict of Interests.

References

1. Velimirovic B. Toxoplasmosis in immunosuppression and AIDS. Infection. 1984; 12(5): 315-7.
2. Gallino A, Maggiorini M, W Kiowski *et a*l. Toxoplasmosis in heart transplant recipients. Eur J Cli Microbiol Infect Dis. 1996;15(5):389-393.
3. Wanke C, Tuazon CU, Kovacs A. *Toxoplasma* encephalitis in patients with acquired immune deficiency syndrome: diagnosis and response to therapy. Am J Trop Med Hyg. 1987;36(3):509-516.
4. Bacigalupo MA, Bazzini P, Farina L, Ius A. Evaluation of three immunoassays for

detection of *Toxoplasma*-specific immunoglobulin G and M. Eur J Clin Chem Clin Biochem. 1996;34: 503–505.
5. Walzer PD, Genta RM. *Toxoplasma gondii*. In: Parasitic infection in the compromised host. Marcel Dekker, Inc. 1989. New York.
6. Ho-Yen DO. Clinical features. In: Ho-Yen DO, Joss AWL, editors. Human toxoplasmosis. Oxford: Oxford University Press; 1992. p. 56-78.
7. Ho-Yen DO. Immunocompromised patients. In: Ho-Yen DO, Joss AWL, editors. Human toxoplasmosis. Oxford University Press; 1992. p. 184-203.
8. Ammassari A, Murri R, Cingolani A, de Luca A, Antinori A. AIDS associated cerebral toxoplasmosis: an update on diagnosis and treatment. In: Gross U, editor. *Toxoplasma gondii*. Berlin: Springer-Verlag; 1996. p. 209-222.
9. Tuuminen T, Seppanen H, Pitkanen EM, Palomaki P, Kapyaho K. Improvement of immunoglobulin M capture immunoassay specificity: *Toxoplasma* antibody detection as a model. J Clin Microbial. 1999;37:270-3.
10. Assmar M, Amirkhani A, Piazak N *et al*. Toxoplasmosis in Iran. Results of a seroepidemiological study. Bull Soc Pathol Exot. 1997;90:19-21.
11. Hoghooghi-Rad N, Afraa M. Prevalence of toxoplasmosis in humans and domestic animals in Ahwaz, capital of Khoozestan Province, southwest Iran. J Trop Med Hyg. 1993; 96(3):163-168.
12. Ghorbani M, Edrissian GH, Assad N. Serological survey of toxoplasmosis in the northern part of Iran using IFA technique. Trans Roy Soc Trop Med Hyg. 1978;72: 369.
13. Takahashi J, Konishi E, Matsumura T. A survey of antibody to *Toxoplasma gondii* among patients of a hospital in Hyogo prefecture, Japan, by enzyme-linked immunosorbent assay. Jpn J Parasitol. 1985;34:87-92.
14. Shim HS, Na YE, Lee YH, Shin DW. *Toxoplasma* antibody titers of general outpatients and pig sera by indirect latex agglutination test. Chungnam Med J. 1991;18:77-85.
15. Taylor MR, Lennon B, Holland CV, Cafferkey M, Community study of *Toxoplasma* antibodies in urban and rural schoolchildren aged 4 to 18 years. Arch Dis Child. 1997;77: 406-409.
16. Excler JL, Pretat E, Pozzetto OB *et al*. Serepidemiological survey of toxoplasmosis in Burundi. Tropenmedizin and Parasitologie. 1988;39:139-141.
17. Konishi E, Houki Y, Harano K, Retno SM, Djoko M, Soetrisno A, Yoes PD. High prevalence of antibody to *Toxoplasma gondii* among human in Surabaya, Indonesia. Jpn J Infect Dis. 2000;53:238-241.
18. Terazawa A, Moljono R, Susanto L, Margono SS, Konishi E. High *Toxoplasma* antibody prevalence among inhabitants in Jakarta, Indonesia. Jpn J Infect Dis. 2003;53: 107-109.
19. Dubey JP, Beattie CP. Toxoplasmosis of animals and man. Boca Raton, FL: CRC Press; 1988.
20. Tenter AM, Heckeroth AR. Weiss LM: *Toxoplasma gondii*: from animals to humans. Int J Parasitol. 2000;30:1217-58.
21. Nateghi Rostami M, Eskandari E, Garoosi Z, Mohajeri N, Rezaian M, Keshavarz H. Serological Study of *Toxoplasma gondii* Infection Using IFA Method in Renal Transplant Recipients. Iranian J Parasitol. 2006;1:31-38.