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Prevalence of Toxoplasma Gondii Antibodies in the Serum of Urban Residents and Ranchers in Tabriz, Iran

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Abstract

Objective: Toxoplasmosis is a common zoonotic infection between man and animal in the congenital form. This pathogenic agent is transmitted to the fetus through the maternal placenta. This infection is acquired through the ingestion of oocysts, which are transmitted by cats or through contaminated meat. Congenital infections can lead to fetal death, pathological changes of the central nervous system (CNS), or eye diseases. The acquired form of the disease often has no symptoms, or is characterizes by general lethargy, swollen lymphatic nodes, and chorioretinitis. Fatal and acute infections are observed in the medically compromised patients or patients with malignancy or tissue plants and AIDS. The purpose of this study is the investigation of the prevalence of antibody of anti-toxoplasma gondii in the serum of urban residents and farmers.

Materials and Methods: A number of 100 blood samples of urban residents and farmers were collected and tested by the enzyme-linked immunosorbent assay (ELISA) method.

Results: Total prevalence of anti-toxoplaxma IgG was 24% in urban residents and 44% in farmers. The prevalence of IgM and IgG positives were higher in farmers than in urban residents. In urban residents, the percentage of IgM and IgG positive was 10%.

Conclusion: Due to the high rate of positive cases among the patients, there should be some regular screening programs to recognize chronic infections which may become acute infections. Serial titration measurement should be performed on these patients and they should undergo antiparasitic treatments.

Keywords: Antibody, Tabriz, Toxoplasma

Introduction

Since the dawn of creation, mankind and animals have had a close relationship. This relationship was based on providing food, protection and guarding of life, and providing the emotional needs of humans. In this regard, in addition to meeting the needs of humans, this close relationship caused problems for humans and sometimes for animals. The most important problem is the common diseases among humans and animals (1-5). Some of these diseases can be fatal for both the human and the animal. Toxoplasmosis is a common disease and its final host is the cat and its intermediate host is the human and other mammals. Toxoplasmosis does not cause important problems in cats, but in the intermediate host, such as humans especially pregnant women and people with weakened immune systems, it creates many complications. Toxoplasma is an obligate intracellular parasite with a global distribution (6-8). Serological data indicates that 20% to 75% in different populations, are chronically but asymptomatic infected in the form of tissue cysts (1,9,10).

As long as the immune system is activated the parasites form cysts (chronic), but in case of loss or impairment of the immune system they are activated and its clinical symptoms become apparent. Given the increasing prevalence of AIDS and cancer in the

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world, the immune system is weakened in most individuals. In addition, in patients with cancer and organ transplant recipients, who use immunosuppressive drugs, toxoplasmosis disease and other secondary infections have the opportunity to emerge and this necessitates further identification of parasites in these individuals (11-15). In this study, the prevalence of toxoplasma infection in ranchers and urban and suburban populations of Tabriz, Iran, was studied in order to gain information on serum levels of parasite and prevalence rate of the disease in these patients. It is hoped that further studies in other provinces of the country will obtain information on the prevalence of this disease. This disease caused miscarriage and much damage in animals (5,9). This article was conducted with the aim to increase awareness of the epidemiology of toxoplasmosis is the city of Tabriz.

Materials and Methods

For sampling of urban residents and ranchers, blood samples were taken randomly from 50 ranchers and 50 urban subjects. Syringe, cotton, and alcohol were provided to the extent necessary. The sample was taken by selecting the best arm vein of the patient and using tourniquet; the sample area was carefully sterilized with cotton and alcohol, and then with a finger the skin was pulled forward and the needle went into the skin and the vein. It is better that the syringe enters the vein with the first attempt. After blood sampling the syringe needle was immediately placed in the syringe and was gently discharged into a blood tube. The samples were immediately transported to the parasitology laboratory. Then, the blood was centrifuged for 15 minutes at 37 °C (2000 cycles for 10 minutes). The samples were checked for hemolysis and lipemia because they had a significant effect on the responses. In this study, some hemolytic samples were discarded and new samples were taken. After centrifugation, serum was poured carefully into micro-tubes and was frozen and stored for enzyme-linked immunosorbent assay (ELISA) testing. During the time until testing, the samples were kept as fresh as possible and if this was not achieved, they were kept in a freezer at -20 °C.

2.3 Materials used and their preparation:

Toxoplasma kit IgM (Trinity kit, Biotech co., USA)

1) Toxoplasma purified antigens: They were cut into 96 microplates (12 cubes of 8), and kept in foils and stable without light and humidity.

2) Calibrator: It contained human serum or defibrinated plasma. Sodium and pen/strep (0.01%) were added as preservative and specific factor printed on the label. The calibrator was used for calibrating the daily absorption device (one vial 0.4 ml), changed constantly in different measuring conditions.

3) Positive control: It contained human serum or defibrinated plasma to which sodium 0.1% and pen/strep 0.01% were added as preservative. Positive control was used in the detection of positive ELISA (one vial 0.4 ml).

4) Negative control: It contained human serum or defibrinated plasma. Sodium 0.1% and pen/strep 0.01% were added as preservative. Negative control was applicable to reading the range of negative control absorption (one vial 0.4 ml).

5) Conjugated Horse radish-peroxidase (HRP): It was prepared to be used and covered by anti-human IgM. Gentamicin and proclin (0.1%) were added as preservative.

6) Serum diluent type 8: ready for use, including anti-human goat and sheep IgG, that competes with absorbing of sera IgG. Protein and proclin (0.1%) were added as preservative (2 bottles of 45 ml).

7) TypeI buffer wash (concentrated × 20): It was diluted in 1 part concentrated with 19 parts deionized or distilled water. It contains tween and proclin.

8) Chromagen/Substrate Solution Type I: Benzidine tetramethylbenzidine (TMB) is ready for use. Until the indicator is used, it must remain closed.

9) Inhibitor solution: it is ready for use and includes H_2SO_4 1 normal solution (a 15-ml bottle), Captia TM, and Toxoplasma gondii IgG

1. Serums, controls, and calibrators were diluted in a ratio of 1 to 20 with serum diluent (10 microliter + 200 microliter SD)

Before diluting serum, controls, and calibrators, they were well vortexed.

2. Washing solution (swashing solution): A volume of washing solution was diluted with 19 volumes of distilled water.

1. 100 microliters of calibrator controls and diluted serum were added to the wells. 100 microliters of serum diluents were poured into blank wells. Wells were incubated for 30 minutes at room temperature (21-25 °C).

2. Contents of all wells were emptied and they were washed 3 times with diluted washing solution. In each step of washing, the wells were dabbed with moisture retention paper. Lack of washing solution and bubbles in the wells during the last rinse was very important. In case of using a washing machine, they were washed 5 times.

3. 100 microliter enzyme conjugate was added to all wells. Wells were incubated for 30 minutes at room temperature (21-25 °C).

4. Washing was performed in the same method as in stage 2.

5. 100 microliters of chromogen TMB was added to all wells. Wells were incubated for 15 minutes at room temperature (21-25 °C).

6. 100 microliters of stopping solution was added to all wells. Absorbance of the resulting color at 450 nm was read.

Results

Investigating the prevalence of toxoplasmosis by ELISA method in urban residents and farmers in Tabriz city and its suburbs showed that from 100 studied patients, 34 patients (34%) had titers of IgG above normal (positive) (Table 1). Among the 50 positive cases of toxoplasmosis in farmers, 22 had

titers higher than normal which was considered positive titer. Moreover, among the 50 subjects from the urban population, 12 subjects had titers higher than normal and were considered positive. In investigating the frequency of toxoplasmosis with ELISA method in urban residents and farmers, it was identified based on IgG. There were 34 positive cases and 66 negative cases among subjects. In analyzing the distribution of anti-toxoplasma IgM antibodies by ELISA method among urban residents and farmers, from a total of 100 subjects studied, 36 patients were positive and 64 were negative (Table 2).

The mean IgM antibody titer in the urban population was 0.11 and in farmers was 0.154. The mean IgG antibody titer in the urban population was 0.243 and in farmers was 0.274. Through studying table 3, it was identified that 15 out of 50 urban residents, 30% of the urban population, had IgM titers of higher than normal. In addition, by studying table 4 it was identified that 21 out of 50 farmers (42%) had IgM titers of higher than normal. Table 5 showed that 12 out of 50 urban residents (24%) had IgG titers of higher than normal. By studying table 6, it was concluded that 22 out of 50 farmers (44%) had IgG titers of higher than normal.

Discussion

The rate of positive antibody titers of anti-toxoplasma IgG in urban residents and farmers of Tabriz by ELISA method were 24% and 44%, respectively. A comparison of this ratio with that gained in the relatively few studies in other regions showed few differences. In the present study which was conducted on 100 patients, 34 subjects (34%) had high anti-toxoplasma IgG titers.

Table 1. Frequency distribution of toxoplasma among urban residents and ranchers based on IgG

	Positive		Negative		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
Urban residents and ranchers	34	34	66	66	100	100
Total	34	34	66	66	100	100

Table 2. The frequency distribution of anti-toxoplasma IgM antibodies in urban residents and ranchers in the city of Tabriz

	Positive		Negative		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
Urban residents and ranchers	36	36	64	64	100	100
Total	36	36	64	64	100	100

Table 3. Percentage prevalence of anti-toxoplasma IgM antibody of the urban population

	Number	Positive percentage
Total	100	30
	100	30

Table 4. Percentage prevalence of anti-toxoplasma IgM antibody of the ranchers

	Number	Positive percentage
Total	100	42
	100	42

Table 5. Percentage prevalence of anti-toxoplasma IgG antibody of the urban population

	Number	Positive percentage
Total	100	24
	100	24

Table 6. Percentage prevalence of anti-toxoplasma IgG antibody of the ranchers

	Number	Positive percentage
Total	100	44
	100	44

Martino et al. also reported that among 41 patients at a transplant center or those who had bone marrow transplant, 94%, before transplantation, were seropositive for toxoplasma (16). In the present study, which was conducted on urban residents and ranchers, 34% had significantly positive titers regarding toxoplasma. This finding was not in agreement with that of the study by Martino et al. (16). This was probably due to the higher prevalence of toxoplasmosis in the areas under study in the study by Martino et al (16). In other words, infections are more common in warm climate regions than in cold climate and mountainous regions and they are mostly found in humid rather than dry regions. This condition is favorable for spores and survival of oocytes in dependent environment. Because the production of spores will stop in temperatures higher than 37 °C and lower than 4 °C, and oocytes remain active in wet soil for more than one year. However, in dry weather and temperatures higher than 66 °C they will lose their power of infectivity. Cultural habits and people's hygiene may also be involved. Generally, infection is less common in cold and mountainous areas with high altitudes and in very hot regions (17-19). Thus, this could account for the differences in positive percentages in this study and the study by Martino et al. in Europe. (16).

In the serological study of toxoplasmosis in humans in Shiraz city and suburbs, the prevalence of antibody was higher in women than men. In the present study, the prevalence of toxoplasmosis among urban residents and ranchers were 24% and 44%, respectively. This difference was statistically significant and showed a relationship between occupation and the risk of toxoplasmosis.

In addition, 10% of the urban studied subjects had

valuable positive IgM and IgG titers and they were considered as positive cases. Furthermore, 28% of farmers were considered positive for IgG and IgM because they were adults. In adults, 10% to 20% of IgG and IgM cases were positive symptomatic patients. These symptoms are often characterized as Paty asymptomatic cervical adeno or cutaneous (20). The positive subjects of this study also showed these clinical symptoms. In a total comparison, the ratios of IgM and IgG positive were higher in farmers than the urban population. This is probably due to more contact with cats and consumption of unsterilized milk in this group.

The chronic form of the disease is the chronic form of IgG positive and IgM negative. In this state, toxoplasma remains in the body as cyst for months and years and this stage is without clinical symptoms. In the urban population, 6% were IgG positive and IgM negative which can be considered as the chronic form of the illness. In farmers, 16% were IgG positive and IgM negative which can be considered as the chronic form of the illness. These people showed no clinical symptoms. In urban population, 18% were IgM positive and IgG negative. In farmers, 14% were IgM positive and IgG negative. This indicated that they were infected with toxoplasma for the first time. These people did not show specific signs of the illness. Some of them had fever. Other studied subjects did not have valuable IgM and IgG titers and were considered as negative cases. In the urban population, 60% did not have valuable IgM and IgG titers and were negative, and 42% of farmers did not have valuable IgM and IgG titers and were considered as negative.

Several studies have indicated that the prevalence of toxoplasma antibodies increases with increasing age (21). This indicated the persistence of antibodies from previous infections and toxoplasma, and acquisition of new toxoplasma antibodies (7). In fact, aging increased exposure to infectious agents, and consequently, the infection rate became higher. There are also some reports that did not show any significant relationship between age and prevalence of infection (22). In the United States, for each year increase in age, antibody prevalence increased 1% (22).

In a study on the infection percentage in different age groups, a significant difference was observed and infections were significantly higher in patients older than 22 years than those under 22 years (23). In a study on bone marrow transplant donors and recipients from 2001 to 2004, toxoplasma serology test was performed on 220 donors and recipients. It was found that 8 donors and recipients were seropositive for toxoplasma. In addition, 59 people were seropositive recipients (high risk) of toxoplasma. Moreover, 12 patients were positive for the transplant and 141 patients were negative donors and recipients of toxoplasma. A total of 67 patients (30%) were identified as high risk for developing toxoplasma. The findings of this study and the present study in respect to the prevalence of toxoplasma were closest (24% of the urban population and 44% of ranchers) in comparison to other studies; other studies showed 30%.

In this study, the relationship between the consumption of raw milk and positive toxoplasma antibodies was considered. They found a significant correlation between the consumption of raw milk and IgG seropositivity. This was consistent with the present study. The probability of consumption of raw milk in farmers was higher and their toxoplasma antibody titer, compared to the urban population, was higher and this matter was confirmed in the present study.

Recommendations

Given the importance of human life and the need for more assistance to protect human health, it is recommended that a general review of cases in which the chronic form is converted to acute form be conducted. Identification of toxoplasma is important in people and should be considered in the country. The Ministry of Health should allocate funds for research on toxoplasma and more accurate methods than ELISA, such as polymeras chain reaction (PCR), isolation of parasites from the blood or body fluids that contain parasites, tissue isolation and exam, and more powerful detection methods should be used. The specificity of the positive PCR result is almost 100% while the specificity of ELISA test is 96.4%. The PCR method is time consuming and expensive; therefore, it is not performed routinely. In less developed areas, previous methods, such as indirect immunofluorescence (IFA) for serological screening for toxoplasma, can be used. In this study, due to time and spatial constraints, the data is valid for the specified time range. For the study findings to be applicable to other regions and time ranges, the study should be extended to consecutive periods, and various locations such as hospitals, universities, pregnant women, and high risk people.

Ethical issues

We have no ethical issues to declare.

Conflict of interests

We declare that we have no conflict of interests.

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References

1.Toxoplasmosis Fact Sheet [Online]. [cited 2002]; Available from: URL: http://www.cdc.gov/ncidod/dpd/parasites/toxoplasmosis/factsht

2.Dobrowolski JM, Sibley LD. Toxoplasma invasion of mammalian cells is powered by the actin cytoskeleton of the parasite. Cell 1996; 84: 933-9.

3.Howe DK, Sibley LD. Toxoplasma gondii comprises three clonal lineages: correlation of parasite

genotype with human disease. J Infect Dis 1995; 172: 1561-6.
4.Ajzenberg D, Cogne N, Paris L, Bessieres MH, Thulliez P, Filisetti D, et al. Genotype of 86 Toxoplasma gondii isolates associated with human congenital toxoplasmosis, and correlation with clinical findings. J Infect Dis 2002; 186: 684-9.
5.Grigg ME, Bonnefoy S, Hehl AB, Suzuki Y, Boothroyd JC. Success and virulence in Toxoplasma as the result of sexual recombination between two distinct ancestries. Science 2001; 294: 161-5.
6.Su C, Evans D, Cole RH, Kissinger JC, Ajioka JW, Sibley LD. Recent expansion of Toxoplasma through enhanced oral transmission. Science 2003; 299: 414-6.
7.Holland GN, Lewis KG. An update on current practices in the management of ocular toxoplasmosis. Am J Ophthalmol 2002; 134: 102-14.
8.Gilbert RE, Gras L, Wallon M, Peyron F, Ades AE, Dunn DT. Effect of prenatal treatment on mother to child transmission of Toxoplasma gondii: retrospective cohort study of 554 mother-child pairs in Lyon, France. Int J Epidemiol 2001; 30: 1303-8.
9.Cornelissen AW, Overdulve JP, van der Ploeg M. Determination of nuclear DNA of five eucoccidian parasites, Isospora (Toxoplasma) gondii, Sarcocystis cruzi, Eimeria tenella, E. acervulina and Plasmodium berghei, with special reference to gamontogenesis and meiosis in I. (T.) gondii. Parasitology 1984; 88: 531-53.
10. Shadde F, Sarvestani RG, Milani MS. Toxoplasma infection in human and dog in Shiraz. J APP Anim Res 1993; 3: 83-9.
11. Mcculloch WF, Braun JL, Heggen DW, Top FH. Studies on medical and veterinary students skin tested for toxoplasmosis. Public Health Rep 1963; 78: 689-98.
12. Gilbert R, Dunn D, Wallon M, Hayde M, Prusa A, Lebech M, et al. Ecological comparison of the risks of mother-to-child transmission and clinical manifestations of congenital toxoplasmosis according to prenatal treatment protocol. Epidemiol Infect 2001; 127: 113-20.
13. Mineo JR, McLeod R, Mack D, Smith J, Khan IA, Ely KH, et al. Antibodies to Toxoplasma gondii major surface protein (SAG-1, P30) inhibit infection of host cells and are produced in murine intestine after peroral infection. J Immunol 1993; 150: 3951-64.
14. Liesenfeld O. Immune responses to Toxoplasma gondii in the gut. Immunobiology 1999; 201: 229-39.
15. Bliss SK, Marshall AJ, Zhang Y, Denkers EY. Human polymorphonuclear leukocytes produce IL-12, TNF-alpha, and the chemokines macrophage-inflammatory protein-1 alpha and -1 beta in response to Toxoplasma gondii antigens. J Immunol 1999; 162: 7369-75.
16. Martino R, Bretagne S, Einsele H, Maertens J, Ullmann AJ, Parody R, et al. Early detection of Toxoplasma infection by molecular monitoring of Toxoplasma gondii in peripheral blood samples after allogeneic stem cell transplantation. Clin Infect Dis 2005; 40: 67-78.
17. Brinkman K, Debast S, Sauerwein R, Ooyman F, Hiel J, Raemaekers J. Toxoplasma retinitis/encephalitis 9 months after allogeneic bone marrow transplantation. Bone Marrow Transplant 1998; 21: 635-6.
18. Luft BJ, Remington JS. Toxoplasmic encephalitis in AIDS. Clin Infect Dis 1992; 15: 211-22.
19. Goebel WS, Conway JH, Faught P, Vakili ST, Haut PR. Disseminated toxoplasmosis resulting in graft failure in a cord blood stem cell transplant recipient. Pediatr Blood Cancer 2007; 48: 222-6.
20. Singh N, Gayowski T, Marino IR. Toxoplasma gondii pneumonitis in a liver transplant recipient: implications for diagnosis. Liver Transpl Surg 1996; 2: 299-300.
21. Holland GN, O connor GR, Belfort R, Remington JS. Toxo plasmosis. In: Pepose JS, Holland GN, Wilhelmus KR, Editors. Ocular Infection and Immunity. Philadelphia, PA: Mosby, Incorporated; 1996. p. 1183-223.
22. Liesenfeld O, Wong SR, Remington JS. Toxoplasmosis is in the setting of AIDS or without AIDS. In: Merigan TC, Bartlett JG, Bolognesi D, Editors. Textbook of AIDS Medicine. Philadelphia, PA: Williams & Wilkins; 1999. p. 225-59.
23. Barragan A, Sibley LD. Transepithelial migration of Toxoplasma gondii is linked to parasite motility and virulence. J Exp Med 2002; 195: 1625-33.

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