

Serological Survey of Toxoplasmosis in Women Referred to Medical Health Laboratory Before Marriage, Northern Iran, 2003-2004

¹Sharif, M., ²A. Ajami, ¹A. daryani, ¹H. Ziaei and ³A. Khalilian

¹Department of Parasitology and Mycology,

²Department of Immunology and Microbiology,

³Department of Biostatistics, Sari Medical School,
Mazandaran University of Medical Sciences, Sari, Iran

Abstract: Toxoplasmosis has world wide distribution and in Iran its prevalence is high. Pregnant women infected with *Toxoplasma gondii* may be prone to abortion, fetal death, still birth or congenital toxoplasmosis. If we know the percentage of immune women, who are going to get married, we can determine the occurrence of abortion, still birth and fetal death due to toxoplasmosis and congenital toxoplasmosis. Women who are going to married and referred to the health center laboratory of different cities of Mazandaran state were studied. The samples comprised of 980 women from Sari, Ghaemshahr, Babol, Nowshahr, Noor and Behshar. Five mL blood was drawn; serum was separated and examined for antibody against toxoplasmosis by indirect immunofluorescence. First of all, 1/50 titer dilution was tested in the cases of positive result, further dilution was prepared and the last dilution was recorded. Serum of 731 samples (74.6%) were positive for toxoplasma antibody. Most of the samples (24 and 37%) were positive in 1:100 and 1:200 dilutions. With increase in the age of women, percentage of positive cases increased (87.9% of the women with age of 30-34 years were positive for toxoplasma antibody). 96.3% of uneducated women were positive for toxoplasma antibody. No difference between women in urban and rural areas, also between employed and housewife women were found. Toxoplasma infection is more prevalent in Mazandaran state than the other states of country. Since the prevalence rate of toxoplasmosis is high in women before marriage, we can conclude that the risk of abortion, still birth and fetal death due to toxoplasmosis and also congenital toxoplasmosis in this state is low. Therefore a health recommendation will prevent the risk of toxoplasmosis in women who are going to marry.

Key words: Toxoplasmosis, *toxoplasma gondii*, seroepidemiology, immunofluorescence, marriage, Iran

INTRODUCTION

Toxoplasmosis is a worldwide distributed zoonotic disease found in humans and many animal species.^[1-3] It is estimated that 20 to 90% of the world adult population, depending on the region, already have had contact with the parasite^[4]. This zoonosis can be found on all of the continents and under different climatic conditions^[5]. Seropositivity is higher in hot and humid areas^[6,7]. Toxoplasmosis prevalence is related to several factors, including cultural level, nutritional habits, age and rural or urban setting^[8,9].

There are several well-known means of transmission. These include eating raw meat or poorly cooked meat containing bradyzoites and the ingestion of oocysts from cat feces in soil, water or food. Infection transmission depends on the level and frequency of exposure to such factors^[10,11]. In people with a well-functioning

immunologic system, the infection usually has no symptoms, except for development of specific antibodies^[12]. Congenital transmission occurs when a woman acquires the infection for the first time during pregnancy and transmits it to her fetus^[13-16]. Congenital infection of human fetus may cause abortion, blindness, mental retardation and other neurological diseases^[16,17]. Positive antibody prevalences in human in Iran are high and have been reported from most geographical areas. In Iran the seroprevalence of toxoplasmosis in adults, especially pregnant woman, has been recorded between 23.7 - 77.3% in many studies^[18-21]. Also incidence of congenital infection is found to be 1200-5250 children per year^[22]. Seroprevalence estimated for non immune girls before marriage varied in different studies^[23,24]. In the present study, we conducted a seroepidemiological study of toxoplasmosis screened for sera anti-toxoplasma antibodies in the woman referring to the Medical health laboratory before marriage in Mazandaran province.

MATERIALS AND METHODS

This descriptive epidemiologic study was undertaken from June 2004 -March 2005 in the women referring to the Medical Health Laboratory before marriage. In accordance to the different studies in the non immune women, 30% (980 individuals) of the seven cities (Amol, Babol, Ghaemshahr, Sari, Behshahr, Noor, Nowshahr) of Mazandaran province were selected for study. Age range of subjected population was 12–40 years old. Blood samples were drawn by venipuncture from the subjects and centrifuge in the laboratory. Sera were used for this study. Serum aliquotes were preserved at –20c until used further. Serum samples were analyzed for antibodies to *T. gondii* by the indirect immunofluorescence antibody technique using commercial toxoplasma antigen (Rayyan teb). Dako, anti-human serum conjugate with fluorescein isothiocyanate was used at a dilution of 1:50 in phosphate buffered saline with 0.1% Evans blue. Each serum sample was first examined at dilutions of 1:50 and 1:100, if the latter dilution gave a positive result, further dilutions were made in order to determine the end point. The films were examined under an Ernst Leitz Weltzlar (GMBH Type 307-143.004) fluorescence microscope, equipped with an Osram HBO 50w-Ac mercury short arc lamp and a combination of exciter filters BG12 and BG3 and 570 mμ barrier filter. Fluorescein isothiocyanate labeled goat antihuman immunoglobulin visible fluorescence of cytoplasmic membrane contrasting with red dyed cytoplasm of parasite regarded as positive.

RESULTS

Seroprevalence data obtained are shown in Table 1. The overall percentage of positive reactions to *T. gondii* in the combined cities amounted 74.6% (731/980) by IFAT. The highest positive rate (84%) was observed in Noor area, the least (69%) in Amol and (68%) in Nowshahr area. However, no significant differences could be demonstrated between the residents of different cities ($p>0.05$). The distribution of antibody titers is shown in

Table 1: Positive rate of toxoplasmosis in the study population

Locality	According to locality of inhabitants	
	Negative (%)	Positive (%)
Amol	59(31)	131(69)
Babol	40(20)	160(80)
Ghaemshahr	20(20)	80(80)
Sari	60(29)	148(71)
Behshahr	30(27.3)	80(72.7)
Noor	15(16)	78(84)
Nowshahr	25(13.6)	54(68.4)

* Positive Titer by IFAT was $\geq 1: 50$

Table 2. The highest number of subjects under study had antibody titer of 1:200 (37.1%) and 1:100 (24.1%). Some of the Socio-demographic characteristics are explained and shown in Table 3. The age distribution of positive reactors is depicted in Table 3. Except in below 15 years old, the correlation of infection with age show statistically differences in these settings, alone or combined. There was a gradual increase in seropositivity with increasing age ($p<0.001$), reaching a peak of 100% in the oldest age group. Comparing the residential status, 76.3% (193/253) of rural and 74% (539/731) of urban areas were positive for antibody, showing no significant differences. The two groups of housewife and employed were compared. 73.2% (183/268) of employed and 74.2% (409/583) of housewife were antibody positive which also indicating no statistically significant difference between. In the present study lower educational level is found as a risk factor for toxoplasmosis, indicating highest positive rate in illiterates ($p<0.05$).

DISCUSSION

The prevalence of Toxoplasma antibodies among the inhabitants of the northern Iran is relatively high (74.6%) in the studied population. It seems that the presence of domestic and stray cats, combined with relatively warm weather and high humidity and also frequent consumption of raw vegetables outside the home, presumably the most important factors associated with the high prevalence of toxoplasmic infection in the areas studied. The percentages of positive results obtained in different studies in northern Iran are higher compared to those obtained 26 years earlier by Ghorbani and Edrissian (55.7%)^[25]. The difference in prevalence reported in the two surveys may be partially due to different sampling techniques and the use of different serological assays and also existence of long years of gap period.

Results of the same studies conducted on the women willing marriage in Tehran and Kerman indicate 31%^[23] and 24.4%^[24] seropositivity, respectively. These significant differences with our findings can be due to environmental favorable condition for existence of parasite and habitual factors and consumption of unwashed fruits and vegetables in Mazandaran province.

Also it is worth nothing that the methodology in the two above mentioned studies were different.

Seroprevalence data obtained from seven cities of Mazandaran province varied 70-80%. Accordingly, there were no apparent differences noticed in the positive rate of toxoplasmosis in subjected persons in mentioned cities ($p>0.05$).

Table 2: Toxoplasma antibody titers in sera of the study populon as examined by IFAT

Locality	No.		Positive			Antibodies titers	
	Tested	No. (%)	1:50	1:100	1:200	1:400	1:800
Amol	190	131(69%)	26(19.8%)	26(19.9%)	50(38.2%)	25(19%)	4(3.1%)
Babol	200	160(80%)	26(16.3%)	32(20%)	70(43.7%)	22(13.7%)	10(7.3%)
Ghaemshahr	100	80(80%)	14(17.5%)	20(25%)	30(37.5%)	12(15%)	4(5%)
Sari	208	148(71%)	34(23%)	44(29.8%)	45(30.4%)	20(13.5%)	5(3.4%)
Behshahr	110	80(72.7%)	15(18.8%)	23(28.8%)	27(33.8%)	10(12.5%)	5(6.3%)
Noor	93	78(84%)	13(16.7%)	16(20.5%)	31(34.7%)	10(12.8%)	8(10.3%)
Nowshahr	79	54(68.4%)	14(26%)	15(27.8%)	18(33.4%)	5(9.3%)	2(3.7%)

Table 3: Toxoplasmosis and sociodemographic factors in the study population

Risk factors	Toxoplasma I g G				
	Negative		Positive		p
	n	%	n	%	
Age (n = 980)					
< 15	30	41	43	59	N-S
15-19	129	28.6	322	71.4	0.000
20 - 24	74	22.8	250	77.2	0.000
25 - 29	12	16	63	84	0.000
30 - 34	4	12.1	29	87.9	0.000
> 34	0	0	24	100	0.000
Residence (n = 980)					
Urban	191	26	538	74	N-S
Rural	59	23.7	192	76.3	
Occupation (n = 851)					
Housewife	174	25.8	409	74.2	N-S
Employed	85	26.8	183	73.2	
Education (n = 826)					
High school and more	145	27.5	383	72.5	<0.05
Primary school	58	21.4	214	78.6	
Illiteracy	1	3.7	26	96.3	

In the present survey, it was shown that there was a significant difference in serological positive rate between younger inhabitants and the adult group with respect to toxoplasmosis. Moreover, the seroprevalence of toxoplasmosis among inhabitants of Mazandaran province in this study by age revealed that positivity was seen to increase with age (59%-100%), which was similar to results reported in Nepal, Bolivia, France, Japan and Penghu Island or Kinmen Island^[26-30].

However, it is acknowledged that seroprevalence increase with age, as seen in studies conducted in various countries^[3]. Frequency of infection increases with older age groups since the probability of an individual coming into contact with one of the transmission routes increases as his or her age increases^[8].

In the current study, no statistical meaningful difference was observed between urban and rural areas for toxoplasmosis seroprevalence. Present data is in agreement with those reports which do not confirm the effect of place of residence on the prevalence of serologic reactions to *T.gondii*. Lebech *et al.*,^[31] in Denmark and Ljungstrom *et al.*,^[32] in Sweden found no differences between rural and urban population in survey of pregnant

women. However, several authors have emphasized the influence of urban versus rural settings in toxoplasmosis. Tayler *et al.*,^[33] found a higher percent of positive reactions in children from a rural area (16.6%) compared to children from urban area (10.2%). Martinez Sanchez *et al.*,^[34] investigating pregnant women in Cuba also found more positive cases in the rural population^[34].

In the present study lower educational level is found as a risk factor for toxoplasmosis, indicating highest positive rate in illiterates. It shows a statistically meaningful difference in relation to level of education ($p < 0.05$).

Taken together, with regard to high seroprevalence rate of Toxoplasma antibody in women willing marriage in Mazandaran province (74.6%), the incidence of congenital toxoplasmosis and toxoplasmosis related abortion is not high. In order to prevent infection, implementation of hygienic recommendation fulfils.

REFERENCES

1. Jackson, M.H. and W.M. Hutchion, 1989. The prevalence and source of Toxoplasma infection in the environment. *Adv. Parasitol.*, 28: 55-105.
2. Yang, H.J., K.N. Jin, Y.K. Park, S. Hong, J.M. Bae and S.H. Lee, *et al.*, 2000. Seroprevalence of toxoplasmosis in the residents of Cheju island, Korea. *Korean J. Parasitol.*, 38: 91-93.
3. Dubey, J.P. and C.P. Beattie, 1988. Toxoplasmosis of animals and man. CRC Press Inc., Boca-Raton, Florida, USA, pp: 1-213.
4. Galvin-Ramirez, M.I., C. Guillen-Vargas, R. Saavedra-Duran and A. Islos-Rodriguez, 1998. Analysis of Toxoplasma gondii antigens with sera from toxoplasmosis patients. *Rev. Soc. Brasil. Med. Trop.*, 31: 271-277.
5. Spalding, S.M., M.R. Amendoeira, C.H. Klein and L.C. Ribeiro, 2005. Serological screening and Toxoplasmosis exposure factors among pregnant women in South of Brazil. *Rev. Soc. Brasil. Med. Trop.*, 38: 173-177.

6. Feldman, H.A., 1974. Toxoplasmosis: An overview. Bulletin of the New York Academy of Medicine, 50: 110-127.
7. Wallace, G.D., 1973. The role of the cat in the natural history of *Toxoplasma gondii*. Am. J. Trop. Med. Hyg., 22: 313-322.
8. Apt, W., E. Thiermann, G. Niedmann and S. Pasmanik, 1973. Toxoplasmosis. Universida de Chile, Santiago, pp: 17-89.
9. Melamed, J., 1991. Retinocoroidite Toxoplasmica. Ph.D. thesis, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte.
10. Amendoeira, M.R.R., 1995. Mecanismos de transmissao da toxoplasmose. Anais da Academia Nacional de Medicina, 155: 224-225.
11. Frenkel, J.K. and J.P. Dubey, 1972. Toxoplasmosis and its prevention in cats and man. J. Infect. Dis., 126: 664-673.
12. Kasper, L.H., 1997. Toxoplasma infection. In Harrison's principals of internal medicine, 14th Ed. McGraw-Hill, New York, pp: 1197-1202.
13. Frenkel, J.k., 1974. Pathology and pathogenesis of congenital Toxoplasmosis. Bulletin of the New York Academy of Medicine, 50: 182-191.
14. Jacobs, I., 1963. Toxoplasma and Toxoplasmosis. Annual review of Microbiology, 17: 429-450.
15. McCabe, R.F. and J.S. Remington, 1988. Toxoplasmosis: The time has come. N.E.J.M., 318: 313-315.
16. Tenter A.M. and A.R. Heckerroth, 2000. Weiss LM. *Toxoplasma gondii* from animals to human. Int. J. Parasitol., 30: 1217-1258.
17. Mervyn, R.H., B.L. Tayler, V. Celia and M.C. Holland, 1997. Community study of Toxoplasma antibodies in urban and rural schoolchildren aged 4 to 18 years. Archives of Disease in childhood, 77: 406-409.
18. Keshavarz, H. and M. Zare, 1993. Toxoplasmosis in pregnant women of Rafsanjan. J. Gillan University of Med. Sci., 2: 28-32.
19. Athari, D.V., 1994. Seroprevalence of Toxoplasma antibodies among pregnant women in Kermanshah. Med. J. Islamic Rep. Ir., pp: 93-96.
20. Alleyassin, F., A. Moatari and F. Azare, 1990. The prevalence of Toxoplasma gondii antibodies in Shiraz. Ir. J. Med. Sci., 15: 13-17.
21. Saffar, M.J. and A. Ajami, 1999. seroprevalence of Toxoplasmosis in pregnant women in Sari. J. Mazandaran University of Med. Sci., 9: 1-5.
22. Gharavi, M.J., 1991. Seroparasitological survey of congenital Toxoplasmosis. Ph.D. thesis. Tarbiat Modarres University, pp: 1-30.
23. Shekarabi, M. and K.H. Khanali, 1999. Serological survey of Toxoplasmosis in women referring to the Health Center Laboratory before marriage. J. Lab. Diag., 1: 31-33.
24. Kamyabi, Z. and M. Attarpour, 1999. Serological study of Toxoplasmosis in women referred to Medical Health Laboratory before marriage, Kerman. J. Kerman University of Med. Sci., 6: 127-133.
25. Ghorbani, M., H. Edrissian and N. Assad, 1978. Serological survey of Toxoplasmosis in the northern part of Iran. Trans. R. Soc. Trop. Med. Hyg., 77: 369-371.
26. Rai, S.K., H. Shibata, K. Sumi, K. Kubota, K. Hirai, A. Matsuoka and T. Kubo, *et al.*, 1994. Seroepidemiological study of toxoplasmosis in two different geographical areas in Nepal. Southeast Asian J. Trop. Med. Public Health, 25: 479-84.
27. Paradisi, F., A. Bartoloni, D. Aquiline, M. Roselli, L.E. Nunez and G. Manzone, 1989. Serological survey of toxoplasmosis in the Santa Cruz region of Bolivia. Trans. R. Soc. Trop. Med. Hyg., 83: 213-214.
28. Barbier, D., T. Ancella and G. Martin-Bouyer, 1983. Seroepidemiological survey of toxoplasmosis in La Guadeloupe, France West Indies. Am. J. Trop. Med. Hyg., 32: 335-342.
29. Suzuki, H., T. Aso, Y. Yamamota and K. Matsumoto, 1988. Seroepidemiology of Toxoplasma infection in two islands of Nagasaki by ELISA. Trop. Med., 30: 129-139.
30. Fan, C.K., C.W. Liao, T.C.H. Kao, J.L. Lu and Su KE, 2001. Toxoplasma gondii infection: Relationship between Seroprevalence and Risk factors among inhabitants in two Offshore Islands from Taiwan. Acta. Med. Okayama. 55: 301-308.
31. Lebech, M., Larsen, S.O. and E. Petersen, 1995. Occurrence of Toxoplasmosis in pregnant women in Denmark: A study of 5402 pregnant women. Ugeskr. Laeger, 157: 5242-5245.
32. Ljungstrom, I., E. Gille, J. Nokes, E. Linder and M. Forsgren, 1995. Seroepidemiology of Toxoplasma gondii among pregnant women in different parts of Sweden. Eur. J. Epidemiol, 11: 149-156.
33. Tayler, M.R., B. Lennon, C.V. Holland and M. Cafferkey, 1997. Community study of Toxoplasma antibodies in urban and rural school children aged 4 to 18 years. Arch. Dis. Child, 77: 406-409.
34. Martinez Sanchez, R., R. Bacallao Gordo E. Alberti Amador and L. Affanso, 1994. Berrio prevalence of toxoplasmosis in pregnant women of the province of La Habana. Rev. Inst. Med. Trop. Sao paulo, 36: 445-450.