

Seroepidemiology of toxoplasmosis in childbearing women of Northwest Iran

Sieroepidemiologia della toxoplasmosi in donne in età fertile in Iran nord-occidentale

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INTRODUCTION

Toxoplasmosis is a worldwide parasitic infection, which can infect animals and humans [1-3]. Prevalence of toxoplasmosis in humans varies in different regions and can increase with age, consumption of raw meat, possession of cats, and low socioeconomic status. Toxoplasmosis can cause serious disease in pregnant women, immunodeficient individuals and organ transplantation candidates [4-6]. *Toxoplasma gondii* (*T. gondii*) is usually transmitted by ingestion of viable cysts in raw/undercooked meat and cysts shed in cat feces [7, 8]. Consumption of undercooked meat was identified as the principle risk factor for *T. gondii* infection in humans, but this does not explain the high seropositivity (24%-47%) in vegetarians [9, 10]. Infection transmission depends on the level of frequency of exposure to such factors [11-13].

Congenital transmission is another route of infection with *T. gondii*. Congenital toxoplasmosis may lead to some severe consequences such as mental retardation, chorioretinitis, cerebral calcification and central nervous sys-

tem (CNS) damage [14-17]. Toxoplasmosis is usually asymptomatic in adults, but the infection acquired during pregnancy may cross the placenta and cause irreversible harm and injury to the fetus and infant. In some cases, abortion occurs. Even if the pregnancy continues, the infection may lead to severe sequelae observed in infancy [5, 18, 19]. In Iran, seroprevalence of toxoplasmosis in adults, especially pregnant women, has been found to be between 23.7-71.3% and incidence of congenital infection is 1200-5250 children per year [20, 21]. Current literature reveals extensive knowledge about toxoplasmosis in most parts of Iran, but few studies were about on the disease's seroepidemiology in Eastern Azerbaijan. The present study advances knowledge as we evaluate epidemiology and seroprevalence of toxoplasmosis in the Eastern Azerbaijan province of Iran.

PATIENTS AND METHODS

The present study is a cross-sectional survey conducted by a joint effort of Department of Immunology, Infectious Disease Research Center and Gynecology Research Center of Tabriz University (Medical Sciences) in Tabriz from July 2009 to August 2010.

Eastern Azerbaijan is one of the main provinces

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of Iran located in the northwest. Apart from the industry, its economy relies heavily on agriculture. This province has 12 cities, of which major ones include Tabriz, Maragheh, Sarab, Ahar, Miane and Marand. Tabriz is the capital city of this province and is where most patients from surrounding cities seek medical service. Assuming a prevalence of 60%, a degree of precision of 4 and 95% confidence interval, the sample size was calculated as 1470 women in child-bearing age. All women in childbearing age seeking prenatal care from February 2009 to August 2010 at the Alzahra Obstetrics and Gynecology Hospital of Tabriz were enrolled in the study.

A total of 1659 healthy women in child bearing age were enrolled to participate in this study. All participants gave informed consent, and the ethics committee at Tabriz University of Medical Sciences (TUMS) reviewed and approved the study protocol which is in compliance with the Helsinki Declaration.

Demographic characteristics were recorded in questionnaires which included age, habitation, educational levels (primary school and lower or high school and higher) and living region (urban or rural). The blood samples were obtained by venous puncture, then centrifuged at 5000 rpm for five minutes. The sera were frozen at -70 °C until analysis.

All samples were examined by Indirect Fluorescent Antibody (IFA) test which is simple to do, has a high benefit-cost ratio and high degree of accuracy. Initially, 1:100 dilution of serum sample was used. Ready-for-use pre-coated slides with antigen were provided from the Pasteur Institute (Tehran, Iran). Polyvalent anti-human immunoglobulin conjugated serums and serum controls were purchased

from Baharafshan Company (Tehran, Iran). Anti-human serum conjugated with Fluorescein Isothiocyanate (FITC) with dilution of 1:20 in phosphate buffered saline (PBS) with 0.1% Evans blue was used. Tested sera were placed on antigen spots then incubated at 37°C for 30 minutes. Afterwards, the slides were rinsed in PBS (PH 7.6) twice for 5 minutes and were air-dried. A drop of FITC-labeled anti-human immunoglobulin diluted in PBS was added to each spot slide and then samples were incubated at 37 °C for 30 minutes, washed, dried and mounted with buffered glycerol. The slides were examined under a fluorescent microscope, equipped with an Osram HBO mercury lamp. FITC-labeled anti-human immunoglobulin was bound to the cell membranes and was contrasted with red-stained cytoplasm of the parasite. Sera reacting positively at dilutions $\geq 1:100$ were considered indicative of previous toxoplasmosis. Statistical analyses were performed using the SPSS statistical package version 13.0 (SPSS Inc, Chicago, Ill, USA). The results are presented as mean \pm Standard Deviation (SD). Then, Fisher exact and chi square tests were used to assess the differences between stages, as appropriate. A P-value less than .05 was considered significant.

RESULTS

The mean age of subjects was 31.89 \pm 8.54 years. Seroprevalence of toxoplasmosis in different cities of Eastern Azerbaijan is shown in Table 1. Although most seropositive cases were reported from Tabriz, highest seropositivity ratio was shown in Maragheh.

Distribution of titer dilutions (1:200, 1:400, and

Table 1 - Seroprevalence of toxoplasmosis in studied population, according to Eastern Azerbaijan cities.

City	Seroprevalence		
	Positive No. (%)	Negative No. (%)	Total No. (%)
Maraghe	178 (10.73)	33 (1.99)	211 (12.72)
Ahar	110 (6.64)	52 (3.13)	162 (9.77)
Sarab	71 (4.28)	15 (0.90)	86 (5.18)
Miane	59 (3.55)	46 (2.78)	105 (6.33)
Marand	45 (2.71)	65 (3.92)	110 (6.63)
Tabriz	435 (26.22)	550 (33.15)	985 (59.37)
Total	898 (54.13)	761 (45.87)	1659 (100)

Table 2 - Frequency of serum dilutions in seropositive subjects.

Dilution	Frequency	
	No.	Percent (%)
1:100	111	12.36
1:200	324	36.08
1:400	244	27.17
1:800	219	24.39
Total	898	100

1:800) is shown in Table 2. In seropositive subjects, the highest percent of serums (36.08%) were seropositive in 1:200 dilution.

Fisher's exact test showed that seropositivity was significantly different between the age groups ($\chi^2=49.14$, DF=3, P<0.001). As the age increased, seroprevalence of toxoplasmosis al-

so increased. Subjects aged between 35 and 40 years had the highest seropositivity of toxoplasmosis, while subjects who are in age range 20-24 years had the lowest seropositivity (Table 3).

Fisher's Exact test showed that seroprevalence of toxoplasmosis was significantly different between subjects with primary school/lower and high school/higher educational levels ($\chi^2=99.79$, DF=1, p<0.001). Seropositivity of toxoplasmosis in subjects with primary school/lower educational level was higher than subjects with high school/higher educational level.

Seroprevalence of toxoplasmosis IgG showed a significant difference between populations living in urban and rural regions (Fisher's Exact test: $\chi^2=74.38$, DF=1, P<0.001). Seropositivity of toxoplasmosis in subjects living in rural region was significantly higher than whom living in urban region (Figure 1).

Table 3 - Seroprevalence of toxoplasmosis IgG in different age groups.

Age Groups (Year)	Seroprevalence of Toxoplasmosis IgG		
	Positive No. (%)	Negative No. (%)	Total No. (%)
20-24	99 (5.97)	174 (10.49)	273 (16.46)
25-29	159 (9.58)	148 (8.92)	307 (18.50)
30-34	270 (16.27)	203 (12.24)	473 (28.51)
35-40	370 (22.30)	236 (14.23)	606 (36.53)
Total	898 (54.13)	761 (45.87)	1659 (100)

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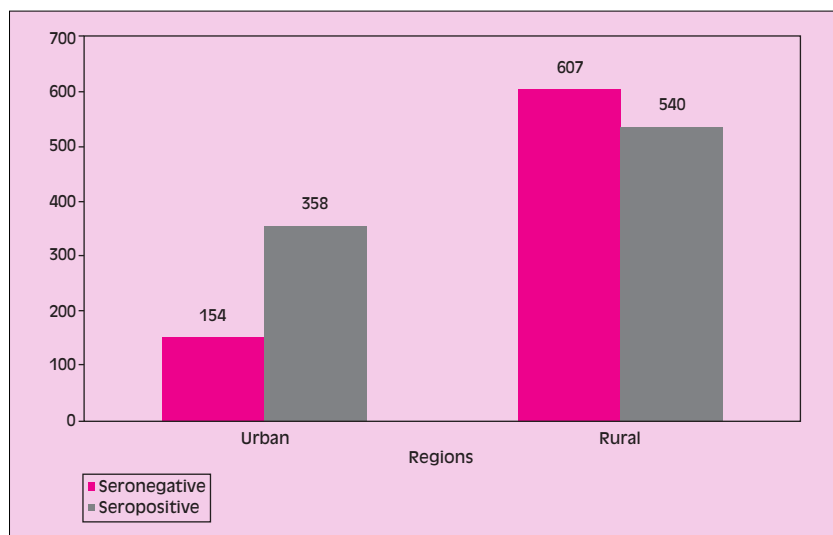


Figure 1 - Seroprevalence of toxoplasmosis IgG according to living regions.

DISCUSSION

Toxoplasmosis has a wide geographical distribution. It is a serious health problem in various countries [3, 22]. Congenital transmission occurs when a woman acquires the infection for the first time during pregnancy and transmits it to her fetus [20, 23]. According to Wong and Remington, 90% of cases with toxoplasmosis are asymptomatic or oligosymptomatic. It is estimated that 20-90% of the world adult population, depending on the region, already have had history of contact with *T. gondii*. Nevertheless, initial infection and subsequent chronic infection are usually benign in immunocompetent subjects.

This zoonosis can be found on all of the continents and under different climatic conditions. Seropositivity is higher in hot and humid areas and its prevalence is related to several factors, including educational level, nutritional habits, age, and rural or urban residence [24-26]. Prevalence of *T. gondii* specific antibody in different parts of Iran depends on several socio-economic, demographic and ecological factors. Several studies in different parts of Iran (Table 4) have shown varying rates of seropositivity [21, 24, 27-30].

Several studies have been conducted concerning seroepidemiology of toxoplasmosis during pregnancy and in the women of childbearing age in Iran and other countries [20, 25, 31]. The frequency of toxoplasmosis acquisition during pregnancy ranges from 1-4 per 1000 pregnancies in several countries, and congenital infection has a prevalence of 0.2-2 per 1000 births [32].

However, because of the importance of maternal immunity in the pre-pregnancy stage, we chose to study the childbearing female populations in Azerbaijan to estimate the disease burden in this area. Seroprevalence of *T. gondii* infection range between 15-77% in different countries [13, 33-35]. In outpatient women of childbearing age, the seroprevalence is between 21.8% to 85% in our country and the neighboring country to our province, Turkey [21, 24, 27, 28, 30, 36, 37].

Consistent with the study of Bobic et al., we found that the prevalence of seropositivity with toxoplasmosis infection increases as the age increases (33). Since the probability of exposure to one of the transmission routes increases as the individual's age increases, it can be concluded that our finding agrees with the current literature. Regarding the few reports about a dissociative relationship between urban and rural areas for toxoplasmosis seroprevalence, several authors have emphasized the influence of living in urban versus rural regions in toxoplasmosis seroprevalence [14, 28, 38, 39]. The underlying reason may be due to variations in the drinking water sanitation, health criteria, and public hygienic problems among rural and urban regions. Importantly, our study showed higher seropositivity of toxoplasmosis in the rural region.

In our study, we hypothesized a direct relationship between education levels and some of behaviors such as washing, cooking, housekeeping; the results support this hypothesis ($\chi^2=99.79$, $DF=1$, $P<0.001$). Finally, increasing the awareness of the impact of female house

Table 4 - Seroprevalence of toxoplasmosis in different regions of Iran.

Region	No. of Subjects	Positive (%)	Test	Date
Gilan & Mazandaran (40)	1779	55.7	IFA	1978
Khuzestan (41)	1806	49.6	IFA	1993
Azerbaijan & Khuzestan (29) 3370	12.8	IFA	1981	
Gilan (Roodsar) (42)	975	86.3	IFA	1994
Malayer (43)	917	41.3	IFA	1994
Hamadan (Women aged 15-45 years) (21)	360	38.9	IFA	2003
Kermanshah (Pregnant Women) (2)	495	32.7	IFA	1994
Kermanshah (Total) (44)	1835	36.4	ELISA	2004
Fars (45)	300	26	IFA	1978
Chaharmahal (Pregnant Women) (16)	394	27.4	IFA	2002

management behavior (cooking, washing vegetables) and maternal immunity, by way of seminars, etc. is highly recommended.

In conclusion, 54.13% of women of childbearing age in the Eastern Azerbaijan province of Iran are seropositive for toxoplasmosis. Increased seroprevalence of toxoplasmosis with age was a predictable result because of increasing time of exposure. Increasing seroprevalence with lower educational level and living in rural region agrees with the latest epidemiological findings due to lower socioeconomic status. Currently, there is no legal obligation for education about methods of minimizing exposure to *T. gondii* in our country. Therefore, our findings indicate the need to design specific prepregnancy educational programs for women of childbearing

age. The data found in the current study point out that due to a high risk of toxoplasmosis in childbearing women, a debate (or forum that fosters discussion) on developing a general screening program for toxoplasmosis in pregnancy in Eastern Azerbaijan and even Iran should be initiated.

Keywords: toxoplasmosis, seroprevalance, female, child bearing.

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SUMMARY

Toxoplasma gondii causes the most common parasitic infection in the world. Congenital transmission, prenatal mortality and abortion are major problems of *T. gondii*. Prevalence of toxoplasmosis is high in Iran, especially in Azerbaijan. The current literature reviewed in this paper reveal results pertaining to various regions of Iran. The present cross-sectional e-study was designed to evaluate the seroprevalence of toxoplasmosis in childbearing women in Northwest Iran. We evaluated 1659 women in childbearing age from several cities in Northwestern Iran (Tabriz, Maragheh, Ahar, Marand, Sarab, Miane) from July 2009 to August 2010. Women aged between 20 and 40 years and seeking prenatal care were enrolled in the study. The subjects' sera were probed with indirect fluorescent antibody (IFA).

A total of 1659 subjects were examined. Titres ranged from 1:100 to 1:800. In all, 899 subjects

(54.13%) were seropositive. The highest frequency of seropositivity was shown in 1:200 dilution (36.08%) and in subjects from Maragheh (84% of 211 subjects). There was a direct linear relationship between seropositivity and age ($p < 0.001$). Also, seroprevalence of toxoplasmosis was higher in subjects with primary school/lower educational level ($p < 0.001$) and subjects living in rural regions ($p < 0.001$).

Overall, more than 50% of women in childbearing age were seropositive for toxoplasmosis in northwestern Iran. Increasing seroprevalence of toxoplasmosis with age was a predictable result due to longer exposure to the parasite. The relationship between increasing seroprevalence and lower educational level as well as living in rural areas is in line with the latest epidemiological findings, which also show such relationships due to lower socioeconomic status.

RIASSUNTO

Toxoplasma gondii è l'agente eziologico di una delle più comuni infezioni parassitarie in tutto il mondo. La trasmissione congenita, la mortalità prenatale e l'aborto rappresentano i principali problemi legati all'infezione da *T. gondii*. In Iran, soprattutto in Azerbaijan, la prevalenza della toxoplasmosi è alta. Gli articoli della letteratura presi in considerazione nel presente articolo riportano dati relativi a diverse regioni dell'Iran. Il presente studio è stato condotto nell'intento di valu-

tare la sieroprevalenza della toxoplasmosi in donne in età fertile in Iran nord-occidentale.

In questo studio osservazionale trasversale abbiamo valutato 1659 donne in età fertile provenienti da diverse città dell'Iran nord-occidentale (Tabriz, Maragheh, Ahar, Marand, Sarab, Miane) nel periodo luglio 2009-agosto 2010.

Nello studio sono state arruolate donne nel range di età 20-40 anni (età fertile) e in attesa di assistenza pre-na-

tale. I sieri di tutti i soggetti sono stati testati con anticorpi indiretti marcati con fluoresceina (IFA). Complessivamente, è stato esaminato un campione di 1659 soggetti. I titoli anticorpali ricadevano nel range 1:100-1:800. La sieropositività è stata riscontrata in 889 (54,1%) soggetti. La frequenza più elevata di sieropositività è stata evidenziata alla diluizione 1:200 (36,08%) e in soggetti provenienti da Maragheh (84% di 211 soggetti). È stata rilevata una relazione lineare diretta tra sieropositività ed età ($p<0,001$). Inoltre, la sieroprevalenza della toxoplasmosi è risultata più elevata nei soggetti con educazione scolastica di livello inferiore

($p<0,001$) e in quelli residenti in aree rurali ($p<0,001$). Una percentuale di donne in età fertile superiore al 50% è sieropositiva per la toxoplasmosi in Iran nord-occidentale. L'aumento della sieroprevalenza per toxoplasmosi in relazione all'età era un risultato prevedibile, da attribuire al maggiore periodo di esposizione al parassita. La relazione tra un tasso di sieroprevalenza più elevato ed educazione scolastica di livello inferiore, nonché la residenza in aree rurali, è in accordo con i più recenti riscontri epidemiologici che evidenziano questa identica relazione in rapporto ad una condizione socio-economica meno agiata.

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