Seroprevalence of Toxoplasma gondii infection among pregnant women in Amol, Northern Iran

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Abstract: Background: Toxoplasmosis is a disease caused by the protozoan parasite Toxoplasma gondii. Congenital transmission may occur when a pregnant mother acquires T.gondii infection for the first time in her life during pregnancy. Detection of anti-Toxoplasma immunoglobulin M (IgM) and IgG is essential for the diagnosis of Toxoplasma infection in pregnant women. The aim of this study was to determine the Toxoplasma antibodies in pregnant women in Amol, *Northern Iran.* **Methods:** In this cross-sectional study, 1057 blood samples were collected from pregnant women. Sera were separated by blood centrifugation at 3000 rpm for 5 min and frozen at -20 °C until use. The samples were tested for IgG and IgM antibody by Enzyme-linked immunosorbent assay (ELISA). **Results:** IgG and IgM anti-Toxoplasma antibodies were positive in 739/1057 cases (69.91%) and 57/1057 cases (5.39%), respectively. The peak age for acquisition of the infection in pregnant females was 26-30 years old. **Conclusion:** About more than half of the married women in the present study were at risk of infection with T.gondii, so preventive method should be considered.

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1. Introduction

Infection by the intracellular parasite Toxoplasma Gondii is often an asymptomatic or a mild clinical disease which is not recognized, Cats and wild Felines are the only definitive host while all other worm-blooded animals including humans are intermediate hosts (1,2,3). Infection is acquired by ingestion of viable tissue cysts in meat or oocysts excreted by cats that contaminate food or water (2-6). However, when a pregnant woman develops a primary T. gondii infection, the parasite may be transmitted to the fetus and cause serious damage (2). The incidence of acquired primary T. gondii infection during pregnancy varies greatly from country to country and ranges from less than 1 to more than 15 per 1,000 pregnancies (2). Association to behavioral disorders like schizophrenia, mood disorders, personality changes and cognitive impairment has recently been affirmed (3). Consequences of primary infection in pregnant women with normal immunity include abortion, blindness, deafness, mental retardation, microcephalus, hydrocephalus and other neurological diseases in the fetus(4). In immune deficient individuals, primary or reactivated infection can result in encephalitis, pneumonia, myocarditis and disseminated infection (5). Considerable variations in seroprevalence of the infection have been reported in diverse regions and different individuals and various times and are chiefly related to factors that expose the population to the infected cysts (6). Age, sex, ethnicity, sanitary conditions, geographic climate, cat or soil contact, patterns of work and behavior, and location and configuration of the community have been main determinant of these dissimilarities (7-9).

Prevention of congenital toxoplasmosis in pregnant women has been based on serological test for Toxoplasma antibodies. In most cases the laboratory diagnosis of acute and latent toxoplasmosis relies on the detection of T. gondii specific IgG and IgM antibodies and the avidity test of T. gondii specific IgG antibodies has also been very helpful in the diagnosis. Many serological tests including the latex agglutination test, ELISA, indirect fluorescence antibody test (IFA) and haemagglutination test have been utilized in the detection of antibodies against T.gondii. There are many

researches about the prevalence of anti-Toxoplasma gondii antibody among Iranian women. Seropositivity have been reported as 33-44% innorthwest (7,8), 22-37% in south and 27-54% in central parts of Iran (10-17). In another study in Chaharmahal and Bakhtyari the seroprevalence of Toxoplasma antibodies among pregnant women using IFA was 27.6 % (15).

The present study was performed to determine the Toxoplasma antibodies in pregnant women in Amol, by ELISA method because of its high sensitivity and specificity, easier technique and lower expanse which is preferred in order to screening Toxoplasma infection.

2. Materials and Methods

This study was carried out in Amol, Mazandaran Province, northern Iran. At the 2006 census, its population was 197,470, in 55,183 families. It is less than 20 kilometres (12 mi) south of the Caspian sea and less than 10 kilometres (6.2 mi) north of the Alborz mountains. The average daily relative humidity is around 84%. Throughout the month of January daytime temperatures will generally reach highs of around 28°C that's 5°C.

3. Patients and blood sampling

The samples were women referred to the central laboratory of Amol for routine pregnancy test. A total of 1057 serum samples were tested at the Amol central laboratory. 3 ml of venous blood sample were drowning from the study group (1057 pregnant women) and anticuagulated with citrated sodium. Two ml of citrated blood were centrifuged at 3000 rpm for 15 minutes and frozen at -20 °C until use °C until use. Serum samples were tested for anti-Toxoplasma IgM and IgG antibodies using Toxoplasma IgM ELISA kit and Toxoplasma IgG ELISA kit (Dia-Pro,Milan, Italy). According to the recommendation of the kit, absorbance levels below 9 was considered as negative, 9-11 assumed as equivocal and above 11 was positive. The samples with equivocal results were retested and were accordingly accepted as negative orpositive. If the second test result was equivocal, the sample was excluded from the study.

4. Statistical analysis

The collected data were coded and analyzed using the Statistical Package for Social Sciences SPSS for Windows version 15. Descriptive statistics were employed to examine antibody status in different age, education and residency groups in the studied population.

5. Results

The average age of these women was 25.8±5.4 years. The overall seroprevalence of toxoplasmosis in pregnant women was 75.02 % (793/1057 cases). IgG and IgM anti-Toxoplasma antibodies were positive in 739/1057 cases (69.91%) and 57/1057 cases (5.39%), respectively. It elucidates that the peak age of T. gondii acquisition in urban areas was less than rural districts 45.7% and 54.3%, respectively.

Table 1: Seropositivity of Anti-Toxoplasma IgG and IgM in relation to gestational age

	IgG POSITIVE		IgG NEGATIVE		IgM POSITIVE		IgM NEGATIVE	
Age	N	%	N	%	N	%	N	%
15-20	89	%12	27	%8.4	7	%12.9	109	%10.9
21-25	192	%26	96	30.2	13	%24.1	275	%27.4
26-30	266	%36	104	%32.8	18	%33.3	352	%35.1
31-35	155	%21	72	%2.7	9	%16.8	218	%21.7
36-42	37	%5	19	%5.9	7	%12.9	49	%4.9
Total	739	%100	318	%100	54	%100	1003	%100

6. Discussion

A wide variability in the prevalence rates of T. gondii seropositivity among pregnant women has been reported worldwide (18). The risk factors that are often associated with acute infection in pregnant women were eating raw or undercooked meat and soil contact. Weaker associations were observed for tasting raw meat during preparation of meals, eating salami, drinking unpasteurized milk and animal contact (19-26). The types of meat products associated with the transmission of Toxoplasma are different in human populations with varying eating habits. Thus, a European multicentre study (27) found that the risk-factors most strongly predictive of acute Toxoplasma infection in pregnant women were consumption of undercooked lamb, beef or game, while a studyconducted in Norway (28) found that Toxoplasma maternal seropositivity was associated with eatingraw or undercooked mutton and pork, and a study conducted among women in California in the USA found that a major infection source was consumption of rare ⁄ medium cooked beef (29).Regional variations in the incidence of Toxoplasma infection rates from one country to another or even within the same country, has been well documented. This variation has been attributed to climate, cultural differences regarding hygienic

and feeding habits (22,23,24, 30-33). The frequency of stray cats in a humid rainy climate favoring the survival of oocysts has contributed to the high Toxoplasma prevalence in Central America (23). Stray cats are widely spread in Amol city, however, the Moderate temperature and wet weather conditions in Amol city and Surrounding villages are ideal for oocyst survival, compared to cooler and hot regions. A decrease in the prevalence of the infection over the past 25 years has been reported in developed countries, In France the prevalence has declined from more than 80% during the 1960s to 54.3% in 1995 (18,19). In Belgium, a major decrease was observed from 70% in 1966 to 47% in 1987 (20). In the United Kingdom (South Yorkshire) a reduction was seen from 22% in 1969 to 8% in 1990 (21).We found an overall T. gondii IgG and IgM antibody prevalence of 75.2% among Pregnant Women in Amol city and around rurals 15–42 years of age in 2011–2012, indicating that approximately 3 in 5 persons in this age group were infected with T. gondii.

We found that the peak age of T. gondii acquisition in urban areas was less than rural districts 45.7% and 54.3%, respectively. Therefore these pregnant women were not at risk for Toxoplasmosis. The rate of seroprevalence of Toxoplasma IgG antibodies in north of Iran was reported 55.7% (34). High seroprevalence rates, reaching 82.2%, have been described previously in Iran (35). An association between rural location of the childhood residence and Toxoplasma infection has also been demonstrated in a study of pregnant women in the UK (30). Contact with soil, gardening and soil related occupations have been shown to be risk factors for toxoplasmosis in a number of studies (29,31,32,33). The association between residence in a small town / village and having toxoplasmosis probably reflects more frequent contact with soil through gardening and farming in rural areas. Because of low socioeconomic condition and low consumption of meat in Amol, the high prevalence of toxoplasmosis may be due to consumption of contaminated water or vegetable.

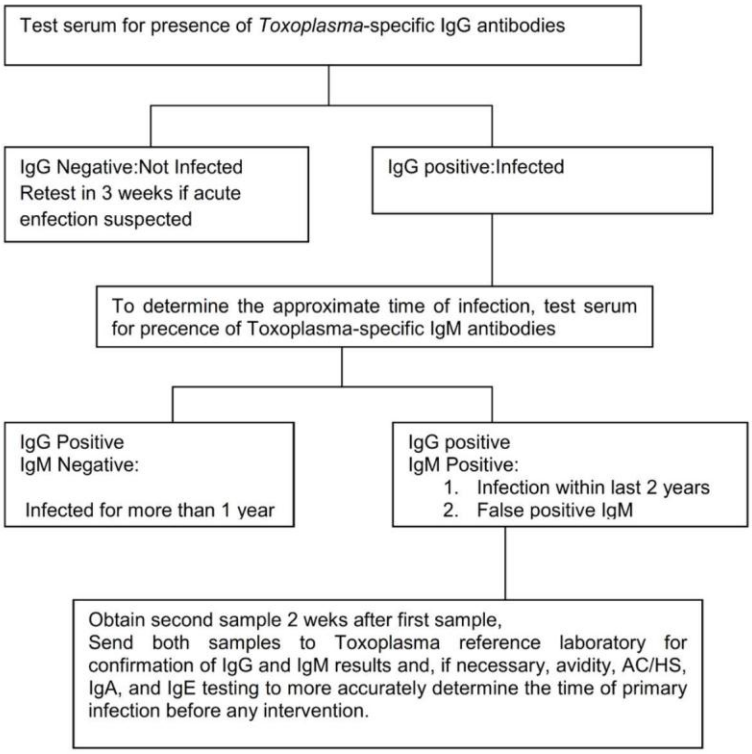

Figure 1. Algorithm for the serodiagnosis of toxoplasmosis in people older than 1 year of age. Adapted from Wilson M, McAuley JM. Toxoplsama. In: Murray PR, Baron EJ, Pfaller MA *et al*., eds. Manual of Clinical Microbiology, 7th Ed. Washington DC: ASM Press, 1999, pp 1347–1382.

Detection of Toxoplasma-specific IgM antibodies has been used as an aid in determining the time of infection, but IgM antibodies have been reported to persist for up to 18 months post Infection (36). A negative IgM with a positive IgG result indicates infection at least 1 year previously. A positive IgM result may indicate more recent infection or may be a false positive reaction. A flow diagram for T. gondii testing is presented in Figure 1.

Given the potential for false-positive results, the true value of IgM testing is in ruling out the presence of acute infection. In other words, negative IgM results are reassuring, whereas positive results should be interpreted carefully, confirmed in a toxoplasmosis reference laboratory, and followed by serial titers at least 3 weeks apart (33,37,38).

Since the U.S. Food and Drug Administration [FDA] has recommended that a solely positive IgM test result should undergo confirmatory testing, avidity specific T. gondii IgG tests have been presented to differentiate between recently whom spiramycin had been recommended because recently acquired infection could not be ruled out in the single serum samples available from each of them (39).

The preventive ways against toxoplasmosis are related to seroprevalence of infection with gestational age and health equipment. Regarding to the result more than half of pregnant women in the present study were at risk of infection with T. gondii, so preventive method should be considered.

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