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Der Pharmacia Lettre, 2016, 8 (7):17-20  
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## Seroprevalence of Toxoplasmosis in Tabriz city-Iran

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### ABSTRACT

Having been known for over 100 years, *Toxoplasma gondii* is one of zoonosis agents. Because of the risk of abortion and congenital defects, detection of contamination with this parasite is important. Evaluation of anti-Toxoplasma IgM and IgG was conducted in referrals of clinical laboratory in Tabriz City during 2015 through chemiluminescence method. Among 7520 referrals for ToxoIgG test, 3054 (40.6%) were positive and 4466 (59.3%) were negative. Of 7159 referrals for ToxoIgM test, 221 (3.08%) were positive, 101 (1.4%) were suspicious, and 6837 (95.5%) were negative. Regarding IgG and IgM values, the cases above 8 u/ml are considered positive. Being IgM-positive is not solely advisable to find out recent infection and other confirmatory tests must also be done. According to the assessments accomplished, the presence of IgM alone in the absence of other antibodies indicates infection acquisition during some recent months in the form of acute disease. IgG often appears two weeks after infection and culminates during one to two months, gradually decreasing after that and lasting for whole life. Since most Toxoplasmosis complications are observed during the last three months of pregnancy and noting the high prevalence of ToxoIgG cases, it is advisable for women at the productive age to be informed of their ToxoIgG test before pregnancy.

**Keywords:** *Toxoplasma gondii*, Tabriz, Chemiluminescence, Seroprevalence

### INTRODUCTION

As an omnipresent protozoan parasite, *Toxoplasma gondii* infects one third of the world's population [1]. *T. gondii* is a coccidian parasite infecting felids as the definitive hosts and warm-blooded animals act as intermediate hosts. With *T. gondii* as the only known species, it is among the commonest animal parasites. In general, Coccidian parasites possess a sophisticated life cycle. Most are host-specific and only spread via an oral-fecal cycle; despite, *T. gondii* is also transmissible through transplacental route and by carnivorousism [2]. Being a major zoonotic and veterinary pathogen, *T. gondii* is recognized as a pathogen in category B priority of the National Institutes of Health, Bethesda, USA. *T. gondii* was first described in the tissues of *Ctenodactylus gundi*, a North African rodent, by Nicolle and Manceaux 100 years ago. The identification of this organism in rabbit tissues was reported in the same year by Splendore in Brazil. Nicolle and Manceaux named the genus *Toxoplasma* because of its bow-like shape [from Greek: toxo= bow or arc; plasma = creature]. It was discovered that other forms of *Toxoplasma* exist; including tissue cysts, but it was in the 1960s and 1970s when this parasite was identified to be a coccidian. Several independent groups identified the cat as the definitive host [1]. The only definitive hosts, in which sexual reproduction of *Toxoplasma* occurs to produce infective oocysts, are cats and other felids. Warm-blooded animals such as humans are intermediate hosts bearing the tissue cysts [3]. Intracellular growth with rapid division of tachyzoites in many tissues distinguishes acute Toxoplasmosis in these hosts. The responses control the acute infection, therefore, some tachyzoites become encysted [principally in the muscles and brain] producing a dormant stage termed the bradyzoite. These cysts contain hundreds of bradyzoites, preserved for years. While eaten by a non-feline carnivore, the cyst-containing tissues release bradyzoites in the intestine, again yielding an infection characterized primarily by rapid tachyzoite growth and finally by encysted bradyzoites. However, if eaten by a

member of the cat family, encysted bradyzoites experience the sexual cycle in the intestine, resulting in oocysts. Ingestion of a sporulated oocyst [ovoid and with the approximate size of  $10 \times 12 \mu\text{m}$ ] by a mammal causes sporozoites release the infection of the intestinal epithelial cells. They develop into rapidly multiplying tachyzoites resulting first in an acute and then a chronic infection with encysted bradyzoites, thereby completing the natural life cycle [4]. In spite of asymptomatic form in normal hosts, *T. gondii* can cause severe disease in people suffering from immunodeficiency. Concomitant Toxoplasmosis has been a major health problem since the HIV–AIDS pandemic, with its worldwide prevalence increasing during the 1980s. Patients afflicted with toxoplasmic encephalitis were documented for the first time in 1992, Thailand and an increasing number of cases has been reported annually, particularly in the northern part of the country [3]. In the case of acquiring primary infection during pregnancy, *T. gondii* may be transmitted to the fetus, causing inflammatory lesions potentially leading to permanent neurological damage, with or without hydrocephalus, and chorioretinitis with visual destruction. The pregnant mother and the infected infant often lack the symptoms, but it is possible for the child to recur chorioretinitis later in life. The best ways to prevent from damages originated from congenital Toxoplasmosis is a controversial matter and recent mutual investigations have clarified the present state of knowledge, but there is a need for prospective, randomized trials [5]. When the mother is infected with *T. gondii* for the first time during pregnancy, the parasite enters the fetal circulation via placenta. The birth incidence of inborn Toxoplasmosis varies from one to ten per 10 000 live births. There is little or no risk posed to the fetus if maternal infection acquired before gestation, except in mothers infected a few months [at most 3] before conception. According to the time during which mother have become infected, frequency of congenital transmission fluctuates considerably. Infection around the time of commencement and within the first 2 weeks of gestation does not result in vertical transmission in women consuming spiramycin, it is while transmission rates are above 60% in the last three months. Transmission frequency and disease severity have a reverse relation. Early maternal infection during the first and second trimesters can cause severe congenital Toxoplasmosis and may result in fetal death in utero and spontaneous abortion. In contrary, late maternal infection in third trimester usually results in newborns appearing normal. The general subclinical infection frequency is 85% in newborns suffering from congenital Toxoplasmosis. Infection is ignored initially, but later babies may be affected with chorioretinitis or delayed growth in the second or third decade of life if untreated. Treating mother during pregnancy can lessen the fetal infection frequency and severity. Spiramycin may reduce the vertical transmission incidence by about 60% [see Management and treatment]. As regards chronic infection, *T. gondii* vertical transmission is only reported in immunocompromised women, experiencing AIDS or receiving immunosuppressive drugs such as corticosteroids. However, vertical transmission in this setting is seemingly quite rare [6].

## MATERIALS AND METHODS

The study has been accomplished people who were attended the pathological laboratories of Tabriz city in Iran. IgM and IgG antibodies from whole blood samples collected using a chemiluminescent kit [DiaSorin LIAISON®, Turin, Italy] was measured. First a little of sample was poured to the cuvette by the left arm of chemiluminescent LIAISON [Germany], then the solution containing magnetic particles was added by right arm, finally it was incubated. The solution was washed three times by washing solution buffer, and then Toxoplasma-Ag added to the cuvette. In the next step, the conjugating solution was added and was re-incubated. Again the solution was washed three times and a starter solution was applied for measurement. First, starter 1 was poured into the vial and the indicator was injected to start quantitative chemiluminescent reactions. After about one-tenth of a second delay, the measurement signal was detected and the measurement has been completed in 3 seconds. Also the age, sex, material status, residential status and education level of patients were investigated by a questionnaire. The research results were analyzed by Chi-square test using SPSS software, version 16.  $P < 0.05$  was considered indicative of a statistically significant difference [7].

## RESULTS

7520 people referred for ToxoIgG and 7159 for ToxoIgM during 2015. Of 7520 referrals for ToxoIgG test, 3054 (40.6%) were positive and 4466 (59.3%) were negative. Among 7159 individuals referring for ToxoIgM test, 221 (3.08%) were positive, 101 (1.4%) were suspicious, and 6837 (95.5%) were negative. The value 8 u/ml was considered positive for IgG and IgM. According to the IgG measurement criteria, 25.5% of males and 41.3% of females referring to the laboratory were infected with *T. gondii*. Based on Chi Square statistical analysis test at  $\alpha = 0.05$ , the incidence of this parasite is meaningfully higher in males ( $P < 0.01$ ). According to the IgM measurement criteria, 7.6% of males and 2.9% of females referring to the laboratory were infected with *Toxoplasma gondii*. Based on Chi Square statistical analysis test at  $\alpha = 0.05$ , the incidence of this parasite is meaningfully higher in males ( $P < 0.01$ ). After reviewing the questionnaires, demographic data was elicited for the individuals referring to the laboratory (Table 1).

**Table 1- Demographic characteristics of Toxoplasmosis infected people in Tabriz 2015**

Parameters	Domain	Percent
Age groups	< 20	0.3
	20-25	21.6
	25-30	52.3
	30-35	25.8
Sex	Female	94.3
	Male	5.7
Education	Less than high school	55.3
	High school graduate	30.2
	University graduate	14.5
Residential status	Urban	69.8
	Rural	30.2
Material status	Single	1.6
	Dead spouse	1.9
	Divorced	3.4
	Married	93.1

## DISCUSSION

*T. gondii* has different prevalence patterns in different societies based on age, geographic region, dietary habits, climate [higher infection rate in hot and humid regions] and the level of contact with felines. The highest prevalence is observed in Sothern and Central America; for example, Haiti has a prevalence rate of almost 100%. The prevalence rate of this parasite is rather high in Iran [up to 70% in Guilan and Mazandaran Provinces] [8-10].

With extreme psychological and physiological influences, pregnancy is an unusual period in a woman's life. Almost the structure or function of all body systems is altered. The immune system is suppressed during this period, making females more vulnerable to some infections [11]. Placental barrier efficiently blocks the entrance of some microorganisms from maternal into fetal blood stream. However, some microorganisms can cross the placenta, leaving destructive consequences to the fetus. Maternal immunoglobulin types IgG1, IgG3 and IgG4 can cross the placenta to protect the fetus with a range of molecules possessing different specificities [12, 13].

Due to the high number of female patients at productive age, being highly valuable during pregnancy, and the adverse effects of infection on fetus, the number of female patients [94.3%] was considerably higher than females [5.7%] in this study. The highest number of female patients was allocated to 25 to 35 year-old pregnant individuals. Anti-Toxoplasma IgM appears during the first week of infection and peaks within one month. Based on the sensitivity of methods, this antibody type is detectable for 2 to 3 months and even one year. IgM assessment is widely used for acute infection diagnosis or diagnosing whether a pregnant woman has acquired the infection during pregnancy or prior to it. The presence of IgM alone is not advisable for detecting the recent infection and other confirmatory tests must be done. IgG and IgM assessment can be achieved through chemiluminescens, IFA and ELISA methods. Based on the assessments, the presence of IgM alone without other antibodies indicates the infection acquired during few recent months and acute form of disease or infection during pregnancy period. IgG usually appears two weeks of infection and peaks during 1-2 months, gradually decreasing after that and often lasting for lifetime. The presence of IgG alone can show chronic infection or recurring disease in an immune-suppressive individual, but along with IgM and IgG, it can be indicative of infection acquired within recent three to six months [8, 9].

In 2007, Nouri and Gharadaghi used ELISA to study Toxoplasma infection in malignant cancer patients with 150 samples. They concluded that noticing the high frequency of positive cases in malignant cancer patients, serum titration should be performed now and then to realize the potential of converting chronic to acute cases [14]. During 2009, Abbasian et al. evaluated the role of *Toxoplasma gondii* infection in testosterone serum level with ELISA analysis on 180 samples. They concluded that there is a meaningful relation between Toxoplasma infection and increased levels of testosterone, increased hair loss, hirsutism, and elevated stature [15].

Dr. Ali Mohammadi et al. studied the seroepidemiology of Toxoplasmosis in women based on pre-marriage tests in 2008. They found that about 43% of marriageable women are serologically Toxoplasmosis-positive, indicating very high importance of this infection in women. The findings of this study are in accordance with our study [16]. Dr. Hashemzadeh and coworkers in the year 2010 while serologic examination of Toxoplasmosis prevalence via ELISA method discovered that there is no significant difference between males and females [17]. In another study by Mahbod and colleagues in pediatrics ward of hospitals between 2000 to 2001, Toxoplasmosis serology was investigated in newborns and almost 34% of infants were reported chronic-Toxoplasmosis-positive by the aid of indirect immunofluorescence method [18]. Arbabi et al. also surveyed the seroepidemiology of Toxoplasmosis in

single females in 2007 and 2008. The average age of their participants was similar to the present study. They understood that the anti-Toxoplasma antibody level was low in these individuals; therefore, the single females are posed to the risk of Toxoplasmosis, which is compatible with the results of this study [19]. In 2007, in Ramsar, Dehghan *et al.* investigated the Toxoplasmosis seroepidemiology by indirect immunofluorescence method in referrals to medical diagnostic laboratories and the results showed that about 32% of referrals were anti-Toxoplasma-positive [20].

Anti-Toxoplasma IgG and IgM in females of northern Iran were surveyed in 2007 by Saeedi *et al.* IgG-positive samples accounted for 48% of their samples, which is similar to our results. They stated that there were not any significant relations between Toxoplasmosis infection and age, education, and habitat, which is consistent with the results gained during our investigation. They also reported that only keeping cats causes increased catching of Toxoplasmosis [21]. In 2010, Xiao and collaborators investigated *T. gondii* seroepidemiologically in China. Their results were indicative of high risk of women exposure to *T. gondii*, probably due to more contact with sources of infection. Their findings are closely comparable with ours [22].

Based on the results obtained, there is no correlation between toxoplasmosis infection and age, education, and habitat. Since most fetal damages are observed during the last trimester of pregnancy and noticing high prevalence of ToxoIgG cases, it is advisable for females on reproductive ages to be informed about their ToxoIgG test results prior to pregnancy.

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