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Full Length Research Paper

Congenital infection with *Toxoplasma gondii*: A case control study of Tehran, Iran

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The prevalence of antibodies in *Toxoplasma gondii* ranges from 24 to 57.7% in Iran. Children who acquire *T. gondii* in intrauterine period are at increased risk of sequels and need prolonged treatment. The aim of the study is to compare serum specific *T. gondii* antibodies (IgM and IgG ELISA) between infants suspected to have intrauterine infections (< 1year) and control infants. This case control study was done in the pediatrics department of Rasul hospital in Tehran (2007 to 2008). We compared specific *T. gondii* antibodies (IgM and IgG) in serum by ELISA in 50 infants (< 1year) suspected to have intrauterine infections based on diagnostic criteria for intrauterine infections (WHO-TORCH) and 30 healthy controls. Mean age in these cases was 4.7 + 3.7 months; and in the controls was 5.3 + 3.1 months. Acute *T. gondii* infection (IgM) was detected in 10% (5/51) of the cases, but none (0/30) in the controls; while previous immunity for *T. gondii* (IgG) was found in 18% (9/50) of the cases and 60% (18/30) of the controls. Although the rate of acute infection was higher in the cases but was not significant (P-value = 0.09), previous immunity (IgG) was significantly higher in the control's healthy group (P-value = 0.00). However, *T. gondii* infection (IgM) was confirmed in at least 10% of the cases. As such, we prefer to consider seropositive (*T. gondii* - IgM) infants (clinically for intrauterine infection) in congenital form, by adding the symptomatic cases with negative IgM and IgG (PCR studies are needed for R/o of intra uterine infection). Probably, *T. gondii* infection is at least the 2nd common cause of intrauterine infection in studied infants with serology (<1 year old), like cases with hearing loss (after CMV). Post natal screening program (serology) may be beneficial for rapid diagnosis, but negative symptomatic cases should be followed up by the PCR study. We recommend prevention and treatment of *T. gondii* in pregnant women for prevention of congenital toxoplasmosis. At least, 1 year treatment is needed in infants (positive IgM) for prevention of its sequels.

Key words: *Toxoplasma gondii*, congenital toxoplasmosis, ELISA (enzyme-linked immunosorbent assay), intrauterine infection, TORCH (*Toxoplasma gondii*, Rubella, Cytomegalovirus, Herpes).

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite. The cat is the only definitive host, but other animals can be infected incidentally. Humans can acquire infection by ingestion of raw or poorly cooked meat containing the *T. gondii* cysts or by ingestion of food or water contaminated with oocysts (Richard et al., 2006). Having knowledge of the prevalence of antibodies in women of childbearing age is important for the prevention of congenital toxoplasmosis (John et al., 2008). The prevalence of congenital *T. gondii* infection can be estimated from the incidence rate of *T. gondii* infection acquired during pregnancy by multiplying the figure for the number of mothers who acquire infection during pregnancy by the transmission rate of the parasite to the fetus (Richard et al., 2006). The prevalence of *T. gondii* risk factors and of previous infections varies from country-to-country (John et al., 2008), while congenital toxoplasmosis occurs almost exclusively as a result of primary maternal infection during pregnancy. Rarely, reactivation of infection in immune compromised women

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during pregnancy can result in congenital toxoplasmosis. Most maternal infections are asymptomatic or they result in mild illnesses (Kravetz and Federman, 2005; Freeman et al., 2005).

Congenital toxoplasmosis infection may be presented as a mild or severe neonatal disease, with onset during the first few months of life, or with sequels or relapse of a previously undiagnosed infection at any time during infancy, or sensorineural hearing loss (SNHL) later in life. The classic clinical presentation of congenital toxoplasmosis is the traid of hydrocephalus, choriore-tinitis and intracranial calcifications, but there is a wide spectrum of manifestation, and more than 75% infected newborns are asymptomatic and free of symptoms at birth; but if untreated, the infection will progress resulting in serious sequels such as intracranial calcifications, chorioretinits, hearing impairment and developmental delay (Freeman et al., 2005; Boyer et al., 2005). Passively transferred maternal IgG antibodies may require months or even a year to disappear from the infant serum. Specific IgM in congenitally infected infants will decrease between 6 months and 1 year. In infants less than 1 year of age, acquired toxoplasmosis is rare and nearly all infections are congenital (Freeman et al., 2005; Boyer et al., 2005; Lebas et al., 2004). The detection of a positive *T. gondii* IgG titre and a positive IgM indirect fluorescent antibody (IFA) or ELISA titre must be presumed to indicate recently acquired infection with *T. gondii* (Armstrong et al., 2004).

The ELISA is capable of detecting 85% of the cases of congenital toxoplasmosis infections in the first few days of life. The most important fact for the clinician is that patients with a positive IgG titre and a positive IgM IFA or ELISA titre must be presumed to have a recently acquired infection with *T. gondii* (Armstrong et al., 2004; Del Castillo, 2004). Prevention of congenital toxoplasmosis is needed by treatment of active *T. gondii* infection in pregnant women. In congenital infection, treatment in the first year of life is associated with diminished occurrence of this complication (Fleury et al., 2004; Boyer et al., 2006; Trenque et al., 2002).

However, prenatal diagnosis of congenital toxoplasmosis is validated by PCR in amniotic fluid against indirect fluorescent antibody assay in mothers. As such, analysis of amniotic fluid by polymerase chain reaction for prediction of congenital toxoplasmosis is useful (Neto et al., 2004; Labm et al., (1988); Thalib et al., 2005; Gharavi et al., 2008; Chabbert et al., 2004; Assmar et al., 2004). Labm et al. (1988) contributed a new PCR assay to the prenatal diagnosis of congenital toxoplasmosis (Labm et al., (1988), whereas *T. gondii* infection is endemic in Iran (18 to 20%). The incidence rate of *T. gondii* in pregnant women in different parts of Tehran is 24 to 34% (Noorbakhsh et al., 2002), which is close the rate of Pakistan and Bangladesh (21%), but higher than the rate of India (7.6%), and more frequent than the rates of United States (0.27%) and France (0.3%) (Richard et al., 2006; John et al., 2008). Moreover, *T. gondii* had some roles in cases with ophthalmic and hearing loss in Iran (Noorbakhsh et al., 2005; Saki et al., 2007; Noorbakhsh et al., 2008). The seroconversion rate in pregnant women in Iran is 71 per 1000, so the higher intra uterine infection is expectable (Noorbakhsh et al., 2002). On the other hand, many intrauterine infected cases are diagnosed in Iran annually (Noorbakhsh et al., 2005).

Aim of the study

The study aims at comparing serum specific *T. gondii* antibodies (IgM and IgG) between infants suspected for intrauterine infections (< 1year) and control infants in the pediatrics departments of Rasul hospital in Tehran (2007 to 2008).

METHODS

This case control study was carried out in the pediatrics department of Rasul Hospital in Tehran (2007 to 2008). Our center is a tertiary care general hospital with 500 active beds. The study's group consists of 51 cases and 30 healthy age matched infants (control). All case and control groups aged less than 1 year old.

The diagnostic parameters were based on WHO criteria for intrauterine infection (congenital rubella syndrome), including the presence of 2 major criteria or 1 major plus 2 minor criteria.

Major criteria

Cataract/glaucoma egg/congenital heart disease, sensory hearing loss and pigmented retinopathy.

Minor criteria

Purpura, splenomegaly, microcephaly, mental retardation, meningoencephalits, radiolucent osteal disease and icter in the first day of birth. This study was approved by the "ethical committee" in Iran Medical University.

Our control group consists of infants who were hospitalized for elective surgery (that is, undescendent testis, hernia, orthopedic, etc). These infants were age matched with our intrauterine infections' (intra uterine infection) group. They all underwent appropriate physical exams for health by expert pediatricians before surgery. We used their extra blood (which was taken for routine blood tests before their surgery) for the serologic tests.

A comparison was done on the serum specific *T. gondii* antibodies (IgM and IgG) between the 51 cases (mean age = 4.7 months + 3.7 month) and the 30 controls (mean age = 5.3+3.1 months). Initially, a questionnaire was completed by an authorized physician for each of the cases and controls, followed by a complete clinical exam. Blood samples (2 ml) of each child were centrifuged and transferred to our research laboratory. The serum was restored in -20°C temperature freezer until the serologic examination was performed. The centrifuge blood specimens were screened using an ELISA assay for *T. gondii* IgM and IgG

Table 1. Serologic results in cases and control.

Positive -Toxo IgG (%)	Positive -Toxo IgM (%)	Age (Mean+SD)	
18 (9/50)	10 (5/51)	4.7 + 3.7 months	Cases
60 (18/30)	none (0/30)	5.3 + 3.1 months	Controls

P value < 0.05 is considered significant.

Table 2. Correlation of age and serologic results in cases.

P-value	Mean age	Serologic results
0.5	4.7 months	Positive -Toxo IgM
--	5.8 months	Negative -Toxo IgM
0.9	5.7 months	Positive -Toxo IgG
--	5.8 months	Negative -Toxo IgG

P value < 0.05 is considered significant.

antibodies.

Serological test

The evaluation of specific *T. gondii* IgM and IgG antibodies were carried out with commercial kits (Biochem; Germany). The results were calculated qualitativelyand interpreted as suggested by the manufacturer.

Statistical analysis

The Student's t test was used to determine significant differences in means for all continuous variables. Chi square values (CI 95%, $p<0.05$) were calculated for all categorical variables. All analyses were conducted using SPSS11.5 software.

RESULTS

The age range of cases (missing = 4) was 1 to 12 months, while the mean was 4.7 + 3.7 months. About 47.2% of the patients were male, while 52.8% were female. The age range of the controls was 1 to 12 months, while the mean was 5.3+3.1 months. Acute *T. gondii* infection (IgM) was detected in 10% (5/51) of the cases, while previous immunity (IgG) was detected in 18% (9/50) of the cases. None (0/30) of the controls had acute *T. gondii* infection (IgM), but 60% (18/30) of the controls had previous immunity (IgG) against *T. gondii*. Acute *T. gondii* infection was higher in cases but not significant (P value= 0.09), while previous immunity (IgG) was significantly higher in controls (P value = 0.000). Serologic results in controls detected acute infection (IgM) in none (0/30) of the control cases, but detected previous immunity (IgG) in 60% (18/30) of the control cases (Table 1). Although, the rate of acute infection was higher in cases, it was not significant in cases (P value = 0.09).

Previous immunity (IgG) was significantly higher in the control healthy group (P value = 0.000), but acute infection was higher in the cases. Mean age of cases with acute *T. gondii* infection (positive IgM) was 4.7 months when compared with 5.8 months in cases without acute *T. gondii* infection. Mean age of cases with previous immunity (positive- IgG) was 5.7 months in comparison with 5.8 months in cases without immunity. Consequently, mean age was not different between the 2 groups (P value: 0.5; 0.9) (Table 2).

DISCUSSION

We observed active (recent) *T. gondii* infection (positive IgM) in 10% (5/51) of the cases (mean age is 4.7 months), but in none of the controls. Children with acute infection (4.7 months) cases were younger than children without acute infection (5.8 months) cases. Previous immunity (IgG), detected in 18% (9/50) of our cases is much lower than 60% in normal infants; and this might be due to the previous immunity of the mother which was transferred from her placenta. As such, some studied cases with negative IgM and IgG (previous immunity) might had been infected (intra uterine) without serologic responses. PCR studies are required for confirmation of infection in negative cases. In infants, less than 1 year of age, acquired toxoplasmosis is rare and nearly all infections are congenital. Usually, infants (= < 1 years) with a positive IgG titre and a positive IgM or ELISA titre must be presumed to have a recently acquired infection with *T. gondii* (John et al., 2008; Trenque et al., 2002; Freeman et al., 2005). So, 10% of the studied cases had active *T. gondii* infection before 5 months. Specific *T. gondii* IgM in congenitally infected infants will decrease between 6 months and 1 year. Probably, after 5 months,

it disappeared and was replaced by IgG. On the other hand, 85% of congenital toxoplasmosis in the first few days of life is detectable by ELISA test. However, 25% of the studied infants, with *T. gondii* infection may not have detectable IgM at birth. Theoretically, some studied cases might have had high, but insidiously decreasing titre of IgM in the time of the study.

Previous immunity (IgG) was significantly higher in normal infants (60 vs 18%; Pvalue < 0.001), while 82% of the studied cases suspected had intrauterine infection and no protection due to absence of transplacental transferring protective IgG antibodies. The mean age of cases with previous immunity was not different from cases without immunity (5.7 vs 5.8 months). Passively, transferred maternal IgG antibodies may require many months or even a year to disappear from the infant's serum. Probably, IgG in 60% of the healthy (control) group transferred from the mother was protective until 5.7 months. Seroprevalence of *T. gondii* in Iranian population is high, and the seroconversion rate in the population that are pregnant is estimated as 60 to 71 per 1000 (Noorbakhsh et al., 2002; Abdi et al., 2008). On the other hand, many cases are diagnosed as intrauterine infection (TORCH cases) annually (Noorbakhsh et al., 2005, 2008; Saki et al., 2007; Noorbakhsh et al., 2008). At least, 1 study in Iran reported ophthalmic disorders due to *T. gondii* infection in children (Saki et al., 2007). Results from the present study are almost similar to the results of the previous study in our center's SNHL cases (Noorbakhsh et al., 2008). Previous immunity (*T. gondii* - IgG) in normal infants was higher than SNHL cases (48 vs 21%; Pvalue < 0.001), while acute *T. gondii* infection (IgM) was detected in none of the controls when compared with 12% of the SNHL cases. However, children with *T. gondii* infected cases in SNHL study were older (50 vs 4.7 months) (Noorbakhsh et al., 2008).

The present study re-emphasizes the importance of intrauterine *T. gondii* infections in many disorders in Iranian children. So, prevention of congenital toxoplasmosis is needed by treatment of active *T. gondii* infection in pregnant women. Risk factors for infection in mothers of infants with congenital toxoplasmosis are defined by Boyer et al. (2005). Treatment of congenital toxoplasmosis in the first year of life could prevent this late sequel (Kravetz and Federman, 2005; Boyer et al., 2006). Treatment of intrauterine *T. gondii* infection after birth is important to minimise the risk of sequels in children (Thalib et al., 2005). About 10 to 17% of infants with congenital toxoplasmosis developed sensory neural hearing loss (SNHL) at the age of 4 months or later (Kravetz and Federman, 2005). Fundamentally, the outcome of treatment for congenital toxoplasmosis was good (Fleury et al., 2004; Boyer et al., 2006), in that the clinical evolution of ocular lesions and final visual function was found in a prospective cohort of congenitally infected children who were identified during monthly maternal prenatal screening. Late-onset retinal lesions and relapse could occur many years after birth, but the overall ocular prognosis of congenital toxoplasmosis is satisfactory when the infection is identified early and treated (Boyer et al., 2006).

If screening test has not been done for pregnant women and their infants with suspected clinical intrauterine infection, then positive Toxo- IgM (<1 year) treatment is necessary. In the presence of each clinical finding in neonates, even with negative serology, treatment is recommended, while in cases with positive IgG, strong possibility of *T. gondii* does not exist. As regards cases with negative results for both IgM and IgG, and cases whose clinical finding strongly considers toxoplasmosis, specific evaluation such as PCR is recommended. It is preferred that seropositive (IgM) in infants should be considered (highly suspicious for intra uterine infection) as a congenital form. In seropositive children, after the first year of birth, the differentiation between congenital infection and acquired infection is very hard. The low predictive value of a positive screening test in populations in which *T. gondii* infection is rare, could result in unnecessary invasive fetal testing or pregnancy termination because of false-positive tests. Prenatal screening could be more easily justified in low incidence populations if the detection and treatment of mothers infected during pregnancy led to lower rates of transmission to the newborn (Freeman et al., 2005; Lebas et al., 2004; Del Castillo, 2004). Boyer et al. identified the risk factors for infection in mothers of infants with congenital toxoplasmosis. They concluded that only systematic serologic screening of all pregnant women at prenatal visits, or of all newborn infants at birth, would prevent or detect a higher proportion of these congenital infections (Boyer et al., 2005). Screening for primary infection with *T. gondii* during pregnancy is not cost-effective in populations with a very low incidence of toxoplasmosis (Kravetz and Federman, 2005, Freeman et al., 2005; Lebas et al., 2004), but neonatal screening in an area with low sero prevalence of *T. gondii* is used to detect 75% of the infected infants born to untreated mothers (Freeman et al., 2005).

Recently, the United Kingdom National Screening Committee reviewed the evidence for prenatal and neonatal screening in support of toxoplasma infection. They concluded that there was insufficient evidence to recommend screening in the United Kingdom (Labm et al., (1988). Seroprevalence of *T. gondii* antibodies in Iranian population are high; so screening program would be useful in pregnant women in our country (Assmar et al., 2004; Hamzavi et al., 2007; Noorbakhsh et al., 2002; Abdi et al., 2008). In pregnant women, without protective antibody, it is better to screen at least once in the first trimester and then twice until the end of the pregnancy. In recent times, infected pregnant women or cases with seroconversion, cord blood evaluation for *T. gondii* antibody is necessary. However, neonatal screening could diagnose infected infants born to untreated mothers

rapidly. In contrast to prenatal screening, newborn screening is relatively inexpensive and efficient (Richard et al., 2006), while year treatment is needed in infants suspected to have intrauterine *T. gondii* infection (positive IgM).

Conclusion

T. gondii infection (IgM) was confirmed in at least 10% of our cases, because we preferred to consider seropositive (*T. gondii*- IgM) infants (clinically for intrauterine infection) in congenital forms by adding the symptomatic cases with the negative IgM and IgG (PCR studies are needed for R/o of intra uterine infection). Probably, *T. gondii* infection is at least the 2nd common cause of intrauterine infection in studied infants with serology (<1 year old) like cases with hearing loss (after CMV). Post natal screening program (serology) may be beneficial for rapid diagnosis, but negative symptomatic cases should be followed up by the PCR study. Conclusively, we recommend prevention and treatment of *T. gondii* in pregnant women for prevention of congenital toxoplasmosis. As such, at least 1 year treatment is needed in infants (positive IgM) for prevention of its sequels.

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