

# Congenital Infection with *Toxoplasma gondii*: A Case Control Study in Tehran, Iran

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## Abstract

**Background:** The prevalence of antibodies to *T. gondii* ranges from 24% to 57.7% in Iran.

**Objective:** To compare serum specific *T. gondii* antibodies (IgM & IgG ELISA) between infants suspected for intrauterine infections (< 1year) and control infants.

**Material & Methods:** This case control study done in pediatrics Departement of Rasul hospital in Tehran (2007-2008). We compared specific *T. gondii* antibodies (IgM & IgG) in serum by ELISA in 50 infants (< 1year) suspected for intrauterine infections based on diagnostic criteria for intrauterine infections (WHO-TORCH) and 30 healthy controls.

**Results:** Mean age in cases was 4.7 months + 3.7 month; and in controls was 5.3+3.1 months. Acute *T. gondii* infection (IgM) detected in 10% (5/51) but none (0/30) of controls; previous immunity for *T. gondii* (IgG) found in 18% (9/50) cases and 60% (18/30) of controls. Although the rate of acute infection was higher in the cases but was not significant (P value = 0.09), but previous immunity (IgG) was significantly higher in the control healthy group (P value =0.00)

**Conclusion:** *T. gondii* infection (IgM) confirmed in at least 10% of our cases. We prefer to consider seropositive (*T. gondii*- IgM) infants which clinically suspects for intrauterine infection, as congenital form. Post natal screening program (serology) may be beneficial for rapid diagnosis but negative symptomatic cases should be followed up by 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.

**Keywords:** *T. gondii*; Congenital toxoplasmosis; ELISA (Enzyme-linked immunosorbent assay); Intrauterine infection; TORCH (*T. gondii*, Rubella, Cytomegalovirus, Herpes)

## Introduction

*T. gondii* is an obligate intracellular protozoan parasite. The cat is the only definitive host, but other animals can be infected incidentally. Human 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 [1]. Having knowledge of the prevalence of antibodies in women of childbearing age is important for the prevention of congenital toxoplasmosis [2].

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 [1]. The prevalence of *T. gondii* risk factors and of previous infections varies from country-to-country [2]. Congenital toxoplasmosis occurs almost exclusively as a result of primary maternal infection during pregnancy. Rarely reactivation of infection in immune compromised woman during pregnancy can result in congenital toxoplasmosis. Most maternal infections are asymptomatic or result in mild illnesses [3,4].

Congenital toxoplasmosis infection may present 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 later in life; Sensorineural Hearing Loss (SNHL) [4]. The classic clinical presentation of congenital toxoplasmosis is the triad of hydrocephalus, chorioretinitis, 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,

and if untreated, the infection will progress resulting in serious sequels such as intracranial calcifications, chorioretinitis, hearing impairment and developmental delay.

Passively transferred maternal IgG antibodies may require months-to-a-year to disappear from the infant's 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 [4-6]. 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* [7].

The ELISA is capable of detection 85% of cases of congenital toxoplasmosis infections in the first few days of life. 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* [7,8].

Prevention of congenital toxoplasmosis is needed by treatment of active *T. gondii* infection in pregnant women. In congenital infection;

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treatment in the first year of life is associated with diminished occurrence of this complication [9-11].

Prenatal diagnosis of Congenital Toxoplasmosis validated by PCR in amniotic fluid against Indirect Fluorescent Antibody Assay in mothers. Analysis of amniotic fluid by polymerase chain reaction for prediction of congenital toxoplasmosis is useful [12-17]. Labm et al. [13] contributed a New PCR assay to the prenatal diagnosis of congenital toxoplasmosis. *T. gondii* infection is endemic in Iran [18-20].

The incidence rate of *T. gondii* in pregnant women in different parts of Tehran is 24-34%.19 which is near to Pakistan and Bangladesh (21%), but is higher than in India (7.6%), and more frequent than United States (0.27%) and France ( 0.3%) [1,2].

*T. gondii* had some roles in cases with ophthalmic and hearing loss, etc in Iran [21-24]. The seroconversion rate in the pregnant women in Iran is 71 per 1000 so the higher intra uterine infection is expectable 19. In the other hand annually many intrauterine infected cases diagnose in Iran [21].

### Goal of study

To compare serum specific *T. gondii* antibodies (IgM & IgG) between infants suspected for intrauterine infections (< 1year) and control infants in the pediatrics departments of Rasul hospital in Tehran (2007-2008).

### Methods

This case control study was carried out in the pediatrics Department of Rasul Hospital in Tehran (2007-2008). Our center is a tertiary care general hospital with 500 active beds. Our study group consists of 51 cases and 30 health age matched infants (control). All case and control groups aged less than 1 years old. Diagnostic parameters 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 eg/ congenital heart disease, Sensory hearing loss, pigmented retinopathy.

Minor criteria: purpura, splenomegaly, microcephaly, mental retardation, meningo encephalitis, radiolucent osteal disease, icter in first day of birth

This study was approved by the Ethical Committee in the Iran Medical University.

Our control group consists of infants who were hospitalized for elective surgery (i.e. undescendent testies, 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.

We compared serum specific *T. gondii* antibodies (IgM & IgG) between 51cases (mean age = 4.7 months + 3.7 month), and 30 controls (mean age = 5.3 ± 3.1 months). Initially a questionnaire was completed by an authorized physician for each case and control, 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 antibodies.

### Serological test

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

### 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 -12 months, Mean = 4.7 ± 3.7 months. 47.2% of patients were male; 52.8% female. The age range of the controls was 1 -12 months, Mean = 5.3 ± 3.1 months.

Acute *T. gondii* infection (IgM) detected in 10% (5/51); previous immunity (IgG) in 18% (9/50) of cases. None (0/30) of controls had acute *T. gondii* infection (IgM); 60% (18/30) of controls had previous immunity (IgG) against *T. gondii*. Acute *T. gondii* infection was higher in cases but not significant (P value = 0.09) but 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 them ,previous immunity (IgG) in 60% (18/30 ) of them (Table 1).

Although the rate of acute infection was higher in cases but not significant (P value = 0.09). Previous immunity (IgG) was significantly higher in the control healthy group (P value = 0.000). Acute infection was higher in the cases. Mean age of cases with acute *T. gondii* infection (positive IgM) was 4.7 months in compare with 5.8 month in cases without acute *T. gondii* infection. Mean age of cases with previous immunity (positive- IgG) was 5.7 months in compare with 5.8 month in cases without immunity .Mean age was not different between 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 cases (mean age 4.7 months) but in none of controls. Cases with acute infection (4.7 months) was younger than cases without acute infection (5.8months).Previous immunity (IgG) detected in 18% (9/50) of our cases is very lower than 60% in normal infants; it might be due to previous immunity of mother which transferred from placenta.

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

P value<0.05 considered significant

**Table 1:** "Serologic results in cases and control".

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

P value<0.05 condiered significant

**Table 2:** "Correlation of age and serologic results in cases"

Some studied cases with negative IgM & IgG (previous immunity) might have 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* [2,4,11].

So 10% of studied cases had active *T. gondii* infection before 5 month. Specific *T. gondii* IgM in congenitally infected infants will decrease between 6 months and 1 year. Probably after 5 month it disappeared and replaced by IgG. In the other hand, 85% of congenital toxoplasmosis in the first few days of life is detectable by ELISA test. 25% of the studied infants, with *T. gondii* infection may didn't have detectable IgM at the birth. Theoretically, some studied cases might had high but insidiously decreasing titre of IgM in time of study.

Previous immunity (IgG) was significantly higher in normal infants (60% vs 18%; P value  $< 0.001$ ). 82% of studied cases suspected had intrauterine infection had not protection due to no transplacental transferring protective IgG antibodies. 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-to-a-year to disappear from the infant's serum. Probably, IgG in 60% healthy (control) group transferred from mother and was protective until 5.7 months. Seroprevalence of *T. gondii* in Iranian population is high. The seroconversion rate in the pregnant population estimated 60-71 per 1000 [19,20]. In the other hand annually many cases diagnosed as intrauterine infection (TORCH cases) [21-24]. At least 1 study in Iran reported ophthalmic disorders due to *T. gondii* infection in children [22]. Results from present study is very close to previous study in SNHL cases in our center [24].

Previous immunity (*T. gondii* -IgG) in normal infants was higher than SNHL cases (48% vs 21%; Pvalue  $< 0.001$ ). Acute *T. gondii* infection (IgM) detected in none controls in compare with 12% of SNHL cases. *T. gondii* infected cases in SNHL study was older (50 month vs 4.7 months) [24].

Present study re-emphasize 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 defined by Boyer et al. [5,10].

Treatment of congenital toxoplasmosis in the first year of life could prevent this late sequel [3,10]. Treatment of intrauterine *T. gondii* infection after birth is important for minimize the risk of sequels in children [14]. 10-17% of infants with congenital toxoplasmosis developed Sensory Neural Hearing Loss (SNHL) at the age 4 month or later [3]. Outcome of treatment for congenital toxoplasmosis is good [9,10]. The clinical evolution of ocular lesions and final visual function 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 that the overall ocular prognosis of congenital toxoplasmosis is satisfactory when infection is identified early and treated [10].

If screening test has not done in pregnant woman, in their infants with suspected clinical intrauterine infection and positive Toxo- IgM ( $< 1$  year) treatment is necessary. In the presence of each clinical finding in neonates even with negative serology, treatment is recommended. In the cases with positive IgG, with strong possibility *T. gondii* does

not exist. Cases with negative results for both IgM and IgG but clinical finding strongly consider toxoplasmosis, specific evaluation such as PCR is recommended.

It is preferred to consider the seropositive (IgM) infants (highly suspect for intra uterine infection) as congenital form. In seropositive children after first year of birth, the differentiation between congenital from 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 [4,7,8].

Boyer et al. [5] 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.

Screening for primary infection with *T. gondii* during pregnancy is not cost-effective in populations with a very low incidence of toxoplasmosis [4,6-8] but neonatal screening in an area with a low sero prevalence of *T. gondii* to detect 75% of infected infants born to untreated mothers [4].

Recently, the United Kingdom National Screening Committee reviewed the evidence for prenatal and neonatal screening for toxoplasma infection. They concluded that there was insufficient evidence to recommend screening in the United Kingdom [13].

Seroprevalence of *T. gondii* antibodies in Iranian population are high. So screening program would be useful in pregnant women in our country [17-20]. In pregnant women without protective antibody, it is better to screen at least 1 time in the first trimester and then two times until to the end of pregnancy. In recently infected pregnant woman or in cases with seroconversion, cord blood evaluation for *T. gondii* antibody is necessary. Neonatal screening could diagnose infected infants born to untreated mothers rapidly. In contrast to prenatal screening, newborn screening is relatively inexpensive and efficient. 1 year treatment is needed in infants suspected for intrauterine *T. gondii* infection (positive IgM).

## Conclusion

*T. gondii* infection (IgM) was confirmed in at least 10% of our cases. We prefer to consider seropositive (*T. gondii*- IgM) infants (clinically for intrauterine infection) as congenital form. With adding the symptomatic cases with negative IgM & IgG (PCR studies is needed for R/o of intra uterine infection) probably *T. gondii* infection is at least 2th common cause of intrauterine infection in studied infants with serology ( $< 1$  years old) like as cases with hearing loss (after CMV). Post natal screening program (serology) may be beneficial for rapid diagnosis but negative symptomatic cases should follow up by 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.

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