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Toxoplasmosis: Seroprevalence in pregnant women, and serological and molecular screening in neonatal umbilical cord blood

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ABSTRACT

Toxoplasmosis is a common zoonotic disease that can also be transmitted from the mother to the embryo, with the risk of congenital infection varying around the world. The aim of this study was to screen pregnant women and their neonates for toxoplasmosis by serologic and molecular methods and assess the impact of risk factors associated with toxoplasmosis on the rate of congenital infection.

This study was conducted at a regional maternity hospital in Arak, the capital of the Markazi Province in Iran, during a period of six months. All selected pregnant women (n = 261) and the corresponding cord blood samples were serologically screened for toxoplasmosis, with seropositive samples also undergoing molecular testing. Demographic data, as well as information related to the risk factors associated with the transmission of the disease, were collected from mothers and their neonates. The detection of anti-*Toxoplasma* antibodies and the extraction of DNA from blood samples were conducted using commercial kits. Results showed that the sera of 87 maternal blood samples (33.3%) and 40 cord blood samples (15.3%) were positive for anti-Toxoplasma antibodies (IgG and/or IgM). Molecular screening of the seropositive samples only identified one positive cord blood sample. In other words, the diagnosis of congenital toxoplasmosis was definitive in only one neonate. There was no significant association between the risk of parasite transmission and neonatal seropositivity ($p > 0.05$). Therefore, the results showed that the prevalence of congenital toxoplasmosis in the studied area was consistent with the global rate and suggest that the implementation of newborn screening and follow-up testing could help reduce the disease risk.

1. Introduction

Toxoplasmosis is a global chronic infection that, according to seroepidemiological studies performed on various populations, infects about 15–85% of the total human population (Pappas et al., 2009). It is a zoonotic disease that is caused by the intracellular parasite *Toxoplasma gondii*. Infection by *T. gondii* has an acute and chronic phase. In immunocompetent people, the acute phase is often asymptomatic. However, congenital transmission occurs during this phase (Dubey et al., 2012). In other words, if infection occurs during pregnancy, the parasite will get a chance to cross the placenta and infect the fetus. When this infection occurs during the first and second trimester of pregnancy, it causes severe symptoms that are detectable at birth. On the other hand, the majority of fetuses that are infected in the third trimester of pregnancy do not display symptoms at birth, but later develop visual impairment or developmental disorders. Therefore, newborns born to mothers diagnosed with acute toxoplasmosis should be examined at the time of birth (Campello Porto and Duarte, 2012). However, definitive diagnosis of congenital toxoplasmosis (CT) in newborns is difficult because it requires the combination of clinical symptom evaluation with serological and molecular testing (Sterkers et al., 2012).

A previous study evaluated the rate of toxoplasmosis in Arak pregnant women but provided no information regarding the effects of the disease on newborns and the rate of CT (Vakil et al., 2014). Thus, the goal of the current study was to perform a cross-sectional screening of toxoplasmosis in pregnant women and their neonates by serological and molecular diagnostic methods and assess the impact of risk factors associated with toxoplasmosis on the rate of CT.

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Fig. 1. The area of study. Map of Iran, Markazi province marked in grey color. The Arak city is marked. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Methods

2.1. Sampling

This cross-sectional study was performed at a maternity hospital of Arak, in Markazi province of Iran, during a six months period (from April to September 2015) (Fig. 1). Informed written consents was obtained from each subject prior to the study. Questionnaires were used to extract demographic data and identify possible risk factors. Blood samples (3 ml) were collected from the women before childbirth and from the umbilical cord after childbirth, using disposable sterile syringes under aseptic conditions (Fig. 2). The sera were separated by centrifugation at room temperature. The sera were separated by centrifugation at room temperature and stored at −20 °C for future use. Blood cells were also collected and stored in separate tubes.

2.2. Serologic assay

Sera were tested for anti-*Toxoplasma* IgG and IgM antibodies using a commercial ELISA kit (Pishtaz Teb Zaman Diagnostics Co., Tehran, Iran) according to the manufacturer's instructions.

2.3. Molecular assay

Seropositive blood samples underwent further molecular analysis using the polymerase chain reaction (PCR). First, blood DNA was extracted with the DNG-PlusTM extraction solution (CinnaGen Co., Tehran, Iran) and confirmed by electrophoresis on a 0.8% agarose gel.

PCR was performed with the standard TOX4 (5′-CGCTGCAGGGAGGAAGACGAAAGTTG-3′) and TOX5 (5′-CGCTGCAGACACAGTGCATCTGGATT-3′) primers, which amplify the REP-529 sequence of the *T. gondii* genome (Homan et al., 2000; Robert-Gangneux and Darde, 2012). Amplification was performed with the Master-Mix Kit (CinnaGen Co.) on an Eppendorf thermocycler (Hamburg, Germany), using the following optimized conditions: An initial denaturation step of 5 min at 94 °C; 35 cycles of 30 min at 94 °C, 30 min at 58 °C, and 30 min at 72 °C, and; a final extension step of 10 min at 72 °C. Reaction products were analyzed by electrophoresis on a 1% agarose gel. Isolated *T. gondii* DNA was used as a positive control and distilled water as a negative control.

2.4. Ethics statement

This study was approved by Ethical Committee of Arak University of Medical Sciences. The ethics committee approval number is: IR.ARAKMU.REC.1394.316. A written informed consent was obtained from all the individuals under study.

Analyses were performed according to the Biosafety Laboratory Experimental and Molecular Pathology protocol.

2.5. Statistical analysis

Statistical analysis was performed by using SPSS software version 16 (SPSS/PC Inc., Chicago, IL, USA). The association between risk factors of *Toxoplasma* transmission was assessed by logistic regression analysis. Quantitative data was presented as mean ± standard deviation and *chi*-square test was used for comparing the proportions of these variables with $p \leq 0.05$ regarded as statistically significant.

3. Results

Table 1 shows various demographic characteristics of the pregnant women included in this study. The majority were in the age range of 21–30 years, lived in urban areas, and had completed college. Table 2 shows the profile of neonates under study. Their majority (63.7%) were born at 39–40 gestational weeks (i.e., full term) and 68.6% of them weighed 3000–4000 g.

Blood samples from all selected pregnant women and cord blood samples underwent serological screening for toxoplasmosis. The results showed that sera from 87 maternal blood samples (33.3%) and 40 cord blood samples (15.3%) were positive for IgG and/or IgM anti-*Toxoplasma* antibodies (Fig. 3). The molecular screening of the seropositive samples gave a positive result only in one cord blood sample (Table 3 and Fig. 4).

Logistic regression analysis revealed that *Toxoplasma* prevalence was not significantly associated ($p > 0.05$) with any of the demographic parameters of pregnant women (Table 1). In addition, there was no significant association between the risk of parasite transmission and neonatal seropositivity ($p > 0.05$) (Table 4).