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Prenatal Diagnosis of Congenital Toxoplasmosis: Validity of PCR Using Amniotic Fluid against Indirect Fluorescent Antibody Assay in Mothers

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Abstract

Maternal infection with *Toxoplasma gondii* acquired during pregnancy may result in congenital infection of developing fetuses. Up to now, decision on informed therapeutic abortion of fetuses suspected of having *T. gondii* infection in Iran has been made based on serological findings in mothers. This might have led to unnecessary abortion of many uninfected children. We evaluated the Polymerase Chain Reaction (PCR) assay on amniotic fluids against serological findings in mothers. PCR results in this study indicated that only about one third of Indirect Fluorescent Antibody (IFA) positive mothers had passed the infection to their children. It was a sigh of relief for the majority of mothers who learned that their infants were uninfected. On the other hand it helped the mothers of infected fetuses out of an awkward predicament by making them able to make a solid decision to terminate the pregnancy or carry their children to term.

Keywords: Toxoplasma gondii*, Congenital toxoplasmosis, Prenatal diagnosis, PCR, IFA, Iran.*

Introduction

Acute *T. gondii* infection at early pregnancy in women without a history of infection may lead to fetal death in the uterus or sever neurological damage (1). Transplacental infection of the fetus occurs in 12% of cases in which the mothers acquire infection during the first trimester. The incidence of transmission increases thereafter to more than 90% when maternal infection occurs during the last weeks before delivery, which is more likely to be asymptomatic, yet may proceed to choreoretinitis later in childhood or in adolescence (1, 2). Effective prenatal diagnosis of congenital toxoplasmosis can permit a decision to terminate the pregnancy at the early stage or initiate the treatment of late – term fetus in uterus. Prenatal diagnosis is commonly performed based on biological and serological tests on fetal blood and amniotic fluid, and ultrasonographic examination of fetus (3, 4). However, at present the only biological method employed here in Iran is isolation of parasite by mouse inoculation of infected materials, e.g. amniotic fluid. This method is confined to a few referral laboratories commonly affiliated to research centers making it unavailable to most of pregnant women seeking a diagnosis. Besides, it can take up to 3-6 weeks to make a diagnosis (5). Consequently, most of the therapeutic abortions or treatment of fetuses in uterus are merely performed based on serological findings in mothers, which may lead to unnecessary abortion of many uninfected fetuses. Polymerase Chain Reaction (PCR) using a fragment within the 35-fold repetitive BI gene has already been introduced as a sensitive method for diagnosis of toxoplasmosis in a variety of different clinical specimens (6, 7). In this paper we present the validity of PCR on amniotic fluid in comparison with IFA method in mothers as a basis for making a decision on therapeutic abortion.

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Materials and Methods

Study group The study included a total of 200 pregnant women referred to the Dept. of Parasitology, Pasteur Institute of Iran, with suspected toxoplasmosis based on clinical findings. The sera from all women were screened for IgG and IgM using IFA. Serological follow up was performed twice a week in positive cases for three successive weeks and then the amniotic fluids from cases that turned positive for IgM or showed a rising IgG titer were selected for PCR.

Serology The sera were first detected for poly Ig (IgG and IgM) by IFA methods and then the positive cases were tested for presence of anti-*T.gondii* IgM by the same method. The antigen used in IFA assay was a product of Pasteur Institute of Iran, which was specified to have 2×10^7 parasite^{-ml}. The conjugate anti-human Ab was supplied by Boehringer Company.

PCR assay DNA extraction was performed with minor changes as described previously (8). Briefly, 4 µl of amniotic fluids were centrifuged at 4000 rpm for 1 min and then the recovered pellets were resuspended in 150 µl of lysis buffer (Applied Biosystem, USA) and 10 µl of a 20 mg/ml proteinase K solution. Suspensions were incubated at 55 ºC for 6 h and then gently extracted with a mixture of phenol /chloroform/ water and chloroform (Applied Biosystem, USA) successively. The DNA was precipitated by two volumes of cold ethanol and then the pellet was resuspended in 20µl of TE (10mM Tris HCl, pH 7.5 and 0.1 mM EDTA). One µl of DNA was used for amplification of BI gene. The reagents including the primers and conditions were those of Assmar et al. (8). Resolution of a band of about 160 bp in size was regarded to be positive.

Mouse inoculation The amniotic fluids were centrifuged at 2000 rpm for 10 min and then the pellets were resuspended in 3 ml normal saline. Amounts of 0.5 ml of each sample were injected interperitonealy into six mice. The mice were sacrificed after 14 days and peritoneal fluids were examined for tachyzoites.

Results

Of the 200 women screened for poly Ig by IFA, 49 cases were positive, among which, 11 cases showed an increasing IgG titer in three weeks follow up and 4 of them had specific anti-*T. gondii* IgM. The antibody titers of ≥ 1/400 for poly Ig and ≥ 1/20 for IgM were regarded positive. Table one detail the serological results. The amniotic fluids from the above 11 cases were tested by nested-PCR for toxoplasmosis and 4 of them turned positive (Fig.1). Three of PCR positive cases that had undergone amniocentesis at early or middle of second trimester, gave birth to healthy children. One of them had begun treatment with spiromycin soon after confirmation of infection, but we were not sure whether the other two were on medication or not. The serological follow-up testing of the infants showed no sign of active infection. The fourth case decided to terminate the pregnancy at the late second trimester. Only one of PCR positive samples was positive in mouse inoculation.

Table 1: Results of serological findings in mothers against *T. gondii* by Poly-Ig (IgG & IgM) IFA test. Significant titers were calculated to be ≥ 1/400 for Ploy-Ig and ≥ 1/20 for IgM antibodies

Type of Ig	Total cases	Negative cases	Seroconverted cases	Non-significant	Significan
Poly Ig (IgG, IgM)	200	49 (24%)	151 (75%)	102 (67%) Ab 1<: 400	49 (32%) Ab ≥1: 400
IgM	151	137 (90.7%)	14 (9.3%)	10 (71.4%) Ab <1:20	4 (28.6%) Ab≥1: 20

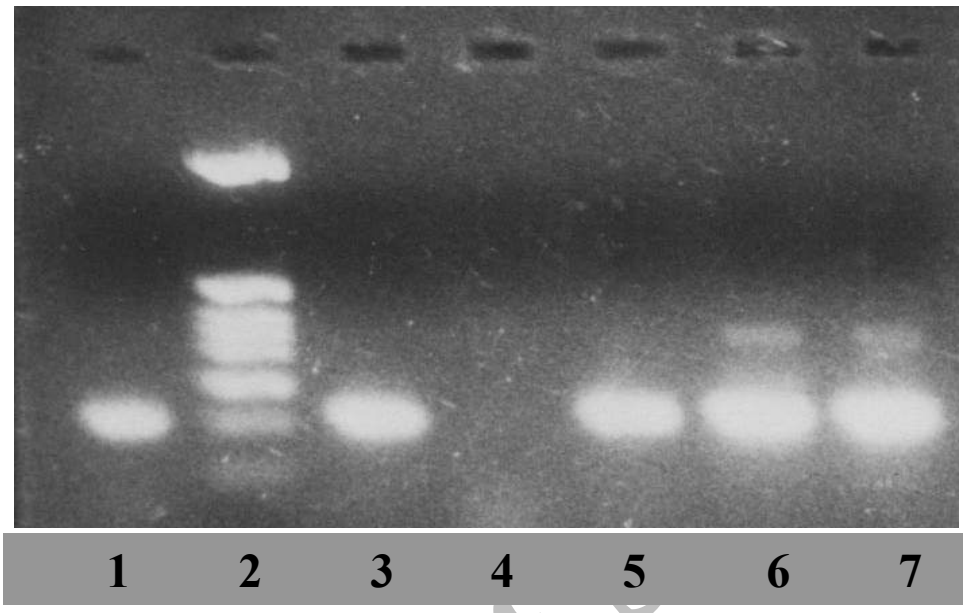

Fig.1: Nested PCR results of amniotic fluids samples from *T. gondii* infected patients. Lines 1, 5, 6, 7 positive specimens; 2, marker; 3, positive control and 4 negative control

Discussion

One third of mothers who acquire a primary *T.gondii* infection during pregnancy transmit the infection to their fetuses (5). Therefore, an antenatal diagnostic method should be employed to screen this fraction. This would help the mothers to make an informed decision on either treatment or therapeutic abortion. In Iran, up to present, most of the decisions on infected fetuses were made based on serological findings in their mothers, which might have led to abortion of many uninfected fetuses. The results obtained in this study present PCR as a reliable method for prenatal diagnosis of congenital toxoplasmosis.

As shown from 11 confirmed seroconverted mothers who either had an increasing IgG titer or IgM in their blood, only four of them transmitted the infection to their offspring. This finding put an emphasis on the issue that making a decision to terminate the pregnancy merely based on serological results in mothers can lead to unnecessarily abortion of many uninfected fetuses. Since PCR is applicable around week of 15 of gestation, when amniocentesis is possible, and would provide a diagnosis within 2 to 3 days of receiving a specimen (9), it would also provides mothers with enough time to begin treatment of infected fetuses in case they seek to carry their fetuses to term. Comparison of the results obtained from PCR and inoculation of laboratory animals, both using amniotic fluids, shows that PCR is more sensitive in detecting parasite. The low number of positive cases by mouse inoculation assay however might either be attributed to viability of *T. gondii* outside the host, which might be adversely affected, by poor transmission conditions or low burden of parasite.

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