ABSTRACT

The antepartum and peripartum maternal infections cause great problems complicating pregnancy. The early diagnosis of the maternal infections causing different pathologies in the newborns is of great importance. For an appropriate judgement, the early diagnosis of Toxoplasma gondii infections acquired during pregnancy is critical for an effective prevention. The common characteristics of these infections are that they may cause abortion, still birth, prematurity, intrauterin growth retardation, congenital malformations, infection of the newborn or normal term live-births. We report here the development of real-time PCR-based assay for detection of T. gondii. Investigation of 300 specimens was carried out using B1 gene region specific primers and probes after the extraction of T. gondii DNA. T.gondii DNA, was found in 4 (1.3%) out of 300 specimens. DNA was not found in the specimens of the remaining 296 patients. Real-time PCR analysis significantly improves the detection of T. gondii in amniotic fluid.

Materials and Methods

The ethics committee of Istanbul University, Faculty of Medicine approved the study design (protocol number 1532/2005).

Patient samples

During the past five years (2005-2010) we tested 300 amniotic fluid samples for detection of congenital T. gondii infections. Patients were selected randomly because we aimed to detect asymptomatic patients. Samples were obtained between the 15th and 18th pregnancy weeks. One mL amniotic fluid samples were collected from pregnant women at the Department of Gynecology, Cerrahpasa and Istanbul Medical Faculties, Istanbul, Turkey.

DNA extraction and real-time PCR quantification

One mL of amniotic fluid were centrifuged (20 min 6000 g) and DNA was extracted from 200 µL pellets with the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) according to the manufacturer’s instructions. In this study, the real-time RT-PCR amplification was carried out in a Stratagene Mx3005P.

Detection of T. gondii B1 gene by real-time quantitative PCR

The forward primer (5’-TGCATCCAACGAGTTTATAA-3’), reverse primer (5’-GGCATTCCTCGTTGAAGATT-3’) and TaqMan (FAM-ATTGCAATAATCTATCCCCATACGATGCATAC-BBQ) probe for real-time PCR amplification were designed with TIB Molbiol (Syntheselabor GmbH, Berlin, Germany) to specifically amplify the T. gondii B1 gene. The target DNA for real-time PCR amplification was
the published sequence of the 35-fold repetitive B1 gene of the 
*T. gondii* RH strain (4).

**Results and Discussion**

In order to determine sensitivity of the TaqMan assay, serial 10-fold dilutions ranging from $10^7$ to $10^3$ toxoplasmic cells per mL of human EDTA blood were prepared. EDTA blood samples without tachyzoites were used for negative controls and water control no-template control (NTC). The standard curve showed a linear range (Fig. 1, $R^2 = 0.968$). *T. gondii* DNA serial dilution limit was 500 to 1 tachyzoites. We were able to detect the B1 gene at a concentration as low as 1 tachyzoite ($C_T = 32.89$) and in a 50-µl reaction volume. The no-template control $C_T$ (NTC) was 40.00 and the 500 tachyzoites limit $C_T$ was 22.88. The number of parasites can be calculated from the $C_T$ values by using diluted *T.gondii* DNA standards.

![Fig. 1. Standard curve for quantification of *T. gondii*. Serial dilutions of *T. gondii* DNA ranging from $10^7$ to $10^3$ copies (500 to 1 tachyzoites). Log fit values: FAM standards (■), RSq: 0.968; FAM (line), $Y = -2.369 \times \log(X) + 39.31$, Eff. = 163.3%.

Investigation of a total of 300 specimens was carried out using B1 gene region specific primers and probes. After the extraction *T. gondii* DNA was found in 4 out of 300 specimens (Table 1).

**TABLE1**

Detection of *T. gondii* using quantitative real-time PCR with TaqMan probes. The table shows the CT value for positive results and negative results.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Real-time PCR $C_T$ value</th>
<th>Result of nested PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37.71</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>37.80</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>38.00</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>34.55</td>
<td>+</td>
</tr>
<tr>
<td>5-296</td>
<td>40</td>
<td>-</td>
</tr>
</tbody>
</table>

Molecular methods are now commonly applied for the diagnosis of infectious diseases (19). Various molecular biology methods have been extensively used in many clinical and research laboratories in order to detect various microorganisms (12). Real-time PCR is a recently invented method of PCR quantification, providing both quantitative measurement of increasing signals of fluorescence parallel to amplification of nucleic acid and chance of specific treatment (5). This method has a low false positive rate, decreasing the risk of contamination with rapid diagnosis with a single tube analysis (13).

Using strand specific probes for detection of infectious diseases, real-time PCR, which has become a preferred method in clinical laboratories, is also a method of choice in routine practice. For example, assessment of viral genome load is critically important both in the determination of the severity and the treatment of disease (3).

Several different types of real-time PCR allow rapid and reliable diagnosis of toxoplasmosis caused by *Toxoplasma gondii* (6, 7). Many studies have shown that diagnosis with real-time PCR is more reliable when the target sequence exists in multi copies compared to single copy (19). Our study was designed to provide clinicians with data concerning the accuracy of real-time PCR analysis on amniotic fluid for prediction of congenital toxoplasmosis.

The method that we used in this study is TaqMan® real-time PCR which aims to amplify *T. gondii* B1 gene based on fluorescence dying. We chose a target sequence with multi copies because our primary aim in this study was the correct detection of parasite without non-specific couplings in test and false positivity (11). *T. gondii* B1 gene is a target location with multi copies that allows reliable diagnosis of toxoplasmosis. Compared to other methods, real-time-PCR is a more rapid and specific diagnostic method. The specificity rate of real-time PCR for the diagnosis of toxoplasmosis has been reported to be between 94% and 100% (1). Various publications reported methods for obtaining *T. gondii* DNA from amniotic fluid and protocols for RT PCR (10, 17, 18). In our study, real-time PCR was positive in 4 out of 300 fetuses. Quantitation of the positive cases was done by comparing with the standard curve. Two of the real-time PCR positive fetuses were missed in utero without confirmation of the diagnosis. Two fetuses diagnosed at the third trimester were serologically and clinically normal after birth. Due to false positivity and negativity of the test, appropriate prenatal counselling is of critical importance. The results showed that low concentrations of *T.gondii* DNA could be detected more sensitively and accurately by real-time PCR using the B1 gene.

**Acknowledgements**

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**REFERENCES**


