PCR VERSUS ELISA IN DIAGNOSIS OF HUMAN TOXOPLASMOSIS IN JEDDAH, SAUDI ARABIA

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Abstract

In this study, polymerase chain reaction (PCR) was compared with detection of specific antibodies to Toxoplasma by ELISA. A total of 70 blood samples from patients with different clinical features were tested, 43/70 (61.4%) were serologically Toxoplasma-IgG positive meanwhile only 16/43 (37.2%) were approved to be positive by PCR. A total of 22 samples (31.6%) were positive by PCR, but only 16 of them were positive by ELISA. So, Tox-IgG indicated catching Toxoplasma infection but not enough with immune modulation to determine acute case, while PCR besides, being valuable in diagnosis showed Toxoplasma parasitemia.
Keywords: Saudi Arabia, Toxoplasma, PCR, IgG-ELISA

Introduction

Toxoplasmosis is a worldwide zoonotic intracellular parasite, T. gondii (Zuber and Jacquier, 1995). Adult-acquired infection was usually mild or asymptomatic (Franzen et al., 1997) but severe in the immunocompromised and children (Wishahy et al., 1971), particularly congenital ones (Abdallah et al., 1994) due to latent or active infection of the mother (Roberts et al., 2001). Primary maternal infection during pregnancy was frequently associated with transmission of T. gondii from mother to fetus (Remington et al., 1995). Transplacental transmission from an infected pregnant to her fetus caused severe complications up to fetal death (Kopecky et al., 2003). Routine serologic diagnosis of toxoplasmosis provided high sensitivity, but specificity varied depending on the test used and false-positive IgM anti-
body results (Hofgärtner et al., 1997). But, even true positive results must be carefully interpreted (Liesenfeld et al., 1997) as IgM antibodies might persist for one year after toxoplasmosis treatment (Francis and Joynson, 1993). Early diagnosis of *T. gondii* in pregnancy must be improved by determination of anti-*Toxoplasma* IgG avidity to differentiate past and present infection (Pelloux et al., 1998). PCR of amniotic fluid was useful to prove or disprove fetal toxoplasmosis (Romand et al., 2001), and to detect infection in lymphadenopathy (Guy and Joynson, 1995). B1 the 35-fold repetitive gene proved valuable PCR target for *T. gondii* detection (Burg et al., 1989).

The present study aimed at evaluating the efficacy of PCR in diagnosis of toxoplasmosis versus ELISA.

**Subjects, Material and Methods**

A total of 70 blood samples from known positive *Toxoplasma*-ELISA patients with different clinical features were used.

DNA was isolated from the samples by a commercial purification system (Wizard Genomic DNA Purified Kit, Promega, Madison, WI) after the manufacturer’s instructions. Final pellets were resuspended in 30 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 7.2) and stored at -70°C until used. PCR was done on all DNA samples targeting the B1-gene of *T. gondii* present in 35 copies and conserved in genome (Burg et al., 1989). The primers were Upstream; 5΄GGAACTGCA TCCGTTCATGAG-3΄ from 694-714, & Downstream; 5΄5’TCTTTAA AGCGTTCGTGGTC-3΄ from 887-868 giving 193 bp amplicon.

NA master mix and amplification: Five microliters of template DNA were added to 50 µl of PCR mixture of 5 µl of 10 x PCR buffer (50 mM Tris-HCl, pH 9.1, 3.5 mM MgCl₂), 8 µl of 1.25 mM deoxy-nucleoside triphosphates, 0.5 µl of *Taq* DNA polymerase (5 units/µl), and 1.5 µl (20 pmol) of the outer primers each. Amplification was done in GenAmp 480 PCR System (Perkin Elmer Cetus). PCRs cycling conditions were at 95°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 55°C for 90 seconds, 72°C for a minute, and extended at 72°C for 10 minutes. Positive DNA control was extracted from RH *T. gondii* strain. PCR mixture without DNA and with DNase-free water was used as negative controls. The amplified DNA was run on agaros gel stained with ethedium bromide.
Results

Fig 1: PCR of 11 cases: lane1: 1k ladder, lane2: water blank, lane3,5,6,7,8,9 & 10: positive samples showed positive bands at 193bp, lane4,11,12 & 13: negative ones.

Table 1: Clinical features of patients versus ELISA-IgG and PCR.

<table>
<thead>
<tr>
<th>Patients’ clinical features</th>
<th>No.</th>
<th>ELISA +ve</th>
<th>PCR +ve</th>
<th>Positive by both</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant women</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Leukemia &amp; T cell deficiency</td>
<td>10</td>
<td>9</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Organ transplant</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>8</td>
<td>7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Others</td>
<td>40</td>
<td>21</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>43</td>
<td>22</td>
<td>16</td>
</tr>
</tbody>
</table>

Discussion

In the present study, there was discrepancy in the ELISA and PCR results. Out of 70 human cases with different clinical features only 43 were ELISA-Toxoplasma-IgG positive and 22 were PCR-Toxoplasma positive. A total 43/70 (61.4%) were ELISA-IgG positive, but by PCR only 16/43 (37.2%) were positive, and 27/43 were negative. Of 22
PCR positive samples, 6/22 (27.3%) were ELISA-IgG negative. Many authors recommended PCR than most serologic techniques (Wilson et al., 1997; Nimri et al., 2004; Sławska et al., 2005). The precise knowledge of acute infection during pregnancy assessed vertical transmission of infections based on counseling, prevention, and treatment (Vimercati et al., 2000).

The diagnosis of T. gondii infection in pregnancy with an antibody-negative serum collected at the beginning of pregnancy or before conception was usually not possible (Jenum et al., 1997) in some other countries such as USA (Li et al., 2000) testing for antibodies to Toxoplasma in pregnancy is performed only in suspected cases. Routine serologic diagnosis of toxoplasmosis gave high sensitivity, but specificity varied depending on the test used and false-positive IgM antibody results (Wilson et al., 1997). Even true positive results must be interpreted with caution (Liesenfeld et al., 1997) because IgM antibodies might persist for ≥1 year after acute infection (Bobic et al., 1991). In the present study, 2 cases were ELISA-positive but PCR negative and vice versa. This might be explained as the positive IgG which al-most the main recommendation by physician indicated Toxoplasma past infection and unreliable to exclude fetal infection (Sławska et al., 2005). The organ transplantation, leukemia and T-cell deficiency affected immunity led to false-serodiagnosis (Liesenfeld et al., 1997).

On the other hand, Abdel-Hameed and Hassanein (2008) stated that the molecular epidemiological studies on Egyptian T. gondii cou-lld be performed directly on infected tissue samples. The nested geno-typing PCR proved fast and highly sensitive method and could be used directly on clinical samples to avoid the time consuming meth-ods required to grow the parasite and to avoid the possible loss of sam-ples or strain select-ion bias during culture. Elsheikha and Xiangro-ng (2008) and Elsheikha et al. (2008) studied the phylogenetic evidence for recombination in sag5 locus in Toxoplasma gondii, conclu-ded that it is a protozoan of worldwide distribution, and the analysis they presented gave important insights into diversity and evolutionary history in T. gondii. They added that experimental evolutionary studi-es were ongoing to improve better knowledge of the population bo-il-ogy and mechanisms involved in recombination in T. gondii. The
question is; did the phylogenetic evidence have in one way or another its input in toxoplasmosis pathogenesis, diagnosis, and treatment?

Generally speaking, up to one-third of the world’s population had evidence of exposure to a chronic infection (Rifaat and Morsy, 1968). Prevalence of human toxoplasmosis ranged from 7.5 up to 95% worldwide. It was 7.5% in Scotland (Jackson and Hutchinson, 1987), 37.4% in Saudi Arabia (Abbas et al., 1986) 50% in USA (Stagno, 1980), 54.0% in Kenya (Griffin and Williams, 1983), 37.5% in Libya (Kassem and Morsy, 1991), 47% in Nigeria (Onadeko et al., 1992), 37% in Jordan (Morsy and Michael, 1980), and 95.5% in Kuwait (Behbehani and Al-Karmi, 1980). Alonso et al. (1984) stated that patients with AIDS developed up to 50% cerebral toxoplasmosis. In Saudi Arabia, perhaps the first human cases were reported in Riyadh (Shoura et al., 1973). Also, toxoplasmosis antibodies were reported among some pilgrims (Morsy and El Dasouki, 1977), in blood donors (Sarwat et al., 1993), and among domestic (Morsy et al., 1994) and wild rodents (Al Dakhil and Morsy, 1996). So, toxoplasmosis represents one of the most important diseases.

In conclusion, rapid and dependable diagnosis of toxoplasmosis is a must. Combination of PCR-positive result and ELISA-IgG-positive result proved the recent infection, and to start treatment even in the lack of sequential follow-up for patients.

References


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