Diagnosis of Toxoplasmosis among couples by immune detection and genetic evaluation of Toxoplasma gondii

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Abstract

In this study, 27 married patients were totally examined for the presence of antibodies against Toxoplasma gondii by using a standard commercial enzyme-linked immunosorbent assay (ELISA) and Polymerase Chain Reaction (PCR) technique was used evaluate the genome of this parasite. Of 27 whole blood of married samples, nested PCR was positive in 22 (77.77%) by got the 96 bp DNA fragment, 9 (40.9%) of them appeared with IgM and 6 (27.27%) of no anti-Toxoplasma, while 7 (31.81%) was positive nPCR results revealed both IgM+ and IgG+ antibodies, and 5 (22.22%) were negative results obtained by ELISA and nPCR.

Introduction

Toxoplasma gondii is a ubiquitous apicomplexan parasites of human and others worm-blooded animals which has been considered as the cause of the most prevalent parasitic zoonosis (1, 2) and is the causal agent of significant morbidity and mortality among human worldwide (3, 4, 5). Congenital toxoplasmosis (CT) contamination occurring early during pregnancy can lead to severe fetal damage. Clinical manifestations depend mainly on when the infection was acquired in utero. Infectivity is highest during the later stages of pregnancy; however, the earlier in gestation is an infection which occurs, the greater the likelihood of severe postnatal sequelae (6). Most cases of acquired Toxoplasma infection are asymptomatic and self-limited; hence many cases remain undiagnosed. The incubation period of acquired infection is estimated to be lasted or continued during 4-21days (7 days on average) (7). When symptomatic infection does occur, the only clinical findings may be focal lymphadenopathy. This involves a single site around the head and neck frequently. Acute infection is rarely accompanied by a mononucleosis-like syndrome characterized by fever, malaise, sore throat, headache and an atypical lymphocytosis on peripheral blood smear (8). The major objective cause is that one third of the world population has been infected with Toxoplasma gondii. The latent toxoplasmosis was recently considered as asymptomatic which has potentially been shown to have serious consequences for physical and psychical health (9). Serological studies showed a considerable difference in the
prevalence of Toxoplasma infection from 0-95% in different parts of the world and indeed between different population groups within the same country (10, 11, 12, 13). In Iraq, Juma and Salman 2011 (14) found that the infection of T. gondii in women was 19.17%. In Tikrit province in Iraq, Al-Doori 2010 (15) showed the presence of infection of about 49 to 95% and higher rate of infection can be seen among those who are 25 to 31 years old particularly women and their husbands. Seroprevalence of T. gondii infection among men rises with age and it did not vary greatly between sexes (16). The prevalence of Toxoplasmosis significantly increased with age and the highest seropositivity rate, 35.4% was found among pregnant women in the age group of 35 to 44 years old in Slovakia (17). The overall seroprevalence of Toxoplasmosis in South African was 29/160 (18.1%). Seroprevalence in males and females were 7/42 (16.7%) and 22/118 (18.6%), respectively and the difference was not statistically significant (p>0.05). The age distribution was 0.63% (1/160) for individuals of 20 years old and below, 10.6% (17/160) for those between 21 and 35 years old and 6.9% (11/160) for individuals who were 36 years old and above (18). The serologic evidence of toxoplasmosis in Ethiopia was found in 60% (39/65) of them. A large number of the seropositives were females (64.1%), while in male was 53.8% (19). The overall anti-T. gondii IgG prevalence in China was 12.3%, the seroprevalence was 10.5% in men versus 14.3% in women (20). This study used PCR to amplify Toxoplasma DNA from the B1 gene to detect T. gondii in whole blood of married patients with confirmed clinical toxoplasmosis and positive specific anti-Toxoplasma (IgG and IgM) antibodies.

Materials and methods

Whole blood samples were clinically and laboratory collected from 27 Toxoplasma infected patients. The samples were collected between November 2010 to May 2011 from a clinical laboratory in Ramadi Hospital.

- IgG and IgM antibodies detection: This assay was performed by two different approaches. One for detection of IgG and another one for detection of IgM specific antibodies against T. gondii antigens in the patient’s serum (Biokit Diagnostics Company, Spain). Detection of IgG and IgM titers in all samples were analyzed for T. gondii by the titer of IgG and IgM antibodies using ELISA kit as described by Biokit Diagnostics Company, Spain. The optical densities (OD) of the samples were measured at 450 nm, using the OD value of the blank well to correct all the OD reading from test wells (Biokit Diagnostics Company, Spain).

- Isolation of DNA: DNA also was extracted from samples using QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer’s instructions.

- Detection of T. gondii by PCR: Detection of T. gondii infections was determined by nested PCR amplification targeting the B1 gene (GenBank: AF146527) (21). The PCR mixture for the nested reaction consisted of 2X PCR master mix solution with a final concentration of 1.5 mM MgCl2, 200 µM of each dNTP and 2.5 U Taq DNA polymerase (Promega, USA); 10 pmol of each primer; and as a template, 2 µl of extracted and 5 µl of treated sample DNA for the first round and 2 µl of a 1:10 dilution of the products of the first amplification for the second round, in a final volume of 25 µl. PCR reactions were performed in a thermal cycler (Techn., UK). First step PCR was 5 minutes at 94ºC, followed by 35 cycles, each cycle consisting of 60 seconds at 94ºC, 30 seconds at the annealing temperature for each primer pair, and 60 seconds at 72ºC. The final cycle was followed by heating for 5 minutes at 72ºC. Primary PCR amplification was performed with outer primers B1F1 (5'-CCGGTTGGGTCCCGCTCTTC-3') and B1R1 (5’-GCAAAAACAGCGGGACAG CGTCT-3’) at an annealing temperature of 54ºC. In the second round, the internal primers B1F2 (5’- CGCCTTCTCTGCTCCTGCT-3’) and B1R2 (5’-TGGGGCGGCGAAGCTCTCTTG-3’) were used at an annealing temperature of 60ºC. Primers were synthesized by Eurofins Genomics India Pvt, Ltd (India). The PCR products were electrophoresed on 1.5% agarose gel in 1x TAE buffer and stained with ethidium bromide (0.5µg/ml). Under a transilluminator with a 100 bp DNA ladder (Promega, USA).

Results

The results of two tests (nPCR, ELISA) showed that 22 pairs were positive nPCR (77.77%), the PCR product of the positive result was 96 bp (Fig. 1), and the positive results of ELISA test 17 (62.96%) (Table 1). The couples 22 (77.77%) who recorded positive results in nPCR analysis were distributed on the patterns of the anti-Toxoplasma antibodies, it was found that 9(40.9%) of them appeared with IgM+ pattern and no the patterns of IgG+ and 6 (27.27%) of no anti-Toxoplasma antibodies. In addition, 7 (31.81%) of a positive nPCR results revealed both
(IgM+ and IgG+) antibodies. Notable, these results showed low significant differences (P≤ 0.05) (Table 2 and Fig. 1).

Fig. (1) DNA amplification by nested PCR for the 96 bp fragment from B1 gene. M: 100 bp DNA ladder, N: negative control, Numbers 1-27: No.s of couples.
Table (1) Prevalence of Toxoplasmosis in couples by two techniques (nPCR, ELISA)

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive</th>
<th></th>
<th>No.</th>
<th>%</th>
<th>Negative</th>
<th></th>
<th>No.</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>nPCR</td>
<td>22</td>
<td>77.77</td>
<td>5</td>
<td>22.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td>17</td>
<td>62.96</td>
<td>10</td>
<td>37.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (2) Nested-PCR analysis distributed by anti-Toxoplasma antibodies in couples

<table>
<thead>
<tr>
<th>Pattern of antibody</th>
<th>Positive</th>
<th></th>
<th>No.</th>
<th>%</th>
<th>Negative</th>
<th></th>
<th>No.</th>
<th>%</th>
<th>Total</th>
<th></th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM(^+)ve</td>
<td>9</td>
<td>40.9</td>
<td>1</td>
<td>20</td>
<td></td>
<td></td>
<td>10</td>
<td>37.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG(^-)ve</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td></td>
<td>25.92</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM(^+)ve &amp; IgG(^-)ve</td>
<td>7</td>
<td>31.81</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td></td>
<td>25.92</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No anti-Toxoplasma Abs.</td>
<td>6</td>
<td>27.27</td>
<td>4</td>
<td>80</td>
<td>10</td>
<td></td>
<td>37.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>100</td>
<td>5</td>
<td>100</td>
<td>27</td>
<td></td>
<td>100</td>
<td></td>
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</tbody>
</table>

Statistical analysis

Cal. \(\chi^2\) = 7.126 ; Tab. \(\chi^2\) = 7.815 ; P≤ 0.05

Discussion

Normally the diagnosis of congenital toxoplasmosis is based on serological demonstration of IgM antibody but may be specific IgM antibodies not present and antibody synthesis is delayed in infants, therefore we added PCR technique to increase the validity of results as was suggested earlier. It is mentioned that PCR for detection of T. gondii had high sensitivity and specificity (22). The serological testing had been one of the major diagnostic for toxoplasmosis, it had many limitations. It might fail to detect specific anti-Toxoplasma IgG or IgM during the active phase of T. gondii infection, therefore, the risk of congenital toxoplasmosis of a fetus might be undetected because the pregnant mother might test negative during the active phase of T. gondii infection, furthermore, the test might fail to detect T. gondii infection in certain immunocompromised patients due to the fact that the titers of specific anti-Toxoplasma IgG or IgM antibodies might fail to rise in this type of patient (23). In our study, there were negative results obtained by both PCR and ELISA 5 (18.51%) rule out an infection in the couples. These 5 couples continued their pregnancies and no congenitally infected newborn were delivered, similar results have been reported in previous studies (24). In our results, negative nPCR and positive IgM antibodies (20%) demonstrated that the acute phase of the disease had passed and they were in chronic phase, and residual IgM detected during prolonged periods of time, even after the end of the acute episode and consequent eradication of parasitemia, also, false positive IgM antibody test results have been reported previously (25, 26). Previous studies have documented that PCR could actually detected T. gondii in blood specimens of women before or during pregnancy (27, 28). Based on this, the presence of Toxoplasma DNA in the maternal blood probably indicates a recent infection or apparent parasitaemia, which is likely to be clinically significant. The clearance time for Toxoplasma DNA from the blood of patients with acute toxoplasmic lymphadenopathy was estimated to be 5.5-13 weeks (29). In this study, the presence of Toxoplasma DNA in the maternal blood and positive IgM antibody 9/27 (40.9%) probably indicates a recent infection or apparent parasitemia or active toxoplasmosis, which was likely to be clinically significant. However, PCR could not discriminated between latent or acute infection. On the other hand, couples who have experienced the birth of congenitally deformed children who recorded positive gene product in nPCR analysis, 7 (31.81%) were revealed to hold IgM\(^+\) and IgG\(^+\). This refers to the presence of latent infection and not taking the treatment because of the absence of cases of abortion during pregnancy, causing congenital birth of
deformed children. The findings lead to the interpretation that chronic toxoplasmosis infection is able to promote positive PCR results when the parasite is found circulating. The diagnosis of primary toxoplasmosis in pregnant women early in the first trimester is of utmost importance in order to offer them early therapy or other interventions to prevent congenital infection of fetuses (25, 26, 30, 31). Our conclusion, we found a relationship between husband and wife in terms of injury, disease toxoplasmosis, especially when determining the genome of the parasite Toxoplasma where we showed that all the couples infected with the existence of cases of abortion in most of the couples and exciting thing that coincides the occurrence of birth defects with positive results for antibodies Toxoplasma (IgM and IgG) and nPCR positive, suggesting sexual transmission probability.

References


