ABSTRACT

Toxoplasmosis is one of the most important of parasitic infections in humans and animals, this parasite caused abortion and death of fetus in humans and mammalian animals. This study aimed to use *Toxoplasma gondii* 70 kDa Heat Shock Protein (Tg HSP 70) and B1 gene for the diagnosis of toxoplasmosis in blood of human by using the technique of polymerase chain reaction assay (PCR). Study were examined (300) blood samples from married patients and (50) blood samples as control for detection the presence of *Toxoplasma gondii* DNA in blood of patients by using Tg hsp70 and B1 gene. The 242 (80.7%) blood samples was given positive results for B1 and (Tg hsp70) compared with control samples who are show a negative results for this test. Also observed in the current study, the infection in female show at ratio rate 136(90.7%) Compared with their spouses show at ratio rate 106(70.7%) this results show no significant different between of theme and highest infection rate by toxoplasmosis show in the 31-35 age group at ratio (90.5%) compared with other age group.

KEYWORD: Toxoplasmosis, gene Tg hsp 70, B1 gene, polymerase chain reaction.

1. INTRODUCTION

Toxoplasmosis is one of the most important of parasitic infections in humans and animals which caused by intercellular parasite called *Toxoplasma gondii*. The most population in world's infected with the toxoplasmosis chronically. Symptoms of toxoplasmosis are usually mild and about 90% of people infected with toxoplasmosis do not show symptoms or mild symptoms. This parasite caused abortion and death of fetus in humans and mammalian animals. The evolution of the severity of the injury in toxoplasmosis depends
on the immune status of the host as well as virulence factors and genetic characteristics of the parasite that play a significant role in the ability to injury in this disease.\textsuperscript{[7]} The diagnosis infections of Toxoplasmosis is very difficult, in humans and animals therefore, using several immunological and molecular technique to determine the Toxoplasma gondii infection in the embryonic fluid and tissue or blood sample.\textsuperscript{[8,9]} Polymerase chain reaction (PCR) is a molecular diagnostic high Sensitivity and specificity used to detected very small quantities of T. gondii in various biological specimens.\textsuperscript{[10,11]} This methods do not depend on the immunological status of the host but depend on DNA targets of Toxoplasma gondii.\textsuperscript{[12,13]} Therefore, present study aimed to use Toxoplasma gondii70 kDa Heat Shock Protein (Tghsp 70) and B1gene for the diagnosis of toxoplasmosis in blood human by use the technique of polymerase chain reaction assay (PCR).

2. MATERIAL AND METHODS

Three hundred blood samples collected during period from July/2015 to December / 2015 from women who are suffering of repeated projections of embryos and their spouses, Distributed across reviewers to AL- Sadr Teaching Hospital in Meyssan province and Private laboratories in Basra province, Iraq.

2.1. Extraction of DNA

The genomic DNA was extracted and purified from whole blood of 300 patients and 50 control according to the manufacturer’s instructions Genomic DNA extraction kit Bioneer. U.S.A.

2.2. Detection of T. gondii by Tg HSP 70 and B1gene

The PCR amplification of the Tg hsp 70 and B1gene by a following primer Tg HSP 70: TgF, (5’AAGGATGCTGGTACCATTGC 3’), TgR 5GTCCGGATCGTTGTGTATTCT3’and primer of Gene B1: B1F(5CTAGGGCACCTTTAATG3), B1R (5CCTGGTAGCTGCGAATG3). The primer design by using the software program of primer 3plus (www. primer 3 plus .com). The PCR mixture for the reaction consisted of PCR master mix mixture (Bioneer. U.S.A) with final concentration (20μl) which contains 5 μl ofmaster mix,2 μl template DNA,1 μl of each forward and reverse primers and 11 μl of nuclease free water to complete the amplification mixture to 20 μl ). The PCR amplification program for Tg HSP70 Gene as follow (1 min at 95°C for one cycle, then 30 cycle of 30 sec at 95°C, annealing tempreature 45 sec at (53°C), 72°C for 1min with one cycle, then final extension of 10 min at 72°C), While the program of B1gene as follow (2 min at 95°C for
one cycle, then 30 cycle of 1 min at 95°C, annealing t empreture 30 sec at (56°C ),72°C for 50 sec with one cycle, then final extension of 5 min at 72°C. The results of PCR was detected after the amplification process. 5 µl from amplification sample was directly loaded in a 1.5% agarose gel containing 0.2 µl ethidium bromide with the addition of loading buffer and DNA size marker as standard in electrophoresis and run at 70 V, then the products were visualized by UV transilluminator until. The DNA was observed and photographed by using gel documentation system.

3. RESULTS AND DISCUSSION

The results of PCR test showed that 242 (80.7%) blood samples from (300) blood samples was given positive results for B1 gene and Tg hsp70 compared with (50) control samples who are show a negative results in this study (Table 1). The product PCR of the positive result for Tg hsp70 and B1gene show in (Fig. 1, 2). The current study also observed in same table, that infection in female show at ratio rate 136(90.7%) Compared with their spouses show at ratio rate106(70.7%) this results show no significant different between of theme, while the current study showed highest rate of infection by toxoplasmosis in the 31-35 age group at ratio (90.5%) compared with other age group with significant different Compared with other age group. The prevalence of Toxoplasmosis significantly increases 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.[14] The development of molecular diagnostic methods has been performed in the laboratory to detect T. gondii DNA in different biological samples by the polymerase chain reaction (PCR) because the serological test which depend on present antibody (IgM,IgG) may be absence this antibodies with infection T. gondii and when the serological tests and clinical symptoms are not evident.[15,16] The PCR technique is a high sensitive to the diagnosis of early onset of toxoplasmosis and do not depend on the immunological status of the host.[17,18] In study by[19] used B1 gene as the target in blood sample of patients to diagnosis of Toxoplasmosis among couples, this studies found relationship between husband and your wife in terms of injury of toxoplasmosis. especially when determining the genome of the parasite Toxoplasma gondii. In Egypt many studies used B1 gene for detection T. gondii from blood, embryonic fluid and tissue of patients with Toxoplasmosis.[20,21] Also[22,23] used used B1 gene to detection Toxoplasma gondii in blood sample. while the study by[24] detect toxoplasmosis in human by used PCR and ELISA technique, showed that a total of 22 samples at ratio (31.6%) from 70 blood samples were positive by PCR, while only 16 of them were positive by ELISA and And
showed that determine Toxoplasma infection by specific antibodies to Toxoplasma by ELISA. not enough for diagnosis this infection while PCR besides, being valuable in diagnosis showed Toxoplasma parasitemia. Tghsp70 is a highly immunogenic protein and a virulence factor in Toxoplasma gondii Under stress conditions and protect the tachyzoites from defiance host.\textsuperscript{25} T. gondii increases induce expression TgHSP70 protein when tachyzoites differentiate into bradyzoites or during brady zoite to tachyzoite interconversion and before death of the host.\textsuperscript{26} The study by\textsuperscript{25} detect Toxoplasma gondii by used (sag3 / 240, gra6 / 700, B1 / 199, Tg hsp70 / 560) and showed, B1, Tghsp70 were presence in intestine, liver, spleen, kidneys and ascitic liquid after 96 hours infection by Toxoplasma gondii. In the study by\textsuperscript{27} show that the Tg hsp70 release into the bloodstream depends on the death of the parasites mediated by the host immune response, whereas the increased Tghsp70 expression in the brain depends on the multiplication rate of the parasite.

Figure (1) Agarose electrophoresis patterns showed PCR amplified products of Tghsp70 gene Lane (L): (2000 bp DNA ladder), Lane: (no. 1-4) Tghsp70 DNA band.

Figure (2) Agarose electrophoresis patterns showed PCR amplified products of B1 gene Lane (L): (2000 bp DNA ladder), Lane: (no. 1-7) B1 DNA band.
Table 1. The distribution positive and negative result for gene B1 and gene Tg hsp70.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exam. No.</th>
<th>B1 &amp; Tghsp70 positive</th>
<th>B1 &amp; Tghsp70 negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>No.(%)</td>
<td>No.(%)</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>150</td>
<td>106(70.7)</td>
<td>44(29.3)</td>
</tr>
<tr>
<td>Females</td>
<td>150</td>
<td>136(90.7)</td>
<td>14(9.3)</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>242(80.7)</td>
<td>58(19.3)</td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-25</td>
<td>84</td>
<td>70(83.3)</td>
<td>14(16.7)</td>
</tr>
<tr>
<td>26-30</td>
<td>76</td>
<td>62(81.6)</td>
<td>14(18.4)</td>
</tr>
<tr>
<td>31-35</td>
<td>84</td>
<td>76(90.5)</td>
<td>8(9.5)</td>
</tr>
<tr>
<td>36-40</td>
<td>40</td>
<td>28(70)</td>
<td>12(30)</td>
</tr>
<tr>
<td>&gt;40</td>
<td>16</td>
<td>6(37.5)</td>
<td>10(62.5)</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>242(80.7)</td>
<td>58(19.3)</td>
</tr>
</tbody>
</table>

4. CONCLUSION

The current study found the presence of gene heat shock protein gene 70 kDa (Tg HSP70), B1 together in women infected with toxoplasmosis and their spouses. The can using gene (Tg HSP70,B1) to diagnosis of *Toxoplasma gondii* in the blood samples of patients with toxoplasmosis.

5. ACKNOWLEDGEMENT

We would like to express my thanks to all the patients who are donated blood samples to make this research possible. We also thank all the person who are taught me patience, strife and pushed me toward success in life and give me all care and happiness.

6. REFERENCE


