Early Diagnosis of Ocular Toxoplasmosis Using Nested PCR Technique for Identification B1 Gene from Blood Samples

Ghada Basi Ali Alomash*1

1. Department of Microbiology and Parasitology, College of Medicine, University of Al-Qadisiyah, Iraq

*Corresponding author: Ghada.Alomashi@qu.edu.iq

Abstract

The most common form of posterior uveitis is the one triggered by the protozoan parasite Toxoplasma gondii namely ocular toxoplasmosis (OT). The current conducted work was aimed at early identifying ocular toxoplasmosis employing the use of a nested polymerase chain reaction (nPCR), for targeted T. gondii related B1 gene using two sets of primers, and a latex agglutination (LA) test as routine serological diagnosis for toxoplasmosis. This study consisted of collecting 62 blood samples from patients with a physician-recognized OT that visited Al-Diwaniyah General Teaching Hospital, Al-Diwaniyah City, Iraq, for eye-health related problems. The findings of the LA uncovered various incidence rates distributed over a classified range of ages, genders, and residential areas (urban vs rural); however, a 41 (66.13%) positive infection rate was revealed in general for all categories. Moreover, the first-run-PCR outcomes demonstrated a 32 (51%) positive identification rate; however, the remaining negative samples were subjected the second-run nested PCR that showed 25 (83%) of positive detection rate of the B1 gene, which, in total, declares that 57 (91%) of the patients were OT-infected. The current study reveals successful use of blood samples for early detection of T. gondii caused uveitis devoting the utilization of the PCR method that targeted the B1 gene.

Keywords: B1 gene, latex-agglutination test, ocular toxoplasmosis, nPCR, Toxoplasma gondii

How to cite this article: Alomash GB (2021): Early diagnosis of ocular toxoplasmosis using nested PCR techniques for identification of B1 gene from blood samples, Ann Trop Med & Public Health; 24(S2): SP24243. DOI: http://doi.org/10.36295/ASRO.2021.24243

Introduction

Toxoplasma gondii, as a protozoan, attacks up to more than 34% of people worldwide. The protozoan can induce some body damages in which OT is considered as a major consequence that affects patients with toxoplasmosis. T. gondii is one of the leading causes worldwide of posterior uveitis. About 2% of people who experience toxoplasmosis have been reported to acquire ocular problems. OT also may exist in both people with or without immunity related issues. Patients of OT, especially those who are immunodeficient, require immediate care for treating retinochoroiditis(1-7). OT appears to be a complicated eye illness with many open issues surrounding the presentation and treatment of the disorder. Over a century, scientists have been operating meticulous clinical evaluation, epidemiological, and protozoal research to establish our recent knowledge about OT. Probable, OT
related retinochoroidal scar was already represented in the mid-19th century as diagnostic signature for the condition. Janku, a Czech ophthalmologist, first, 1923, identified OT as one of the newly born condition clinical properties of toxoplasmosis of the congenital origin; however, the OT illness was also indicative as recognized sign of adult toxoplasmosis (6).

In the past, health scientists sought—and have very considerable success to identify OT on the basis of clinical criteria including lesion location, the extent of the inflammatory process, the first presentation age, the forms of infection and complications. Whether in immunosuppressed and immunocompetent cases, or whether the disorders are developed congenitally or posnatally, no differentiation can be established between different disease groups, regarding toxoplasmo retinochoroiditis. Recent OT-related debates also center on the effective function of multiple \textit{T.gondii} strains, the host conditions, appropriate diagnosis and medical intervention (i.e. efficacy of corticosteroids) and the value of antiparasitic prevention procedures. OT is under-diagnosed, and, usually, an erroneous ocular condition is concluded other than the existence OT infection; therefore, improved attempts must be introduced to lighten the burden of the OT disorder. In a short process, an insufficient approach is the conventional treatment that targets vision preservation without a curative alternative and re-occurrence avoidance, and this is considered as a non-vital solution for the condition. Necrotising retinochoroiditis is known to be the standard involvement of OT and to such a point that additional testing analysis often isn't required. However, there are significant clinical differences even when this is the most common occurrence of OT, and the interpretation of the OT may quite be difficult. Understanding of multiple OT submissions is essential to physicians, and exposure to OT alterations can provide guidance into the processes of the illness. On the other hand, OT can be presented by different lesions such as punctate outer retinal toxoplasmosis, a multiple lesion condition that results from the effects of some protozoal and host factors inducing changes in the retinal deep layers and the retinal pigment epithelia, neuroretinitis, characterized by edema and hard exudates of the optic nerve, scleritis, and some complication such as spill-over condition that is manifested by granulomatous lesions (8-12).

The current conducted work was aimed at early identifying ocular toxoplasmosis employing the use of a two-run polymerase chain reaction (PCR) method targeted \textit{T. gondii} related B1 gene using two sets of primers, an abundant gene with 35 times of repetition in the genome of this protozoan (13), and a latex agglutination (LA) test. Successful early diagnosis maintains appropriate early medical intervention to avoid further damages to the ocular tissues.

**Patients and methods**

**Patients**

The investigation consisted of collecting 62 blood samples from patients with an ophthalmologist-recognized OT that visited Al-Diwaniyah General Teaching Hospital, Al-Diwaniyah City, Iraq, for eye-health related problems. The samples were ethically (under the release of patient consents) collected during June, 2018 to October, 2019. The blood samples were sterile-syringe-collected and divided into EDTA tube for PCR and gel tube for serological test LA.
DNA extraction and PCR procedures

The extraction of the protozoan DNA was carried out using an RNase-, 20µg/ml, based commercial kit with employing its protocol for fulfilling the process.

The current conducted work was aimed at early identifying ocular toxoplasmosis employing the use of a two-run PCR method targeted *T. gondii* related B1 gene using two sets of primers, table 1, and an LA test.

**Table 1: The sequence of two sets of primers for the B1 gene**

<table>
<thead>
<tr>
<th>PCR round</th>
<th>Sequence</th>
<th>Direction</th>
<th>PCR product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>5’- GGA ACT GCA TCC GTT CAT GAG-3’</td>
<td>Forward</td>
<td>193</td>
</tr>
<tr>
<td></td>
<td>5’-TCT TTA AAG CGT TCG TGG TC-3’</td>
<td>Reverse</td>
<td></td>
</tr>
<tr>
<td>Nested</td>
<td>5’-TGC ATA GGT TGC AGT CAC TG-3’</td>
<td>Forward</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>5’-GGC GAC CAA TCT GCG AAT ACA CC-3’</td>
<td>Reverse</td>
<td></td>
</tr>
</tbody>
</table>

For the positive control of the PCR, *Toxoplasma* RH strain tachyzoites was employed along with the clinical samples. Two runs of the PCR were performed as the first set of the primers, which included, for the total volume, 25µl, of the PCR reaction, each dNTP at 250µM, Tris based HCl at10mM with pH at 8.3, KCl at 50mM, MgCl<sub>2</sub> at 1.5mM, each primer direction at 10pmol, Taq-DNA polymerase at 1U, and DNA of each sample at 100ngas, and the thermocycler step condition are outlined in table 2.

**Table 2: the first run for the first set of primers**

<table>
<thead>
<tr>
<th>Steps</th>
<th>Temperature</th>
<th>Time</th>
<th>No of cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>94°C</td>
<td>4 min</td>
<td>36</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94°C</td>
<td>45s</td>
<td>45</td>
</tr>
<tr>
<td>Annealing</td>
<td>57°C</td>
<td>45s</td>
<td>45</td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>45s</td>
<td>45</td>
</tr>
<tr>
<td>Final extension</td>
<td>72°C</td>
<td>2min</td>
<td>36</td>
</tr>
</tbody>
</table>

The second round (nested) of the PCR was performed using the same procedures and conditions of the thermocycler, except for the annealing temperature that was 62°C, and employing the negative PCR samples from the first round. For the electrophoresis of the PCR products, 2%-agarose gel pretreated with a visualizing dye was used followed by detecting the findings under a UV-based documentation system.

Statistical analysis: Chi-square test was used to compare for the results.

**Results**

**Serology**

The findings of the LA uncovered various incidence rates distributed over a classified range of ages, genders, and residential areas (urban vs rural); however, a 41 (66.13%) positive infection rate was revealed in general for all categories. Table 3 displays the details of incidence rates regarding different ranges of age, genders, and urban vs rural residential areas.

<table>
<thead>
<tr>
<th>Age</th>
<th>No.6</th>
<th>Gender</th>
<th>Affected eye</th>
<th>Residence</th>
<th>Serological Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>Male</td>
<td>Female</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>5-10</td>
<td>22</td>
<td>10</td>
<td>12</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>11-15</td>
<td>13</td>
<td>5</td>
<td>8</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>16-20</td>
<td>15</td>
<td>5</td>
<td>10</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>21-25</td>
<td>8</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>&gt;26</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

Moreover, the first-run-PCR, figure 1, outcomes demonstrated a 32 (51%) positive identification rate.

Figure 1: Image of agarose gel electrophoresis for the B1 gene 1st round PCR. M: DNA ladder, Positive PCR product, 193bp,
However, the remaining negative samples were subjected the second-run PCR, figure 2, that showed 25 (83%) of positive detection rate of the B1 gene.

![Image of agarose gel electrophoresis for the B1 gene 2st round PCR. M: DNA ladder, Positive PCR product, 96bp, at lanes, 1 to 5.]

The total detection rates of the PCR for both rounds are declared that 57 (91%) of the patients were OT-infected, table 4.

Table 4: Detection of B1 gene for T. gondii by PCR in ocular infection patients with clinical indications of toxoplasmosis

<table>
<thead>
<tr>
<th>PCR</th>
<th>No.</th>
<th>Positive</th>
<th>negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st round</td>
<td>62</td>
<td>32 (51%)</td>
<td>30</td>
</tr>
<tr>
<td>2nd round</td>
<td>30</td>
<td>25 (83%)</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>57 (91%)</td>
<td>5</td>
</tr>
</tbody>
</table>

Discussion

As well-known targets of T. gondii, ocular tissues, more likely, retina and optic nerve, were reported, here, to be the main affected entities in the body. This was confirmed via the presence of only OT symptoms and signs without other reactions mentioned; elsewhere, in the body of patients that the blood samples were collected from. Moreover, according to the ophthalmologists who clinically examined those patients, highly recognized ocular lesions, refer to
OT, were visualized plus treatment used was successful in improving OT symptoms in 7 to 10 days. This means that using serological and \textit{B1}-based PCR tests were useful in early detecting of OT via the use of blood samples (13).

In most instances, acute infection is identified with similar IgM and/or IgA antibodies, while IgG antibodies with high avidity may be observed when the infection has arisen over the preceding four months. For validity of serological testing for toxoplasmosis detection, the antigen(s) used in the given test must also be taken into consideration. Also now, many experiments are widely presence commercially employing whole parasite cell lysates. Nonetheless, provided the severe acute toxoplasmosis clinical symptoms are often derived from the extremely replicative tachyzoite rather than cyst-bradyzoite stage. In recent serological tests, a number of methods have been used to distinguish acute from latent toxoplasmosis utilizing recombinant antigens. One of the most widely utilized recombinant antigens used in ELISA studies is the SAG-1 tachyzoite surface major antigen. The immunoblot technique, unlike ELISA, makes it possible to assess antigen-specific immunoreactivity, which is incredibly useful when analyzing multiple anatomical compartments, such as serum and aqueous humor. Simultaneously, in reaction to antigen presentations, this strategy permits differentiation at the Ig-class level(14,15,6).Successful diagnostic steps were made due to the use of LA test in the present investigation, and this agrees with the work findings of Liu \textit{et al.}(16) who detected significant positive results of using agglutination tests for identifying the presence of \textit{T. gondii} based antibodies, IgG and IgM. For more confirmative evidence, it has been found that utilizing serological tests for detecting OT is important due to the trustfulness in the successful findings via unveiling the presence of IgG in sera of Korean patients (17).

The results from the current work revealed improved detection of the OT by using the first-round and the nested PCR methods. This agrees with Farhadi \textit{et al.}(18) who found that, with first-round PCR, the outcome was 58%, almost similar to the current work score of 51%, of their samples withB1-gene based 193bp piece. Interestingly, the nested-PCR was able to increase the successfulness of the OT detection using the inner primers with a total positivity rate of 88%, close to the present study finding of 91%. Moreover, it has been found that a B1-gene based multiplex PCR method was successful revealing 11 positive results out of 13 patients(19).Regardless the type of samples, blood or ocular fluids, were taken in previous studies, PCR methods that employed the use of B1 gene as a molecular target enhanced the detection of OT (20,21).

The current study reveals successful use of blood samples for early detection of \textit{T. gondii} caused uveitis by the utilization of the PCR method that targeted the B1 gene.

\textbf{References:}


10.1086/338827.


