Study of *Cutaneous leishmaniasis* using polymerase chain reaction technique in west of Karbala rural sites

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**Abstract:**

This work was aimed to study the most common forms of leishmaniasis, *Cutaneous leishmaniasis*, and its types using Polymerase Chain Reaction (PCR) technique in west of Karbala governorate rural sites during the period between Sep. 2015 to Apr. 2016.

Leishmaniasis infection is considered as a great medical problem in most countries on the world faced the infected persons causes deformity in the manifestation site, the study included 91 infected persons by cutaneous ulcers diagnosed primarily as Leishmaniasis.

The results of the present study revealed that the male infections (71.42%) were more than the infected female (28.57%) with a significant differences \(P<0.05\). To inappreciable diagnosis of Leishmania types in west Karbala rural sites have employed PCR technique to samples. Also the results have revealed the appearance of two types of *L*. parasite causing the manifestation. In PCR test bundle length of about 650 base pair (bp), (77 samples) belongs *L.* major and 750 bp (12 samples) belongs *L*. tropica were obtained.

An attempts was carried out to find suitable vaccine for this disease, in order to study the immune response using lymphocyte transformation assay. The ratio was 8.9% for *L*. tropica, 19.4%, for *L*. major with a significant differences of \(P<0.05\) for control sample (5.9%).

**Keywords:** Leishmania, *L. cutaneous*, *L. tropica*, PCR diagnosis.

**INTRODUCTION**

The Leishmaniasis infection (Oriental Sore) is considered a great medical problem in most countries of the world facing the infected persons which causes deformity in the manifestation site\(^{[2]}\). Risk factors include poverty, malnutrition, deforestation, and urbanization\(^{[3]}\). World Health Organization (WHO) classified this disease as one of biggest six parasitic disease infect the human being\(^{[4]}\).

Leishmaniasis is a vector-borne disease that is transmitted by sandflies and caused by obligate intracellular protozoa of the genus Leishmania, it spread by the bite of certain types of Phlebotomus (sandflies)\(^{[2;3]}\). Infections in humans are caused by more than 20 species of *Leishmania*\(^{[4;5]}\). It can appears in three main types: cutaneous, mucocutaneous, or visceral leishmaniasis\(^{[6]}\). The different species are morphologically indistinguishable, but they can be differentiated by isoenzyme analysis, molecular methods, or monoclonal antibodies. The cutaneous form is the most common form, which causes an open sore at the bite sites, which is only healed after few months to a year and half, leaving an unpleasant-looking scar tissue\(^{[7]}\), presents with skin ulcers, skin sores which could be erupted in weeks to months after the person is bitten by infected sandflies which transmit the infection from infected mammals reservoirs as human, rodents and animals.

The parasite lived as a amastigote phase\(^{[10]}\). Parasite were isolated, by injection of the Promastigote phase\(^{[10]}\). The samples were preserved for DNA extraction.

**Materials and Methods**

Samples were collected from 91 patients living in west Kerbala rural area during the period from September, 2012 to April 2015. They were having sore skin infection diagnosed by dermatologist as Cutaneous *L*. . The samples were preserved for DNA extraction. Blood smear from infected persons were examined to ensure the presence of Amastigote phase, Biphasic media (Novy, McNeal – Nicolle NMN medium), were used in order to culture and growth the parasites under the microscope\(^{[10]}\). Additionally, visceral form could be diagnosed by blood tests\(^{[11]}\). The disease can be transmitted between human beings by the vector or by blood transfusion\(^{[12;13]}\).

*L. cutaneous*. can be diagnosed in the hematology laboratory by direct visualization of the amastigotes (Leishman-Donovan bodies)\(^{[10]}\), using Buffy-coat preparations of peripheral blood or skin lesions on a slide to make a thin smear then stained with Leishman stain or Giemsa stain. Amastigotes are seen within blood and spleen monocytes or, less commonly in circulating neutrophils\(^{[11]}\).

Taking tissue samples is often painful for the patient and identification of the infected cells can be difficult. So, other indirect immunological methods of diagnosis are used, including enzyme-linked immunosorbent assay, antigen-coated lipstics, and direct agglutination test. Although these tests are readily available, they are not the standard diagnostic tests due to their insufficient sensitivity and specificity. So several different Polymerase Chain Reaction (PCR) tests are available for the detection of Leishmanial DNA,\(^{[13]}\) which is a specific and sensitive diagnostic possible procedure. Till this work, no real active vaccine for humans available\(^{[9]}\). But researchers know that people whom recover from cutaneous Leishmaniasis are protected against future infections; for example people in the middle east have transfused fluid from lesions of infected people to non-infected people using thorns\(^{[5;6]}\). This process is called "leishmanization"\(^{[7]}\). Some experiment using genetically-modified live attenuated parasite vaccines was conducted in the 1990s and 2000s. Other experiment had used synthetic peptides and naked DNA\(^{[13]}\).

Some attempts carried out in Iraq to produce vaccine against *L. cutaneous* by exposing the children to sand-fly bite at their buttock in order to immunized them against the disease in the future. However, another researches have been conducted to produce suitable vaccine in rodents lab-animals by using killed or attenuated vaccine., these attempts represent the 1st generation works. Moreover there are some trials to produce the 2nd generation vaccine using parasitc DNA trial level\(^{[16;17;18;19;20;21]}\).
Infected solution with antibiotic (Crystalline penicillin 1000000 IU +1 gm Streptomycin sulphate) were injected in one leg sole and phenol saline solution in the other leg sole, after twenty hrs. sole pad thickness were measured, the deference in thickness represent the cellular response[23]. The percentage of lymphoblast were calculated according to the following equation:

Percentage of lymphoblasts formation = \( \frac{\text{No.of Lymphoblasts}}{\text{Total no.of Lymphocytes}} \times 100 \)

The analysis of biostatistics was done by SPSS 10.10.

**RESULTS**

Table 1 explain the distribution of Leishmaniasis infection between males and females of the present study. Sixty five cases of the infected samples(71.42%) were males, while the females (26 cases) were less affected (28.57%).

Table 1: Distribution of Leishmaniasis infection depended on age and sex.

<table>
<thead>
<tr>
<th>Age categories (years)</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>1≥</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1-5</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>6-10</td>
<td>6</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>11-15</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>16-20</td>
<td>13</td>
<td>6</td>
<td>19</td>
</tr>
<tr>
<td>21-25</td>
<td>8</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>26-30</td>
<td>15</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td>31-35</td>
<td>14</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>36-40</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>41-45</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>46-50</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>51≤</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>65 (71.42%)</td>
<td>26 (28.57%)</td>
<td>91</td>
</tr>
</tbody>
</table>

The blood smears were showing small, round bodies with indistinct cytoplasm. Occasionally, amastigotes may be seen lying free between cells(fig. 1).

To detect parasite type causing Leishmania, PCR technique were used, we get bundle length at 650 bp for type *L. major*, 79 cases (Fig.6); and 750 bp for type *L. tropica*, 22 cases (Fig.7).

Lymphocyte transformation assay test using putrefied Lipophosphoglycan in 10 experimental rats reveals that, the ratio was 8.9% for *L. tropica* ; 19.4%, for *L. major*, there is a significant differences p<0.05 while for control sample was (5.9%) (Table: 2). Results of late hypersensitivity test in 15 experimental rats revealed that the toe average thickness of hind limb injected by *L.* antigen was 1.18 mm, and to hind limb injected by saline-phenol solution was 1.24 mm, while for control gp. was 0.43mm by significant differences p<0.05 (table: 3).
vaccination against this disease and propose scientific scheme to control it\cite{21}. Results of this work have revealed that the percentage of male infection (71.42\%) was higher than female infection (28.57\%); within a ranging age between 16 to 30 years. These results were compatible with other works conducted by several researchers\cite{23,25}. In agreement with Willy et al.\cite{23}, the presence of higher percentage of infection among the males was explained by higher incidence of exposure of the male to causative vectors.

In this work PCR technique were used which considered the best accurate, modern, sensitive method in diagnosis and typing and serotyping, immunological reactions of the causative agent even the contaminated blood sample was very little (in nano gram)\cite{25}.

**REFERENCES**


