Detection of Cutaneous Leishmaniasis species via PCR in Salah Adeen and Baghdad provences

Maysaa Ibrahim Al-Jubori¹, Abd Alrahman A. Al-Tae², Mohammad A. Al-Faham³
¹Department of biology, college of science, Tikrit University, Tikrit, Iraq
²Department of microbiology, college of medicine, Iraqia University, Baghdad, Iraq
³Department of microbiology, college of medicine, Baghdad University, Baghdad, Iraq

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Corresponding Author:
Name: Maysaa Ibrahim
E-mail: maysaa.aljubory@gmail.com
Tel:

ABSTRACT

Background: Leishmaniasis is a parasitic diseases that are spread worldwide due to various species of Leishmania, which infect mammals diversity as well as human. L. tropica, L. major, and L. aethiopica which is common causes of cutaneous Leishmaniasis in Salah Adeen and Baghdad provences.

Material and Methods: The present study was conducted to investigate the prevalence of cutaneous Leishmaniasis and to identify Leishmania parasites by using polymerase chain reaction (PCR) in some endemic areas of Iraq. A total of 117 samples of patients with suspected cutaneous Leishmaniasis were collected in different age groups. And both sexes 73 male and 44 females patients.

Results: PCR results showed the percentage of infections 62.39% of males while 37.60% of females. The average age was 23.35 years (the range, from 1- 60 years), with the highest percentage of cases in the age group 1-4 years and the lowest rate in the age group (40-60 years). The highest infection was by L.tropica of L.major and lowest infection caused by Laethiopica, where is considered first revealed in Iraq.

Conclusion: The study found that males were more likely to be infected than females. The study revealed that polymerase chain reaction (PCR) is the most effective and sensitive method for detecting types of Cutaneous Leishmaniasis.

Introduction

Leishmaniasis of the most important vector-borne diseases of humans, which is due to many species of Leishmania, mostly zoonotic. Cutaneous Leishmaniasis (CL) is a widespread parasitic disease that can causes serious health problems in the communities throughout the Mediterranean regions and the Middle East, including Iraq [1]. CL has been growing worldwide in both incidence and range, mainly due to the increased human migration. This movement contributes to Leishmanial infections in low or nonendemic areas. The importance of studying the genetic variation of Leishmania is mainly attributed to its binding with the epidemiological aspects of the disease, such as geographical location, clinical forms, pathogenicity, virulence, antigenic variation and drug resistance, among others [2].

This parasite is transmitted by females from two genera of sand fly, Phlebotomus and Lutzomyia, which are of medical importance as the only quality vectors for Leishmania species that causing Leishmaniasis to humans [3]. As with many parasites, the classification of the genus Leishmania is very complex because the limitation and definition of species are difficult to identify. The exact identification of these species are clinically important and epidemiologically. Leishmania parasites have similar morphology and sometimes cause similar clinical manifestations[4]. CL caused by L. tropica usually appears as dry, small lesions, located mainly on the face, which after healing, leaving permanent scarring and the inability of a serious, while ulcer because of a L. major leads to the production of single or multiple "wet" sore [5].
The symptoms of Leishmaniasis can be range from moderate, self-healing cutaneous lesions to fatal visceral cases. The absence of a human vaccine, increasing resistance to the presently used drugs and their serious side effects, urge the need to research of Leishmaniasis [3].CL due to L. tropica complex is present in many parts of Asia, Africa, Mediterranean Europe and the southern region of the former Soviet Union. Urban Cutaneous Leishmaniasis is thought to be an anthroponosis while the rural cutaneous Leishmaniasis is zoonosis with human infections that occur only sporadically. The reservoir hosts in L. major are rodents. L. aethiopica is endemic in Ethiopia and Kenya. This disease is a zoonosis with rock and tree hyraxes serves as reservoir hosts. The vector for the old world cutaneous Leishmaniasis is the Phlebotomus sand fly. The epidemiology of Leishmaniasis due to L. tropica has not been illustrate fully. The disease is often described as being urban and anthropogenous, and most infections occur in and around quite densely populated cities. However, the rarity of cases in some foci and sudden occurrence of small outbreaks of disease in semi-urban areas suggest that the disease may be zoonotic in some cases [6].Diagnosis of CL is based on both clinical features (supported by epidemiologic data) and laboratory tests. Several diagnostic methods have been described with significant differences in diagnostic accuracy, including parasitological direct assay (microscopy, anatomy, parasite culture) and / or indirect test with serological and molecular diagnostics [7].Modern molecular characterization techniques have used polymerase chain reaction (PCR) to amplify parasite DNA from host tissues. PCR-based methods, have been demonstrated as a powerful tool for directly detecting Leishmania clinical specimens as well as for characterization of parasites that are highly sensitive and specific compared to conventional methods and provide results in one or two working days. With advances in molecular techniques, a number of molecular markers and protocols have been reported to detect or identify Leishmaniasis at different taxonomic levels (genus, complex, and species) has been reported [1].PCR is a commonly used technique for typing and pathogen detection, has already been taken for use for Leishmania spp. The materials and biopsy Skin obtained by skin scrape have been used for PCR in different studies [8].There are several methods of laboratory diagnosis of Leishmaniasis including parasitological, molecular, immunological and by using experimental animals [9].The aim of the study to diagnose the pathogen, epidemiology as a first study, is advanced in our country and for the purpose of providing means to reduce infection with this disease.

Materials and methods

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Materials and methods
Under sterile conditions and examined through polymerase chain reaction (PCR), among these 62.39% were male and 37.60% were female. The median age was 23.35 years (the range, 1 year - 60 years), Where highest percentage of cases to the age group 1-40 years old and less infection rate to the age group (40-60 years).

The present study reported the diagnosis of three species of *Leishmania* by using PCR method, as in Fig (1), which include *L. tropica* (100 bp) , *L. major* (99 bp) and *L. aethiopica* (154 bp). Which is the first appearance in Iraq for *L. aethiopica*. All patients enrolled in this study had characteristic signs and symptoms of CL, from small erythematous papules to nodules and ulcerative lesions.

### Table 1: Percentage of Infection with Cutaneous Leishmaniasis According to Location, The Sexes and the Age Groups.

<table>
<thead>
<tr>
<th>Location</th>
<th>Aldhuluiya</th>
<th>Balad</th>
<th>Aldujail</th>
<th>Tikrit</th>
<th>Sammarra</th>
<th>Baghdad</th>
<th>total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. sample &amp; percentage</td>
<td>42 (35.89%)</td>
<td>24 (20.51%)</td>
<td>11 (9.40%)</td>
<td>15 (12.82%)</td>
<td>10 (8.54%)</td>
<td>15 (12.82%)</td>
<td>117</td>
<td>100%</td>
</tr>
<tr>
<td>Male</td>
<td>27</td>
<td>13</td>
<td>7</td>
<td>11</td>
<td>6</td>
<td>9</td>
<td>73</td>
<td>62.39%</td>
</tr>
<tr>
<td>Female</td>
<td>15</td>
<td>11</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>44</td>
<td>37.60%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age groups</th>
<th>1-10</th>
<th>11-20</th>
<th>21-30</th>
<th>31-40</th>
<th>41-50</th>
<th>51-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>3</td>
<td>13</td>
<td>11</td>
<td>8</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Female</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

Discussion

Cutaneous Leishmaniasis (CL) appears to be a major public health problem and endemic in Iraq, Kuwait, Iran, Afghanistan, and other places in the Middle East [13]. The present study is performed in Salah Aldeen province and Baghdad in Iraq, Where it had the highest rate of infection appeared in Aldhuluiya while lowest rate infection was in Sammarra (Table 1), the reason might that Aldhuluiya have massive population and increased the numbers of the vectors due to found suitable condition.

In the present study was among 62.39% were male and 37.60% were female, because of the nature of the male work abroad unlike females, which resides most of the time at home and this will be the incidence in males larger.

According to Jamal et al. 2013, the percentage was significantly higher among males (70.58%) than females (29.42%). One possible cause of high male incidence may be their tendency to take off shirts while sleeping during the hot summer, which may make them more susceptible to sand fly bites. On the other hand, females are cold and keep themselves covered in a sheet that is thought to give them protection from sand fly bites [14].

In the present study, the infection were observed among all age groups. The average age was 23.35
years (the range, 1 - 60 years), with the highest rate of cases in the age group (1-40) years old and lowest infection rate in the age group (40-60) years. Due largely to the presence of this age group in the places that allow the transmission of this disease easily among people, such as schools and children's play area and the workplace for employees.

The current study has been recorded the infection caused by \textit{L.tropica}, \textit{L. major} and \textit{L. aethiopica}. Several studies have shown that differences between the agents of CL in old world (\textit{L.major} & \textit{L.tropica}) may be related to various factors, like biological and morphological characteristics of the parasite.

In this study, \textit{L. aethiopica} was first recorded in Iraq. The diagnosis of CL is traditionally based on microscopic demonstration of \textit{Leishmania} in swabs or scraped. However, this method usually lead to low sensitivity, and in atypical forms, CL may be ignored due to of similarity with other dermal diseases. Thus, it is necessary to apply specific and sensitive diagnostic methods as PCR technique [15]. In the present study, we investigated the benefits of PCR as a tool for the detection of \textit{Leishmania} parasites in lesion aspirate specimens [16].

Diagnosis of CL although is clinically experienced of practitioner obvious in an endemic area, confirmed by using different techniques. In most CL-endemic countries, diagnosis is mainly based on epidemiological and clinical criteria, the existence of single or multiple nodular/ ulcerative skin lesions can be considered an indicator of Cutaneous Leishmaniasis causes skin sores depending on the parasite species, which can be topical in a particular place on the body (mostly on the face or limbs) or spread [17].

The best advantages of PCR-based methods are the high ability to detect the low amount of \textit{Leishmania} DNA in a variety of clinical specimens, such as blood, skin biopsy, bone marrow puncture, or samples prepared from Giemsa-stained slides even in paraffin block. Proper diagnosis and identification of the \textit{Leishmania} species is important for assess prognosis and appropriate treatment description [18] [19].

Cutaneous Leishmaniasis is increased in endemic and non-endemic areas due to the geographical expansion of the disease population migrations; and changing of international travel patterns [20].

The diagnostic procedure is the only remedy to determine the \textit{Leishmania} species in hospital as well as at the community level in endemic areas, similarity signs have occurred due to other parasites, and can cause the confusion in the diagnosis of species identification. [21].

References
للكشف عن أنواع اللشمانيا الجلدية في محافظتي صلاح الدين وبغداد

المستند: إستخدام ال PCR للكشف عن أنواع اللشمانيا الجلدية في محافظتي صلاح الدين وبغداد

الملخص

داء اللشمانيا هو مجموعة من الأمراض الطفيلية المنتشرة عالميا والناجمة عن أنواع مختلفة من اللشمانيا، والتي تسبب مجموعة متنوعة من

الشذرات بالضفة إلى الإنسان. العواعد وطرق العمل: أجريت الدراسة الحالية للتحقق من انتشار داء اللشمانيا الجلدي في بعض المناطق المستوطنة في العراق. بلغ مجموع العينات 117 عينة والتي تم جمعها من المرضى المشتبه بإصابتهم باللشمانيا الجلدية بمختلف الأعمار والجنسين وتشمل 73 مريضاً من الذكور و44 من الإناث.

النتائج: أظهرت نتائج PCR إن نسبة الإصابة 62.39% في الذكور في حين أن النسبة 37.60% في الإناث، وكان متوسط العمر 23.35 سنة.

الاستنتاجات: واستنتج من العمل الحالي أن الذكور أكثر إصابة باللشمانيا الجلدية من الإناث. وكشفت الدراسة الحالية أن تفاعل PCR هو أكثر الطرق فعالية وحساسية للكشف عن أنواع داء اللشمانيا الجلدي.