Cutaneous leishmaniasis in patients referred to the Pasteur Institute of Iran during 2003-2006
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Citation

Abstract
Cutaneous Leishmaniasis (CL) is a one of major health problems world wide. In Iran it is endemic in many parts of Iran. In the present study the frequency of CL were assessed in patients referred to the Department of Parasitology, Pasteur Institute of Iran in Tehran.
Initial personal and clinical information including age, gender, occupation, place of residence and number of lesions all were recorded in a questionnaire sheet. Patients with ulcerative skin lesions were examined for CL by using both direct microscopic examination and culture methods. Leishman body was observed in 43.4% out of 322 patients by direct smear and 45.34% by cultivation. In this study some cases of none healing leishmaniasis were observed. Most of patients have traveled to Kashan city that is one of the endemic areas for CL located in center of Iran. The results indicated that although CL is endemic in many parts of Iran and most of cases are diagnosed by direct microscopy, no significant differences were found in compare to culture method. It is recommended that parasitological and clinical information of CL could be useful before treatment.

INTRODUCTION
Leishmaniasis is endemic in 88 countries through out Africa, Asia, Europe, and North and South America. There are estimated 12 million cases world wide, with 1.5-2 million new cases each year (WHO, 2006). The parasite enters the human host with the bite of the sand fly and is pulled into macrophages by ingestion. Leishmania parasites are able to survive in acidic environment of the lysosome and become amastigote forms which cause disease in humans and affects cellular immunity (Talari, 2006). Parasite begins as an erythromatous papule at the site of sand fly bite on exposed parts of the body. Cutaneous leishmaniasis (CL) is still considered an important health problem in many parts of the world, especially the Mediterranean region, and almost all countries of the Middle East, including Iran (Talari, 2006; Momeni, 1994). In Iran the prevalence of disease is high in some provinces, including Isfahan (Salimi, 2000), Shiraz (Moaadab et al., 1993), Khorasan (Javadian et al., 1967), Khouzestan and Kerman (Nadim, 1971).

Although this disease dose not cause mortality, but a prolonged period of lesions, great expense of treatment , and side effects of available drugs, it has created many problems (Momeni, 1994; Talari, 2006). Diagnosis of CL is important for initiating appropriate clinical management and treatment of this disease. Microscopy may be the simplest method of achieving but diagnosis by cultivation has advantages. This study was performed in order to the abundance and characteristics of cutaneous leishmaniasis in patients referring to the Department of Parasitology, Pasteur Institute of Iran (IPI) during 2003-2006.

MATERIAL AND METHODS
PATIENTS AND SAMPLES
This descriptive study was carried on all patients clinically suspected of having CL who were referred to the Department of Parasitology for laboratory confirmation. The diagnosis of CL was based on clinical presentations and a positive parasitic smear and culture. For each case having cutaneous lesions, a questionnaire was completed to record the necessary information such as name, age, gender, address, location of ulcer on the body, data and place of acquiring the disease, previous travel history or work address. A total of 322 patients with skin lesions, suspected to CL were examined. For patients exhibiting more than one lesion, a detailed examination of each lesion was performed in order to choose the site of sample extraction.

DIRECT SMEAR
Generally samples were obtained only from those sites which showed the most indurate margin. The lesion was cleaned of debris with saline solution. Purulent or necrotic
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Ulcers were treated with particular care, and debris was removed before sampling. None of patients had received any antileishmanial chemotherapy treatment prior to diagnosis. Samples for parasitological diagnosis were dermal scrapings of the active indurate margins of lesions or dermal scraping of the bottoms of the ulcers. Skin scrapings from the edge of the lesion were obtained from each patient and was smeared on a slide for staining with Giemsa and examined microscopically for presence of amastigotes.

CULTURE METHOD

The samples were aspirated from edge of the skin lesions and cultured in liquid phase (normal saline) of Novy-Macneal-Nicole (NNN) media. The culture was incubated at 25°C and checked for growth of Leishmania promastigotes and supervised every day using an inverted microscope for 28 days (Ramirez et al., 2000; Romero, 1999).

RESULTS

The results indicated the amastigotes in skin smears and the frequency of CL in 322 patients was 45.3% (146 positive cases). Infections with CL were observed in 57.7% (83) males and 43.1% (63) females. Although, the highest rates (%28.8) of infection were recorded in both genders in 21-30 years age group, the lowest rates (8.9%) were observed in the age group of >50 years (Fig. 1).

Figure 1

Figure 1: Frequency of cutaneous leishmaniasis by age and gender.

Single lesions were observed in 45% of patients, appearing a round popular plaque with a diameter of 4-80 mm. In addition, double lesions were shown in only 23% of patients, however 32% represented multiple lesions between 3-7 (Fig. 2).

Figure 2

Figure 2: Percentage frequency of CL lesions in patients.

Detailed location of lesions was shown in Table 1. Hand and arm was the most commonly affected sites (48.6%).

Table 1: Lesions location of CL in patients referred to IPI

<table>
<thead>
<tr>
<th>Location</th>
<th>Number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Face and neck</td>
<td>40</td>
<td>27.3</td>
</tr>
<tr>
<td>Hand and arm</td>
<td>71</td>
<td>48.6</td>
</tr>
<tr>
<td>Legs</td>
<td>33</td>
<td>22.6</td>
</tr>
<tr>
<td>Other parts of body</td>
<td>2</td>
<td>1.37</td>
</tr>
</tbody>
</table>

All samples were scraped and aspirated from the edges of skin lesions and cultured in liquid phase of NNN media. After regular examination of the overlay, 146 isolates were grown in the culture media (Fig. 3).

Figure 3

Figure 3: Frequency of cutaneous leishmaniasis according to methods of diagnosis.

Residence, workplace, travel history of patients were important data for determining the location where the infections might have taken place. The highest infection rate (19.9 %) was recorded in patients who are traveling or living in Isfahan and Kashan . Lowest rate (3.4 %) was recorded in
patients living in Ghom, Fars, and Khouzestan provinces (Table 2).

**Figure 5**
Table 2: Frequency of CL by place of residence and travel history

<table>
<thead>
<tr>
<th>Name of province</th>
<th>Endemic area</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fars</td>
<td>Shiraz &amp; Jahrom</td>
<td>5</td>
</tr>
<tr>
<td>Ghom</td>
<td>Ghom</td>
<td>5</td>
</tr>
<tr>
<td>Ilam</td>
<td>Dehloran</td>
<td>16</td>
</tr>
<tr>
<td>Isfahan</td>
<td>Isfahan &amp; Kashan</td>
<td>29</td>
</tr>
<tr>
<td>Kerman</td>
<td>Bam</td>
<td>7</td>
</tr>
<tr>
<td>Khorasan</td>
<td>Mashhad</td>
<td>22</td>
</tr>
<tr>
<td>Khouzestan</td>
<td>Ahvaz</td>
<td>5</td>
</tr>
<tr>
<td>Semnan</td>
<td>Damghan &amp; Shahrod</td>
<td>21</td>
</tr>
<tr>
<td>Tehran</td>
<td>Tehran &amp; Varamin</td>
<td>12</td>
</tr>
<tr>
<td>Yazd</td>
<td>Yazd</td>
<td>6</td>
</tr>
<tr>
<td>Other countries</td>
<td>Afghanistan</td>
<td>20</td>
</tr>
</tbody>
</table>

Leishmania parasite was isolated from the unhealed lesions of a woman who had lesion in her face within period of about 2.5 years. She lived in Sabzevar (Khorasan province), where the CL is endemic (Fig. 4).

**Figure 6**
Figure 4: Unhealed CL lesion from a patient from Khorasan

In this study the location of lesions were on the hands and arm (48.6 %), face (27.3 %), legs (22.6 %) and other parts of the body (1.37 %). Because the phlebotomies attack the exposed area of the body to suck the blood, the lesions mostly appears in the hands, face and legs and upper parts of the body, which may be due to the outdoor sleeping on the terraces or yards without using bed net during summer.

In conclusion, the results indicated that although CL is endemic in many parts of Iran, and the frequency of non leishmanial skin lesions is high, and because of misdiagnosing CL lesions, performed parasitological smear isn’t an adequate method and must used parallel with culture and other techniques. A timely and definitive diagnosis of CL is important for initiation appropriate clinical management and treatment of this disease. Microscopy may be the simplest method of achieving these, but undiagnosed cases associated with this method show its inadequate sensitivity.

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