The recent emergence of Leishmania tropica in Jericho (A'riha) and its environs, a classical focus of L. major

Article in Tropical Medicine & International Health · August 2004
DOI: 10.1111/j.1365-3156.2004.01268.x · Source: PubMed

CITATIONS 56
READS 76

9 authors, including:

Amer Al-Jawabreh
LRU-Jericho
42 PUBLICATIONS 623 CITATIONS

Abedelmajeed Nasereddin
Hebrew University of Jerusalem
118 PUBLICATIONS 2,089 CITATIONS

Jan Schwenkenbecher
14 PUBLICATIONS 553 CITATIONS

Fikry Barghuthy
12 PUBLICATIONS 187 CITATIONS

Some of the authors of this publication are also working on these related projects:

Surface active germanium complexes View project

Leishmaniasis View project

All content following this page was uploaded by Amer Al-Jawabreh on 24 September 2014.
The user has requested enhancement of the downloaded file. All in-text references underlined in blue are added to the original document and are linked to publications on ResearchGate, letting you access and read them immediately.
The recent emergence of *Leishmania tropica* in Jericho (A’riha) and its environs, a classical focus of *L. major*

A. Al-Jawabreh1,2, L. F. Schnur3, A. Nasereddin3, J. M. Schwenkenbecher1, Z. Abdeen4, F. Barghuthy5, H. Khanfar6, W. Presber1 and G. Schönian1

1 Institute of Microbiology and Hygiene, University Medicine, Charité, Berlin, Germany
2 Islah Medical Laboratory, Islah Charitable Social Society, Jericho, Palestine
3 Hebrew University-Hadassah Medical School, Jerusalem, Israel
4 Department of Community Health, Faculty of Medicine, Abu-Deis, Palestine
5 Faculty of Public Health, Al-Quds University, Abu-Deis, Palestine
6 Department of Science and Technology, Al-Quds University, Abu-Deis, Palestine

**Summary**

Between 1997 and 2002, 49 strains of *Leishmania* were isolated from the cutaneous lesions of Palestinians living in and around Jericho. A polymerase chain reaction (PCR) amplifying the ribosomal internal transcribed spacer 1 (ITS1-PCR) was applied to their cultured promastigotes and to 207 individuals’ skin scrapings spotted on filter-papers, 107 of which proved positive for leishmanial DNA. Species identification was performed by restricting the ITS1-PCR amplification products from the cultured promastigotes and the amastigotes in the scrapings with the endonuclease *Hae* III. Of the 49 cultures, 28 (57%) were *L. major* and 21 (43%) were *L. tropica*. Of the 107 dermal samples tested directly, 53 (49.5%) were infected with *L. major*, 52 (48.5%) with *L. tropica* and two remained unidentified. This is the first time *L. tropica* has been exposed in the population of the Jericho area and on such a large scale. The itinerant behaviour of some of this population precludes categorically declaring that *L. tropica* has recently become established in this classical focus of *L. major*. For this and although 88.2% of the cases of *L. tropica* claimed not to have travelled out of the vicinity of Jericho, local infected sand fly vectors of *L. tropica* must be caught, identified and, if possible, shown to harbour infections, and, if one exists, an animal reservoir host should also be exposed to endorse whether the cases caused by *L. tropica* were imported or autochthonous.

**Keywords** *Leishmania major*, *Leishmania tropica*, ITS1-PCR, filter papers, Jericho area

**Introduction**

The District of Jericho (Figure 1, map 1), with the City of Jericho as its administrative centre, is the geographical area encompassed by the town of Nablus, which is just north but not part of it, the northern end of the Dead Sea in the south, the River Jordan in the east and the town of Ramallah, which is just west but also not part of it. The southern part of the Jordan Valley, which is a typical focus for zoonotic cutaneous leishmaniasis (ZCL) caused by *Leishmania major* (Schlein et al. 1982, 1984; Arda & Kamal 1983; Al-Jawabreh et al. unpublished data), runs through it. The City of Jericho itself is small and rural and not a typical urban city. It has a constant and plentiful supply of water, which gives it the appearance and ambiance of a desert oasis.

The ZCL is a reportable disease. In this part of the Jordan Valley, the animal reservoir host is the fat sand rat, *Psammomys obesus* (Schlein et al. 1984) and the vector the female of the sand fly species *Phlebotomus papatasi* (Schlein et al. 1982, 1984).

Diagnosis of CL at the regional clinic in Jericho was performed mainly by microscopical examination of dermal tissue smears stained with Giemsa’s stain and the culture of dermal tissue aspirates and biopsies in rabbit blood-agar medium. This classical method lacks maximum sensitivity and still requires identification of the infecting species of *Leishmania*. In human cases, lesions caused by *L. major* self-heal and bestow immunity to re-infection. Therapy, if applied, is usually by Pentostam® administered intra-lesionally. Treatment of CL caused by *L. tropica* is sometimes less successful. Based on the assumption that *L. major* transmitted by *Ph. papatasi* is the only cause of human CL in this region, massive spraying in the entire Jericho District for 40 days at, both, the beginning and end of the sand fly season, which are in April and September, is the adopted method of control. The severity of some cases, slow response to treatment and only partial efficacy of the control strategy have raised uncertainty concerning the cause of some of the cases of CL encountered.
Methods

Target group and study area

Patients with lesions suspected of being CL, who were referred to the Islah Medical Laboratory in Jericho between June 1997 and December 2002, represented all the inhabited areas of the District of Jericho. This included the city, the refugee camps, the villages and the Bedouin encampments. Because of the poor response to treatment, species other than L. major were suspected of being a cause of CL in this focus. Forty-nine strains were isolated by culturing dermal tissue aspirates in rabbit blood-agar semisolid and NNN media and identified by employing a polymerase chain reaction (PCR) amplifying the parasites’ ribosomal internal transcribed spacer 1 (ITS1-PCR). Also, 107 cases were diagnosed by using the ITS1-PCR directly on dermal tissue aspirates spotted on filter papers.

DNA Extraction

DNA from cultured strains of Leishmania was extracted as described by Van Eys et al. (1992). Disks of filter papers with dermal tissue aspirate were aseptically punched out of clinical samples. To avoid DNA contamination, 10 disks were punched out of clean filter paper immersed in 70% ethanol before punching out the next clinical sample. Two disks were placed in 250 μl lysis buffer (50 mM NaCl, 50 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.4); 1% Triton X-100 and 200 μg proteinase K/ml) and incubated for three hours or overnight at 60 °C. Lysates were then subjected to phenol–chloroform extraction as described by Meredith et al. (1993). The DNA pellets were dried, using a speed vacuum dryer (savant, speedvac 100) for 5–10 min and re-dissolved in 100 μl TE buffer (10 mM Tis and mM EDTA pH 7.5). The extracted DNA was then purified, using Nucleospin® Extract (Macherey Nagel GmbH & Co. KG, Dueren, Germany). The 30 μl samples were kept at −20 °C until used.

PCR Amplification

A PCR was used to amplify the ribosomal internal transcribed spacer 1 (ITS1) region, which separates the genes coding for the ssu rRNA and L5.8S rRNA, using the primers LITSR and L5.8S for PCR-ITS1 as described by El Tai et al. (2000) and Schoenian et al. (2003). Amplification reactions were performed in volumes of 50 μl. Three microlitres of genomic DNA or 2 μl of PCR product (for re-amplification) or 3 μl genomic DNA (for the DNA extraction control) were added to a PCR Master Mix, containing 200 μM of each dNTP, 1.5 mM MgCl₂, 1 unit Taq polymerase, 25 pmol of each primer and 2.5% DMSO as an enhancer. The samples were overlaid with sterile, light mineral oil and amplified as follows: initial denaturation at 95 °C for 2 min followed by 35 cycles of denaturation at 95 °C for 20 s, annealing at for 53 °C for 30 s for both primer pairs, LISTR/L5.8S and HβG-F/HβG-R, and extension at 72 °C for 1 min. This was followed by a final extension cycle at 72 °C for 6 min. The PCR was run in a Perkin Elmer Cetus 9600. PCR amplifications products were run electrophoretically in 1% agarose at 100 V in 0.5X TBE buffer (0045 mM Tris-borate, 1 mM EDTA) and

Figure 1

Map 1 shows the distribution of Leishmania major (the first number) and L. tropica (the second number) in the District of Jericho. Map 2 shows the distribution in the City of Jericho and its immediate vicinity: the triangle = a case caused by L. tropica and the oval = a case caused by L. major.
visualized under UV light after staining with ethidium bromide (0.5 μg/ml) for 15 min. DNA from the international reference strain of L. tropica (MRHO/MN/83/MNR-6) was run alongside each sample as a positive control and sterile distilled water was the negative control. For each diagnostic sample, an internal control for PCR inhibition was included where the same amount of purified DNA as in positive controls was added to these samples. The primer pair HβG-F (5’-GAA GAG CCA AGG ACA GGT AC-3’)//HβG-R (5’-CAA CTT CAT CCA CGT TCA CC-3’), specific for human β-globin, was used as a DNA extraction control to check true negativity of the ITS1-PCR, under the same conditions described above for ITS1-PCR. All negatives were subject to DNA extraction control.

To obtain enough material for subsequent restriction analysis the ITS1-PCR products were re-amplified, using the same primer combination and PCR conditions as for the first round of amplification.

Restriction analysis

Each ITS1-PCR amplification product (15 μl) was digested at 37 °C for 2 h with 1 μl of either the two restriction enzymes HaeIII and MnlI without prior purification, using the conditions recommended by the supplier (Hybaid GmbH Heidelberg, Germany). The restriction fragments were run electrophoretically in 2% agarose gel at 100 V in 1x TBE buffer and visualized under ultraviolet light after staining with ethidium bromide (0.5 μg/ml).

Results

All classical stages of the development of leishmanomas were seen among the cases: from small erythematous papules through nodules and to ulcerative lesions; whereas unusual clinical manifestations such as the sporotrichoid pattern, i.e., subcutaneous nodules developing along lymphatics, hyperkeratosis, i.e., thick adherent scale and leishmaniasis recidivans also known as lupoid leishmaniasis were not. It was very difficult and even impossible to discern if cases were caused by L. major or L. tropica by the clinical picture. However three severe cases led to the suspicion that they were not caused by L. major, as they were different from the classical stages of CL generally seen in the vicinity of Jericho. The three cases displayed common features. They all presented single lesions, two of which were on the nose and one on the chin. Development was slow and all three only sought medical advice 6–12 months after the first appearance of the lesion. All three lesions resisted antimony treatment and took 6 months or more to heal leaving scars. The lesion on the chin and one of those on the nose were caused by L. tropica. Of the 68 cases caused by L. tropica, 60 (88.2%) said they had not travelled out of the vicinity of Jericho in the last 3 months. The other eight cases were people who came either from the hilly regions around Jerusalem or cities like Ramallah and Jenin. Of the 57 cases caused by L. major, 43(75.4%) also said they had not travelled out of the vicinity of Jericho in the last 3 months.

Most cases had single lesions on the cheek or arm (Table 1). Most of the patients (67%) sought medical intervention in <3 months of the appearance of the lesion as Jericho is a hyperendemic region and the inhabitants are well aware of CL, which is also partially owing to the education campaigns conducted by Islah Medical Center in Jericho over the last few years.

Forty-nine strains were isolated by culturing dermal tissue aspirates in either rabbit blood–agar semisolid medium or NNN medium. Of the 207 dermal clinical samples spotted on filter papers checked by the ITS1-PCR, 107 were positive, indicating a 52% positivity rate. Amplification of the leishmanial DNA present, in this case the 300–350 bp ITS1 amplicon, and its subsequent digestion with the endonuclease HaeIII did enable detection of the parasites and identify the species of Leishmania to which they belonged. The restriction patterns obtained for L. major, L. tropica and L. infantum (not shown), another species of Leishmania present in the Middle East, were clearly different and enabled the identification of the

Table 1 Comparison of the clinical features of cases of CL caused by Leishmania major and L. tropica in the district of Jericho

<table>
<thead>
<tr>
<th>Duration (months)</th>
<th>L. major (%)</th>
<th>L. tropica (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤3</td>
<td>34 (87.2)</td>
<td>33 (72.2)</td>
</tr>
<tr>
<td>3–6</td>
<td>15 (38.5)</td>
<td>9 (19.2)</td>
</tr>
<tr>
<td>&gt;6</td>
<td>0 (0.0)</td>
<td>5 (10.6)</td>
</tr>
<tr>
<td>Total*</td>
<td>39</td>
<td>47</td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forehead</td>
<td>5 (6.7)</td>
<td>8 (9.4)</td>
</tr>
<tr>
<td>Chin</td>
<td>4 (5.3)</td>
<td>9 (10.5)</td>
</tr>
<tr>
<td>Eye</td>
<td>2 (2.6)</td>
<td>4 (4.7)</td>
</tr>
<tr>
<td>Nose</td>
<td>2 (2.6)</td>
<td>6 (7)</td>
</tr>
<tr>
<td>Ear</td>
<td>5 (6.7)</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>Cheek</td>
<td>15 (20)</td>
<td>22 (25.9)</td>
</tr>
<tr>
<td>Arm</td>
<td>17 (22.7)</td>
<td>24 (28.2)</td>
</tr>
<tr>
<td>Leg</td>
<td>17 (22.7)</td>
<td>10 (11.8)</td>
</tr>
<tr>
<td>Neck</td>
<td>2 (2.6)</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>Total*</td>
<td>75</td>
<td>85</td>
</tr>
<tr>
<td>No. of lesions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>26 (45.6)</td>
<td>40 (61.5)</td>
</tr>
<tr>
<td>2</td>
<td>15 (26.3)</td>
<td>11 (16.9)</td>
</tr>
<tr>
<td>≥3</td>
<td>16 (28.0)</td>
<td>17 (26.2)</td>
</tr>
<tr>
<td>Total*</td>
<td>57</td>
<td>65</td>
</tr>
</tbody>
</table>

* Figures represent the total number of patients who answered the question related to each clinical feature.
isolated strains at the species level (Figure 2). Fifty-three (49.5%) of the filter paper samples contained DNA of L. major and 52 (48.5%) DNA of L. tropica. The parasite species of the amplified DNA samples from two (2.0%) of the filter paper samples remained unidentified. Of the 49 cultured, characterized and identified strains, 21 (43%) were L. tropica and 28 (57%) were L. major. Cases caused by L. major and cases caused by L. tropica were both found in almost all the inhabited areas of the District of Jericho.

Discussion

Restriction analysis of the ITS1-PCR positive samples revealed the co-appearance of L. major and L. tropica in Jericho and its immediate vicinity (Figure 1). The existence of L. major in the lower Jordan Valley, including the margins of Jericho, and the human CL it causes as zoonoses has been well-documented (Schlein et al. 1982, 1984; Al-Jawabreh et al. 2004). The presence of L. tropica in the human population of the Jericho area is a novel finding, although it has been recorded as causing human cases of CL in a focus at a higher altitude just east of Jerusalem (Schnur et al. 2004), infected with L. tropica were present in the area during the sand fly season and, if there is one, also in the infected animal reservoir host.

What is significant is that even before and since the Israeli Army took over the ‘West Bank region’ people travelled between the hills and the Jordan Valley, yet cases of CL caused by L. tropica were extremely rare throughout the whole District of Jericho, including the mountainous regions. Now, more and more cases caused by L. tropica are being seen in many foci of this part of the Eastern Mediterranean region (Klaus et al. 1994; Anis et al. 2001; Nimri et al. 2002; Jacobson et al. 2003; Schnur et al. 2004) so there has definitely been a change. However, this, it appears, has taken place in the hills rather than in the Jordan Valley. So far, there has been no evidence of Ph. sergenti existing in and being a potential vector of L. tropica, in the City of Jericho and its environs, although Schlein et al. (1984) found it 10–15 km south of Jericho at Ein Gedi on the western shore of the Dead Sea and in the Arava, which is even further south.

Prior to 1998, the diagnosis of Palestinian cases of CL was restricted to microscopical examination of smears stained with Giemsa’s strain, which did not lend itself to the identification of different species of Leishmania. Later culture and PCR-based diagnosis and leishmanial species identification directly from dermal scrapings on filter
papers were introduced. This is the first study in the District of Jericho to use new molecular biological diagnostic technology and to such an extent. Species identification is a vital step in the diagnostic procedure, especially in areas where more than one species of Leishmania occurs. This impinges on control strategy, therapy and determining the epidemiology and dynamics of the disease. The presence and increasing numbers of human cases caused by L. tropica are a definite change compared with the past when all cases from this area, from which parasites were isolated and identified, were shown to harbour L. major and none were shown to harbour L. tropica.

Acknowledgements

We thank Mrs Hanaan Al-Jawabreh of the Islah Medical Laboratory, Jericho, who helped collect the dermal samples and leishmanial isolates. This work was supported by grants of the Deutsche Forschungsgemeinschaft (Scho 448/6-1-3) and by a fellowship of the Deutscher Akademischer Austauschdienst (DAAD) to A. Al-Jawabreh.

References


Acknowledgements

We thank Mrs Hanaan Al-Jawabreh of the Islah Medical Laboratory, Jericho, who helped collect the dermal samples and leishmanial isolates. This work was supported by grants of the Deutsche Forschungsgemeinschaft (Scho 448/6-1-3) and by a fellowship of the Deutscher Akademischer Austauschdienst (DAAD) to A. Al-Jawabreh.

References


Acknowledgements

We thank Mrs Hanaan Al-Jawabreh of the Islah Medical Laboratory, Jericho, who helped collect the dermal samples and leishmanial isolates. This work was supported by grants of the Deutsche Forschungsgemeinschaft (Scho 448/6-1-3) and by a fellowship of the Deutscher Akademischer Austauschdienst (DAAD) to A. Al-Jawabreh.

References