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ORONASAL LEISHMANIASIS CAUSED BY A PARASITE WITH AN UNUSUAL ISoenzyme PROFILE

MUNTAZER IBRAHIM, AHMED SULIMAN, FASSAL A. HASIB, EL TAHIR A. O. KHAZIL, DAVID A. EVANS, ARSALAN GHARAZMI, and AHMED M. EL HASSAN

Leishmaniasis Research Group, Institute of Disease Control, University of Khartoum, Khartoum, Sudan; National Public Health Laboratory, Khartoum, Sudan; Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, London, United Kingdom; Centre for Medical Parasitology, University of Copenhagen, Copenhagen, Denmark

ABSTRACT
A 45-year-old Sudanese man from western Sudan presented with oronasal leishmaniasis of three years duration. He had no history of previous kala-azar or cutaneous leishmaniasis. The parasite isolated from the oral mucosa was characterized by isoenzymes using 12 enzymes and by polymerase chain reaction amplification of kala-azar DNA using species-specific primers. The specific primers gave products indistinguishable from those of the Leishmania donovani complex. However, the isoenzyme profile showed a symbiotic pattern which was significantly different from the symbionesis previously reported in the Sudan and the Ethiopian region.

Visceral,1 cutaneous,2 and mucosal1 leishmaniasis are endemic in the Sudan. Mucosal leishmaniasis is the least common; approximately 70 cases have been reported since the first case was described by Christophorides 80 years ago.3 The disease affects the mucosa of the upper respiratory tract and/or the oral mucosa and is usually not preceded or accompanied by skin lesions. Mucosal leishmaniasis may follow treated kala-azar; or, rarely, both kala-azar and mucosal leishmaniasis may occur in the same patient.4 Based on the epidemiology of the disease and its association with kala-azar, Sudanese mucosal leishmaniasis was suspected of being caused by Leishmania donovani, the cause of visceral leishmaniasis in the country.5,6 In a previous report, L. donovani and L. major were identified in mucosal leishmaniasis lesions by Southern blot and a specific DNA probe.7 Using the polymerase chain reaction (PCR) and species-specific primers, parasites from patients with mucosal leishmaniasis were characterized as L. donovani 1.5,8

In this paper, we describe a Sudanese patient with mucosal leishmaniasis caused by a parasite with a symbiotic pattern that has not been described previously in humans.

MATERIALS AND METHODS

Parasite isolation and isoenzyme characterization. Tissue material obtained by punch biopsy was used for parasite culture. Tissues were immersed briefly in alcohol and washed thoroughly in RPMI 1640 medium and homogenized in a sterile tissue homogenizer. The homogenized tissue was injected into culture bottles containing blood agar plus RPMI 1640 medium and 10% fetal calf serum. Primary cultures were eventually expanded in larger volumes of media for subsequent use in isoenzyme analysis.

Isoenzyme typing was carried out as described by Le Blancq and others.9 Briefly, parasites were harvested by centrifugation and lysed by freeze-thawing. The lysate was stored as beads in liquid nitrogen until use. Twelve enzymes were tested in a thin slice electrophoresis system in which well-characterized Leishmania reference strains known to occur in the region (Table 1) were included along with the isolated parasite. The enzymes tested were aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), e

![Table 1](image)

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
<th>Enzyme</th>
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<tr>
<td>M96M/35000/E5l30HIII</td>
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<tr>
<td>M96M/35000/E5l30HIII</td>
<td>LCH 56</td>
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![Figure 1](image)

**FIGURE 1.** Amplification of kala-azar of four Leishmania isolates from Sudan (lanes 3-6). Lanes 1 and 7, DNA marker (4X174 Hae III digest); lane 2, negative control (no DNA); lane 3, M96M/35000/E5l30HIII (L. donovani); lane 4, M96M/35000/E5l30HIII (isolated from the patient with mucocutaneous leishmaniasis); lane 5, M96M/35000/E5l30HIII (L. major). Valets on the left are in basepairs.
Figure 2. Isoenzyme patterns of polymorphic loci for a, aspartate aminotransferase; b, 6-phosphogluconate dehydrogenase; c, nucleoside hydrolase; and d, proteinase isoenzyme of Leishmania species. Lane 1, MHOI/SD/94/Adel; lane 2, MHOI/SD/94/Adel; lane 3, MHOI/SD/94/Adel; lane 4, MHOI/SD/94/Adel; lane 5, MHOI/SD/94/Adel; lane 6, MHOI/SD/94/Adel; lane 7, MHOI/SD/94/Adel.

Amplification of DNA with species-specific primers. Leishmania total DNA was extracted from 10 ml of parastomal culture by conventional phenol-chloroform extraction and minicircle kinetoplast DNA (kDNA) was amplified using the L. donovani species-specific primers DBS and AIS (kindly provided by D. C. Barker, Molexis Laboratories, Cambridge, UK) as described by Smyth and others.11

Results

Clinical history and presentation. A 45-year-old man from El Obeid in western Sudan presented with oral swelling, bleeding gums, a sensation of fulness in the mouth, and difficulty in mastication of three years duration. The condition had developed shortly after the patient was on a visit to Libya. There was no previous history of cutaneous lesions or kala-azar. At examination, the patient was afebrile and looked well. The liver and spleen were not palpable. The mucosa covering the palate floor of the sphen, cheeks, and gums was swollen, red, and fissured. The success of right nostril showed a similar appearance but the e was no nasal perfor-
A biopsy of the palate stained with hematoxylin and eosin showed a monocellular infiltrate inflammatory reaction consisting of lymphocytes, macrophages, and plasma cells. Scattered in the infiltrate were several discrete epithelial granulomas. Leishmaniasis parasites were found in a Giemsa-stained impression smear of the biopsy but no parasites were seen in the sections. (Hemoglobin, which is usually seen in lymphocytes and plasma cells) The biopsy was positive for Leishmania parasites. The direct agglutination test result for Leishmania antibodies was negative. Bone marrow aspiration did not show Leishmania parasites. The direct agglutination test result for Leishmania antibodies was negative. The bone marrow aspiration was negative. A leishmanin skin test result using L. infantum antigen (kindly supplied by Dr M. Gramiccia, Instituto Superiore di Sanita, Rome, Italy) was negative.

Purative characterization. A PCR amplification of Leishmania DNA using species-specific primers directed against a circle of the parasite kinesin-like DNA gave a product size of approximately 800 base pairs (bp) corresponding to members of the L. donovani complex. The amplicon of Leishmania DNA produced a pattern that was indistinguishable from those obtained with other isolates from the region. This species specificity of the isolation by 12 polymerase enzymes displayed significant differences from thezymodemes present both in the Sudan and the Ethiopian region at four enzyme loci, namely ASAT, GPDH, PEPD, and NPH (Fig. 2). The difference at the ASAT locus, which is the main site of polymorphism for members of the L. donovani group in Sudan, was pronounced and characterized by its remarkably slow mobility in the gel (Fig. 2a, lane 7).

DISCUSSION

The parasitc isolate from this patient has not been de- scribed before in patients with mucosal or other forms of leishmaniasis in the Sudan. Previous isolates in the area from this series are Leishmania infantum and Leishmania major by Southern blot and specific DNA probe and by PCR using species-specific primers. The only parasite characterized by enzymatic analysis was shown to be Leishmania major (40–48), the same parasite that causes visceral leishmaniasis in the southern Sudan, among the M. lehmnaite tribes of Kedorot State in the western part of the country, and in the eastern Sudan.

It is worthy of note that the results of the PCR analysis of DNA from this species-specific primers the parasite in the patient described here was indistinguishable from L. donovani. It was only through its isoenzyme profile that it was possible to distinguish the parasite from L. donovani. From the history of the patient, we were not able to tell whether he was infected in Sudan or elsewhere. Therefore, there is a need to isolate and characterize more parasites from mucosal and other forms of leishmaniasis, particularly in western Sudan, by both PCR and isoenzyme analysis. The characteristic band mobility, partly at the ASAT locus, does not resemble any other isoenzyme described in human, suggesting that this is a new species and indeed a unique one.

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REFERENCES


