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Clinicoepidemiologic pattern of cutaneous leishmaniasis and molecular characterization of its causative agent in Hajjah governorate, northwest of Yemen

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ABSTRACT

The clinicoepidemiologic profile of 143 cases (93 males and 50 females) with cutaneous leishmaniasis from 18 villages of Hajjah governorate, Yemen was studied. Dry-type lesions were seen in 98.6% and wet-type lesions in 1.4% of patients. Lesions were localized in all cases with different morphological patterns. Microscopic examination of Giemsa-stained slit smears revealed amastigotes in 74.1% of patients with dry-type lesions and 0% in patients with wet-type lesions. The burden of the parasites in the lesions was high indicating active transmission of the disease. Most cases were from villages with moderate altitude range (8001–1600 m). All age groups were affected, but most cases were seen in ages from 5 to 15 years. *Leishmania* species identification was done for all cases by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The biopsic material was scraped from both Giemsa-stained and methanol-fixed smears. The molecular characterization of *Leishmania* species revealed *Leishmania tropica* as the causative agent of cutaneous leishmaniasis in Hajjah, Yemen. The risk factors associated with the transmission of the disease and recommendations for improving case detection were discussed.

1. Introduction

Leishmaniases are among the most important and resurging vector-borne protozoal diseases. The leishmaniases are second only to malaria in terms of number of people affected (WHO, 2010). Cutaneous leishmaniasis (CL) is endemic in many regions of the Old World. It is caused by different dermotropic *Leishmania* species (*L. tropica, L. major* and *L. aethiopica*) and occasionally by viscerotropic ones (*L. infantum* and *L. donovani*).

Leishmaniasis is the most prevalent skin infectious disease in Yemen (Al-Kamel, 2015). It is responsible for 60% of Disability Adjusted Life Years (DALYs) lost due to tropical-cluster diseases prevalent in Yemen (WHO, 2008). CL is the common form of leishmaniasis in Yemen causing disfiguring skin sores and sometimes disabling in severe lesions. The clinical pattern of the disease in some cases shows variation in the severity and duration and low response to treatment in some cases (Khatri et al., 2006). Published research available does not reflect the true incidence of the disease (Sarnelli, 1933; Rioux et al., 1986, 1989; Pratlong et al., 1995; Khatri et al., 2006, 2009). *L. tropica* is known as the common causative agent of CL in Yemen (Khatri et al., 2006, 2009). However, this species has also been identified as causing severe visceral infections (Magill et al., 1993, 1994). The inability to determine the species of *Leishmania* limits the knowledge associated with the dynamics of disease transmission such as the presence of a reservoir host(s) and vector(s) responsible for the transmission of the disease which, in turn, will assist establishing a control strategy. The routine method using microscopic examination of Giemsa-stained smears for the diagnosis of *Leishmania* does not enable species identification. Molecular identification of *Leishmania* species in Yemen has been adopted in few studies. Khatri

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et al. (2009) identified *L. tropica* from methanol-fixed smears using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 85.80% of the cases from eight governorates of northwestern Yemen including Hajjah governorate. In addition, the authors pointed out that *L. tropica*, *L. infantum* and *L. donovani* were found sympatric in Hajjah governorate. Mahdy et al. (2010) identified *L. tropica* from the archives of the Central Health Laboratory in Sana'a Yemen based on the sequencing of the ribosomal internal transcribed spacer 1 (ITS1) locus.

Reports from the Yemen Ministry of Public Health and population show a progressive increase of leishmaniasis from 1009 cases in 2007–2475 in 2012. Continuous surveillance of the disease is an essential component of control or elimination program. Accordingly, this is an in-depth study in 18 villages in Hajjah governorate to monitor the clinical status of cutaneous leishmaniasis and to identify the causative *Leishmania* species.

2. Materials and methods

This is a passive case detection-13-month study of 143 cases of CL (93 males and 50 females) seen at the dermatology clinic of Saudi Hospital at Hajjah City between March 2013 and March 2014. All patients were from Hajjah governorate. For all reported cases, details of the history, physical findings, laboratory data, treatment and follow-up were recorded with special emphasis on residency in endemic region, history of travelling to other endemic areas. The diagnosis was based on clinical features and parasitological examination. In some cases, part of the smear slides was methanol-fixed for comparison with Giemsa-stained smears for molecular typing of parasites.

2.1. Description of the study area

Hajjah governorate is located in northwestern Yemen with an area of about 8288.3 km^2 and altitude ranging from 0 to 2400 m. It lies between LAT. 15° 30′ N–16° 37′ N, and LONG. 42° 30′ E–43° 45′ E. (Fig. 1). The governorate is characterized by three topographical zones based on altitudes (Almisly, 2008): Tihama lowland (0–800 m); West highland (801–1600 m) and Interior highland (1601–2400 m).

2.2. Parasitological diagnosis

The parasitological diagnosis was based on microscopic examination of Giemsa-stained skin slit smears (Amro et al., 2012) taken from the edge of the lesions (some times more than one smear was taken). If amastigotes were not seen after microscopic examination using 1000 oil immersion field (OIF), the smear was considered negative. According to Ramírez et al. (2000) amastigote density was quantified using a semi-quantitative scale; +, 1 amastigote/whole slide to 1 amastigote/OIF in a total of at least 100 OIF; ++, 2–10 amastigotes/OIF in a total of at least 50 OIF; +++, 11–20 amastigotes/OIF in a total of at least 50 OIF; ++++, >21 amastigotes/OIF in a total of at least 10 OIF.

2.3. Molecular identification of Leishmania species

Human lesion smear samples (Giemsa-stained or methanol-fixed) were scraped from the slide and collected in 1.5 ml Eppendorf tubes. DNA was extracted using the Qiagen DNA mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA was eluted in 200 μl elution buffer and kept at −20 °C for PCR assay.

2.4. PCR amplification

A PCR assay was used to amplify the ribosomal internal transcribed spacer 1 (ITS1) followed by restriction fragment length polymorphism (RFLP) using HaeIII restriction enzyme according to Schönian et al. (2003). The set of forward primers LITSR (5′-CTGGATCATTTTCCGATG-3′) and L5.8S (5′ TGATACCACTTATCGCACTT-3′) was used to amplify 321 bp of rDNA including parts of 3′ end of 18S rDNA gene, complete ITS1 and part of 5′ end of 5.8S rDNA gene. Amplification reaction was performed in a volume of 50 μl containing 2.5 mM of each dNTP, 1.5 mM $MgCl_2$, 5 units GoTaq flexi polymerase, 30 uM of each primer and 5 μl of sample. Each PCR reaction included a positive control (DNA from a reference strain: *L. tropica*–WR1063-3/27/2010) and a negative control (water). The reaction was done in a thermocycler with the following steps: initial denaturation at 95 °C for 2 min, followed by 34 cycles each, consisting of denaturation at 95 °C for 20 s, annealing at 53 °C for 30 s, and extension at 72 °C for 1 min followed by a final extension cycle at 72 °C for 7 min. PCR products were analyzed using a 2% agarose gel stained by GelRed (Biotium) and visualized by UV-light transilluminator.

2.5. RFLP analysis of amplified ITS1

ITS1-PCR products were assigned to RFLP analysis. The PCR products were digested with the restriction endonuclease HaeIII (New England Biolabs, Ipswich, MA, USA) for species identification. The reaction was incubated for complete digestion at 37 °C for one h. The produced restriction fragments were separated by electrophoresis on 2% agarose gel and were visualized under UV light. DNA fragment sizes were determined by 1 Kb DNA ladder and compared with the reference strain of *L. tropica* –WR1063-3/27/2010.

2.6. Ethical approval

The study was approved by the Faculty of Science and Education, Hajjah University. No information on the patients has been presented in this research.

3. Results

All cutaneous leishmaniasis cases were Yemeni nationals originating from 18 villages of Hajjah governorate (Fig. 1). Dry-type lesions were seen in 98.6% (N = 141) of the patients and wet-type lesions in 1.4% (N = 2). The geographical distribution of CL cases was presented in Table 1. Most of the cases originated from three villages with moderate altitude range of the West highland (801–1600m) namely Kohlan-Afar; Al' Sharagi and Hajjah City. The percent of cases from individual villages were 30.1%, 23.1% and 16.1% respectively. Very few cases (range from 0.7% to 2.1%) were recorded from villages of the Tihama lowland region (0–800m) and the Interior highland (1061–2400m). The age group of patients ranged from less than five years to more than 15 years. Most of the cases were in subjects aged 5–15 years for males and females. The infection rate among males was higher than females and the sex ratio was 1.86:1 (Table 1). The number of lesions per patient ranged from 1 to 16. Most of the patients had single lesion (N = 107, 74.8%); 23 (16.1%) had two lesions, 4 (2.8%) had three lesions, 6 (4.2%) had four lesions, two (1.4%) had five lesions, and one (0.7%) had 16 lesions. The size of the lesions varied from 0.5 to 6 cm. The types of lesions in the majority of patients were localized with different morphological patterns (Fig. 2). The lesions were located on the face in 75.6% of patients; on the upper extremities in 18.9% of the patients; on the lower extremities in 5.5% of the patients. Giemsa-stained smears showed *Leishmania* amastigotes in 106 patients and no amastigotes were detected in 37 smears (two of which were