Acta Tropica 154 (2016) 125–132

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Clinical and epidemiological features of leishmaniasis in northwestern-Argentina through a retrospective analysis of recent cases

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ARTICLE INFO

Article history:
Received 14 May 2015
Received in revised form 27 October 2015
Accepted 13 November 2015
Available online 21 November 2015

Keywords:
Cutaneous leishmaniasis
Mucocutaneous leishmaniasis
Visceral leishmaniasis
Leishmania (V.) braziliensis
Leishmania (L.) amazonensis
Leishmania (L.) infantum

ABSTRACT

Leishmaniasis is a parasitic disease caused by hemoflagellates of the genus *Leishmania* and is transmitted to humans by the bite of infected phlebotomine sandflies. Depending on the *Leishmania* species, the disease has different clinical forms including cutaneous, mucocutaneous, and visceral manifestations. Previous studies performed in endemic zones of northwestern-Argentina, during epidemic outbreaks, have been important for detecting patients suffering from the acute phase of the disease, but have not given a complete representation of the clinical and epidemiological features in the region. Furthermore, due to the resurgence of leishmaniasis worldwide and in particular the large increase of international tourism to the region, it seems pertinent to update the current epidemiological and clinical profile of leishmaniasis in northwestern-Argentina. Here we present a retrospective analysis of 95 *Leishmania* positive cases, presenting between 2000 and 2014. Patients were derived from hospitals and diagnosed in our lab at the University of Salta, located in a non-endemic area in Salta, Argentina. We detected numerous extensive mucocutaneous cases (34/95, 35.8%) distinct from mucosal affected patients, some instances originating in locations with no previously reported human cases. Additionally patients suffering from concomitant diseases, besides leishmaniasis, were assessed. These included Chagas disease, syphilis, deep mycoses, tuberculosis, toxoplasmosis and intestinal parasitosis. This study updates the clinical and epidemiological features of leishmaniasis in northwestern-Argentina, and discusses the implications and management strategy for patients who acquire the disease in this region.

1. Introduction

Leishmaniasis is a neglected vector-borne disease caused by *Leishmania* hemoflagellates and is associated with poverty; 350 million people are considered at risk of contracting leishmaniasis, and some 2 million new cases occur yearly (World Health Organization, 2010). Depending on the parasite species and the immune response of the host, the disease can manifest as cutaneous (CL), mucocutaneous (MCL), and visceral (VL) forms (Convit et al., 1993; Silveira et al., 2004).

In the Americas, leishmaniasis endemic areas extend from Mexico to Argentina (Reveiz et al., 2013). CL and MCL forms, referred to as American Cutaneous Leishmaniasis (ACL), are variable in terms of their clinical presentation and course ranging from asymptomatic to localized, sometimes self-healing cutaneous

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http://dx.doi.org/10.1016/j.actatropica.2015.11.008
0001-706X/

lesions to severe mutilating (MCL) lesions or diffuse cutaneous lesions (Silveira et al., 2004). VL, also present in the region, is characterized by prolonged fever, hepatomegaly, splenomegaly, and it is usually fatal if not treated adequately (Romero and Boelaert, 2010).

ACL is endemic in ten provinces of northern-Argentina, and the incidence has been increasing annually since 1980 (Salomon et al., 2012). Orán and San Martín departments (Salta province), located in northwestern-Argentina (NWA), reported 53.1% of the total number of ACL cases recorded in the country, both are considered hyper-endemic areas (Salomon et al., 2008). Exposure to the sandfly vector has been positively associated with recent ecological disturbance (deforestation), and with periurban vegetation (Salomon et al., 2008). *Leishmania (Viannia) braziliensis*, *L (V.) guyanensis* and *L. (Leishmania) amazonensis* are commonly isolated from human ACL cases although *L. (V.) braziliensis* is the main agent associated with ACL outbreaks and subsequent MCL cases (Barrio et al., 2009; Frank et al., 2003; Marco et al., 2005). With respect to VL, most of the human cases are from Misiones province, where *L. (L.) infantum* (syn. *L. chagasi*) has been isolated from humans, dogs and the sandfly vectors (Acardi et al., 2010). In some northwestern-areas of the country (Santiago del Estero and Salta provinces), rare VL cases have also been reported and the permissive vector *Lu. longipalpis* described (Bravo et al., 2013), *My. migonei* has also been implicated as a putative vector (Salomon et al., 2010).

Except for scanty reports of ACL cases (Casero et al., 2010; Romero et al., 2004), there are very few accurate clinical descriptions of CL and MCL lesions in northern-Argentina. This could adversely affect case detection or lead to clinical misdiagnosis and inadequate treatment (particularly regarding MCL). In the light of the resurgence of disease worldwide, in areas not previously thought to be endemic, and specifically the increase of foreign tourists to the region, there is an urgent need to update the current clinical and epidemiological profile of leishmaniasis in NWA. In this context, we present a retrospective analysis of 95 confirmed *Leishmania* positive case-series. We describe the clinical forms, implicated species, and a differential diagnosis suggesting an optimized case detection and management strategy for diagnosed individuals. We also report on concomitant infections associated with patients infected with leishmaniasis.

2. Methods

2.1. Study population and data collection

The study population initially comprised 346 suspected leishmaniasis cases, referred from Señor del Milagro and San Bernardo hospitals in Salta city, Argentina. The patients were assessed at the laboratory of microbiology (lab-UNSa; Facultad de Ciencias de la Salud, Universidad Nacional de Salta), during the period 2000–2014, for a confirmatory diagnosis. Only patients with positive parasitological diagnosis (microscopy and/or culture) for *Leishmania* were included in the analysis. Patient data was collected by means of a semi-structured questionnaire and hospital clinical records. Structured questions derived personal, and demographic information, and open questions epidemiologic and clinical data. Briefly, questions encompassed the reason for consultation, place of exposure (contact with the vector), occupation at the time of infection, antecedents of the current disease, and other pathological antecedents. Particular note was taken of data collected outside of known endemic areas. All patients provided written informed consent and were subjected to a physical clinical examination. Suspected cases were referred for laboratory diagnosis (below), and a blood sample was also taken for Chagas' disease serodiagnosis. At the hospitals, further laboratory tests were undertaken to detect bacterial and fungal superinfections, and other concomitant pathologies (including mycoses, syphilis, toxoplasmosis and tuberculosis).

All confirmed cases were referred for treatment, provided by the Argentinian Ministry of Health. The first-line therapy in this region is meglumine antimoniate, (20–850 mgSb^{5+}/day) administered twice daily by intramuscular injections over a period of 3 weeks for CL or 4 weeks for MCL (Ministerio de Salud Pública, 2004).

2.2. Leishmania diagnosis

2.2.1. Microscopic examination

Dermal scrapings were taken from ACL suspected lesions, stained with May Grünwald-Giemsa, and amastigotes visualized microscopically according to published protocols (Barrio et al., 2007). All described diagnostic procedures (smears, cultures and PCR) were performed in triplicate.

2.2.2. Culture

Material from the peripheral edges of suspected CL and MCL lesions was aspirated by syringe containing 0.5 mL of sterile proline balanced salt solution supplemented with 100 U/mL penicillin and 50 μg/mL streptomycin, seeded into USMARU culture medium supplemented with 20% defibrinated rabbit blood, and incubated at 23 °C (Barrio et al., 2009).

2.3. Leishmania species identification

A sample of skin/mucosal lesion was placed in a microcentrifuge tube containing 300 μL TE, boiled for 10 min, and stored at −20 °C until use. Polymorphism Specific–PCR (PS-PCR) was performed for the identification of *Leishmania* species (Barrio et al., 2009). In more detail, samples were thawed prior to use, centrifuged at 14,000 rpm for 1 min, and 10 μL of supernatant used for the reaction. PS-PCR was performed in two-stages: Initially, using primers V1–V2 and L1–L2 for subgenera identification (*V. (Viannia)* and *L. (Leishmania))*, and secondly, species specific primers, as reported by Barrio et al. (2009). For suspected VL cases (based on clinical examination), bone-marrow aspirates were performed followed by microscopic examination of smears, culture and DNA extraction as described above. Nested-PCR was performed with VL specific primers, targeting the cytb-gene for confirmatory diagnosis and subsequent DNA sequencing for species identification (Barrio et al., 2012).

2.4. Anti-T. cruzi antibodies serology

Plasma samples were obtained by centrifugation of 20 mL EDTA anticoagulated blood and stored as 2 mL aliquots at −80 °C. Anti-T. cruzi antibodies analysis was performed using a recombinant enzyme-linked immunosorbent assay (recombinant ELISA v.3.0, Wiener lab, Argentina), following the manufacturers protocol.

2.5. Statistical analysis

Categorical variables are described in detail below (2.5.1) and included gender, clinical forms, activity in relation to exposure and infection, *Leishmania* species, geographical place of infection and presence of concomitant infectious pathologies. Frequencies and association of variables were assessed by Pearson's Chi-square test (IBM® SPSS® Statistics Version 21). Continuous variables (patient age and lesion age) described by means, medians, standard deviation and range were compared by the *T* test or Mann–Whitney test, depending the normality of distribution (GraphPad Prism® Software Version 5.01). Differences were considered statistically significant if *p*-values were <0.05.