

American cutaneous leishmaniasis in children and adolescents from Northcentral Venezuela

Olinda Delgado¹, Sylvia Silva¹, Virginia Coraspe¹, Maria A. Ribas¹, Alfonso J. Rodriguez-Morales^{1,3,4*}, Pedro Navarro² and Carlos Franco-Paredes⁵

¹Immunoparasitology Section, Tropical Medicine Institute, Universidad Central de Venezuela, Caracas, Venezuela

²Tropical Medicine Section, Tropical Medicine Institute, Universidad Central de Venezuela, Caracas, Venezuela

³Experimental Institute JWT, Los Andes University, Trujillo, Venezuela

⁴Public Health Division, Universidad Central de Venezuela, Caracas, Venezuela

⁵Div. Infectious Diseases, Emory University, Atlanta, GA, USA.

*Corresponding author email: alfonsorm@ula.ve

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Abstract. American Cutaneous Leishmaniasis (ACL) comprises a broad range of cutaneous manifestations caused by different *Leishmania* species which may produce severe and chronic sequelae in adults. However, it has been suggested that ACL may show different clinical and epidemiological features in children and adolescents that need to be further elucidated. We evaluated the epidemiological features of ACL in a cohort of pediatric patients from Northcentral Venezuela between years 1997 and 2005. Mean age of patients was 9 years old, with a mean clinical evolution of 3 months. Lesions were located mostly in extremities. Forty patients (93%) were positive by MST, 97.7% by IFAT and 48.8% by smear. MST values tended to be related to patients' age, higher values being recorded in older patients ($p=0.153$).

INTRODUCTION

In Latin America, American Cutaneous Leishmaniasis (ACL) is caused primarily by members of subgenera *Leishmania* (*Leishmania*) and *Leishmania* (*Viannia*) (Davies *et al.*, 2000). ACL comprises a broad range of cutaneous manifestations which may produce severe and chronic sequelae in adults (Davies *et al.*, 2000; Hepburn, 2001; Murray *et al.*, 2005). In the pediatric literature the clinical spectrum and burden of ACL in children is still limited reported. Furthermore, there have been described significant differences between management of leishmaniasis in this population compared to adults (Ebrahim, 2000; Palacios *et al.*, 2001; Ampuero *et al.*, 2005). In contrast to what occurs in Visceral Leishmaniasis (VL), where approximately 65% of cases, reported in countries such as India, Sudan, Eritrea, Saudi Arabia and Ethiopia, have been described in children less than 15 years old,

and in Venezuela 67.7% of the VL patients are less than 4 years old (80.6% younger than 15 years) (Zerpa *et al.*, 2003); in ACL the extent of this estimate in children is less known. Therefore, VL has been the only leishmaniasis syndrome extensively studied in children due to the significant associated mortality, this disease has one of the highest mortalities if untreated. (Costa *et al.*, 1998; Ebrahim, 2000; Palacios *et al.*, 2001; Ampuero *et al.*, 2005). In the case of ACL more studies are necessary to establish the real burden of disease. It has been suggested that ACL in children and adolescents has a different clinical and epidemiological spectrum compared to adults; (Costa *et al.*, 1998; Ebrahim, 2000; Palacios *et al.*, 2001; Ampuero *et al.*, 2005; Minodier *et al.*, 2005). In consequence, we have been interested in describing these features in children and adolescents in Northcentral Venezuela, where American cutaneous leishmaniasis is endemic.

MATERIALS AND METHODS

We evaluated the epidemiological features of ACL in a cohort of pediatric patients from Northcentral Venezuela (Figure 1) between years 1997 and 2005 seen at the Tropical Medicine Institute of the Central University of Venezuela in Caracas, the country capital city. From approximately 2,000 patients seen at our Institution during the study period, a total of 43 children and adolescents with the clinical diagnosis of ACL were identified (2.15%; Fleis quadratic 95%CI 1.58%-2.91%).

Different diagnostic methods were used to confirm the clinical diagnosis, Montenegro Skin Test (MST), Indirect Immunofluorescence Test (IFAT) and smear. The Montenegro skin test was performed by a standardized technique, injecting killed promastigotes intradermally. An induration of 5 mm or more is considered a positive test. For this skin testing, the antigen was prepared from a pool of *Leishmania* spp., grown during eight days in agar blood medium, washed in PBS 0.02 M, pH = 7.4 and resuspended in physiological saline containing 0.5% phenol at a concentration of

1.5×10^6 promastigotes/ml. This antigen was stored at 4°C until use. Both, antigen and control material (physiological saline containing 0.5% of phenol), were administrated in a dose of 0.1 ml intradermally on the volar surface of both forearms and reading was done after 48-72 hours, using the ball-point pen method (Sokal, 1975; Ali & Ashford, 1993; Shiddo *et al.*, 1995; Mansueto *et al.*, 2007). In the case of the immunofluorescent antibodies test, the antigen was prepared using whole promastigotes of an isolate of *Leishmania* spp. from a patient proceeding from the locality of Chuao, Aragua State, according to the technique described by Pappas *et al.* (Pappas *et al.*, 1983). A reaction was considered positive when more than 50% of the organisms showed complete peripheral fluorescence (serum dilution titers for a cut-off of 32 dilutions).

Statistical analysis

Clinico-epidemiological features of ACL among these patients were studied and described. Correlations between immuno-diagnostic tests and clinico-epidemiological



Figure 1. Geographical origin (%) of children evaluated with ACL and ubication of the Tropical Medicine Institute (Caracas).

features (e.g. MST and patient's age), were calculated, with a 95% level of confidence (p significant <0.05), using the statistical software Epi Info v.6.0 and SPSS 10.0®.

RESULTS

From the total number of evaluated patients (43), 32 (74%) were female and 11 (26%) male. The mean age was 9.2 years old (± 4.8 , SD) (range 1-17 y-old); 35% were adolescents and 35% school-age children (6-12 y-old); 97.7% proceeded from endemic zones; 81% from Miranda State, 7% from Vargas State and 7% from Aragua State, among others (Figure 1).

These patients had a mean clinical evolution of 2.9 months (± 2.4) of their lesions. Most patients presented just one lesion (86%), which were located mostly in extremities (45% in legs, 19% in arms, 13% in hands; 9% cases were in the face). These corresponded to non-nodular cutaneous ulcers without associated significant lymphadenopathy.

Out of the total, 40 (93%) patients were positive by MST, 97.7% were positive by IFAT and 48.8% were positive by smear. Then, with the combination of these diagnostic tests, ACL was confirmed in 97.7% patients.

MST values tended to be related to patients' age ($r^2=0.0867$, $F=2.184$, $p=0.153$) (Figure 2A), with a mean of 9 mm in the group of 13-24 months, 10.4 mm in the 2-5 y-old group, 12.1 mm in the 6-11 y-old and 10.5 mm in the 12-18 y-old (Figure 2B). In the case of IFAT there were no significant differences neither a trend in this regard of age (Figure 3).

All cases were treated successfully with Meglumine antimoniate (20mg/kg/day) in two divided doses given intramuscularly during 28 days.

DISCUSSION

There is a growing interest in defining the epidemiological and clinical features of ACL in children and adolescents compared to adults (Carrada-Bravo, 1984; Palacios *et al.*, 2001; Campos-Munoz *et al.*, 2007; Oliver *et al.*, 2007; Tuon *et al.*, 2008), particularly for the still lacking of epidemiological studies in the New World on cutaneous leishmaniasis in pediatric settings.

New findings support the hypothesis that humans (particularly adults) could serve as a source of infection for children (Carrada-Bravo, 1984; Ampuero *et al.*, 2005; Ampuero *et al.*, 2006; Campos-Munoz *et al.*, 2007;

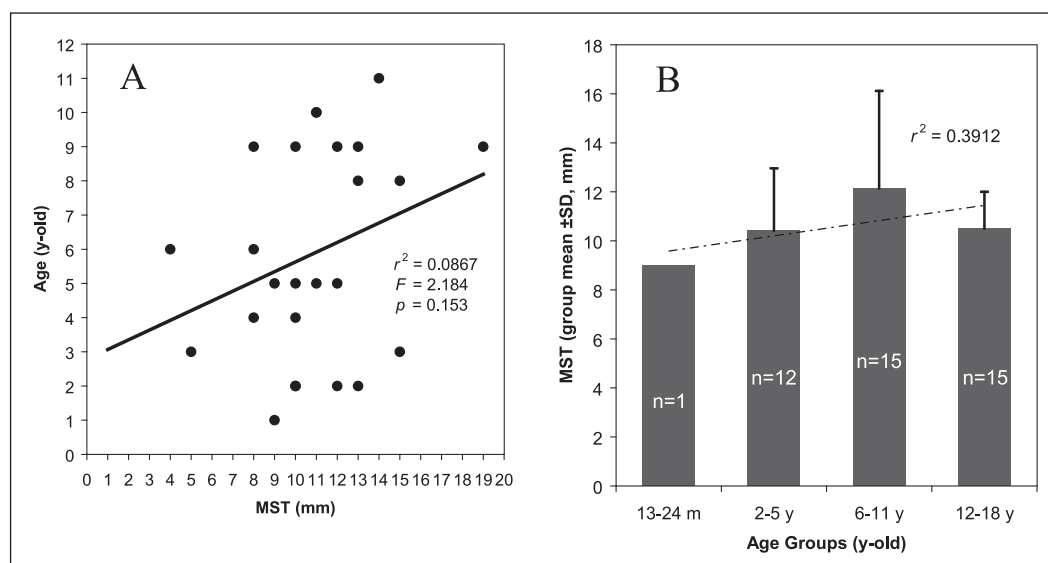


Figure 2. Correlation between MST values (in mm) and the patients' age (A) and age-groups (B) (y-old) in children with ACL.

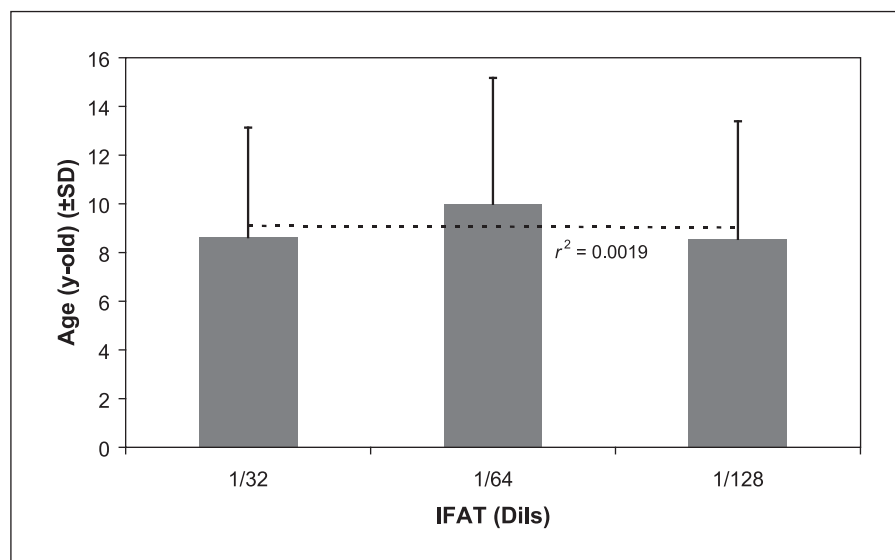


Figure 3. Correlation between age (y-old) and IFAT (dilutions) in children with ACL.

Oliver *et al.*, 2007; Tuon *et al.*, 2008). In a matched case-control study carried out in Corte de Pedra, Bahia, Brazil, an endemic area of *L. brasiliensis*, children from 0 to 5 years (with and without ACL) were selected and matched by age and place of residence. The presence of a family member with a history of cutaneous leishmaniasis in the year prior to the appearance of the disease in the child was found to be an important risk factor (Ampuero *et al.*, 2005).

As we observed in our study, the immune response may differ in adults and children, and be related to the age, especially if adults with a long history of residence in the endemic area had previously experienced infection and children not (Pappas *et al.*, 1983; Shidlo *et al.*, 1995; Palacios *et al.*, 2001; Tuon *et al.*, 2008). Those older children showed higher values of MST (delayed-type hypersensitivity, cellular immunity [TH1 and T CD8 cytotoxic cells]), which is related to time of exposure in endemic zones where these children live, but there are not significant different values of IFAT dilutions (humoral immunity) according to age. In the case of MST, prior disease evidenced by a typical scar has been shown to be associated with a more marked delayed-type hyper-

sensitivity response to leishmanin and a lower number of parasites in subsequent lesions (Palacios *et al.*, 2001).

In conclusion, we identified that most disease, in children and adolescents with American cutaneous leishmaniasis, tend to affect extremities (77%) but also the face in some cases. In a recent study in adults with cutaneous leishmaniasis it was found that patients had just one lesion in 17% (herein in children this figure was 86%). In that study the extremities were affected in 20% (Delgado *et al.*, 2008). We also identified that skin testing for leishmaniasis varies according to age-groups in an endemic population in Northcentral Venezuela. These preliminary findings have implications for the diagnosis and management of leishmaniasis in children and adolescents in endemic areas of leishmaniasis in South America.

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