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Atypical manifestations of tegumentary leishmaniasis in a transmission area of *Leishmania braziliensis* in the state of Bahia, Brazil

L.H. Guimarães^a, P.R.L. Machado^a, E.L. Lago^a, D.J. Morgan^b,
A. Schriefer^a, O. Bacellar^a, E.M. Carvalho^{a,*}

^a Serviço de Imunologia, Hospital Universitário Professor Edgard Santos, Universidade Federal da Bahia, R. João das Botas S/N, Canela 40110-160, Salvador, Bahia, Brazil

^b Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, 100 N. Greene Street, Lower Level, Baltimore, MD 21201, USA

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Summary American tegumentary leishmaniasis (ATL) can occur in different forms, classically categorised as cutaneous leishmaniasis, mucosal leishmaniasis, diffuse cutaneous leishmaniasis and disseminated leishmaniasis. We analysed the presence of atypical manifestations (vegetative, verrucous, crusted and lupoid) among a cohort of patients presenting to the Health Post of Corte de Pedra, Bahia, Brazil. Among 1396 patients diagnosed with ATL in 2005–2006, 35 patients (2.5%) presented with atypical manifestations of the disease. Of these patients, 14 were pregnant women, 2 were co-infected with HIV and 19 had no co-morbidity or other apparent risk factors for the development of atypical ATL. The latter 19 patients were the focus of this study. They were predominantly adult males, frequently presenting with facial lesions [$P < 0.001$; odds ratio (OR) = 17.5, 95% CI 6.1–52.4] and had higher rates of treatment failure with antimonial therapy ($P < 0.001$; OR = 327, 95% CI 45–6668) compared with patients with classic ATL attending in the same period. Thirteen cases healed with amphotericin B, introduced after failure of three or more courses of antimony, suggesting that amphotericin B should be considered as the drug of choice for all patients diagnosed with atypical ATL.

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1. Introduction

Leishmania (Viannia) braziliensis is the most important causal agent of American tegumentary leishmaniasis (ATL) in northeast Brazil. Cutaneous leishmaniasis (CL) is most commonly characterised by a single or few well delimited ulcers

* Corresponding author. Tel.: +55 71 3237 7353;
fax: +55 71 3245 7110.

E-mail addresses: edgar@ufba.br, imuno@ufba.br (E.M. Carvalho).

with elevated borders on the lower limbs.^{1,2} Mucosal leishmaniasis occurs in approximately 3% of CL patients, involving the nasal mucosa in the majority of cases.^{3,4} Disseminated leishmaniasis (DL) has been emerging in the endemic area and the number of documented cases has increased by more than 10-fold in the last 20 years.⁵ DL is characterised by the presence of ten or more acneiform papules and ulcerated skin lesions in at least two different parts of the body.⁵ We have recently reported that pregnancy was associated with large, exuberant and exophytic cutaneous lesions.⁶ In this study, we describe the clinical characteristics of 19 cases with atypical ATL without apparent risk factors for this form of disease. The clinical manifestations, immunological response and response to antimonial therapy in patients with atypical ATL were compared with patients with classic ATL.

2. Materials and methods

2.1. Subject selection and clinical evaluation

All patients presenting to the leishmaniasis clinic of Corte de Pedra, Bahia, Brazil, with a diagnosis of ATL between January 2005 and December 2006 were included in the study. The diagnostic criteria were parasite isolation in culture or positive delayed-type hypersensitivity (DTH) reaction with *Leishmania* antigen and compatible histopathological findings. Atypical ATL was defined by the presence of unusual lesions such as vegetative, verrucous, crusted and lupoid lesions. This group was compared with patients with classic CL and DL considered here as classic ATL. Clinical data, such as possible co-morbidities and use of immunosuppressive drugs, were collected from all patients. Serological tests for HIV, hepatitis B virus (HBV), hepatitis C virus (HCV) and human T-cell lymphotropic virus type 1 (HTLV-1) were performed as well as tests of blood glucose levels, blood urea nitrogen and hepatic enzymes. Written informed consent was obtained from all adult patients and from the parents or guardians of minors, and all patients photographed gave permission to publish the pictures.

2.2. Diagnosis of leishmaniasis

DTH skin testing was performed with *Leishmania* antigen as described previously.⁷ The test was considered positive

when the induration was ≥ 5 mm. A 4-mm punch biopsy was performed at the border of skin lesions and a fragment was analysed in the Pathology Department of the Professor Edgard Santos University Hospital, Salvador, Brazil. Parasite culture was performed from samples obtained by needle aspiration.

2.3. Immunological studies

Cytokine levels were determined in the supernatant of peripheral blood mononuclear cells after 72 h of stimulation with *L. braziliensis* antigen as described previously.⁸ IFN γ and TNF α were measured using the ELISA sandwich technique (R&D Systems, Minneapolis, MN, USA) and the results were expressed in pg/ml on the basis of a standard curve generated by the use of recombinant cytokines.

2.4. Treatment

All patients were treated with meglumine antimony at a dose of 15–20 mg/kg/day i.v. for 20–30 days. Patients who presented with two consecutive treatment failures were treated with a third course of meglumine antimony i.v. plus pentoxifylline 400 mg orally three times a day for 20–30 days or amphotericin B 0.5 mg/kg/day i.v. three times weekly until a total dose ranging from 1.0 g to 1.5 g.

3. Results

During the 24-month period, 1396 patients were diagnosed with ATL in the clinic, 35 (2.5%) of whom presented with atypical ATL. These patients were divided in three groups: pregnant women ($n = 14$), as described in a previous study⁶; patients co-infected with HIV ($n = 2$); and patients with atypical ATL ($n = 19$) without clinical co-morbidities. The latter 19 patients were predominantly young adult males (84%) (Table 1). The majority of cases had three or less lesions. In the patients with atypical ATL, the lesions were exophytic, without clear borders and were sometimes crusted. They also had a tendency to bleed when touched (Figure 1). The DTH reaction to *Leishmania* antigen was positive in the majority of patients (Table 1), and the frequency of positive culture for *Leishmania* was consistent with previous

Table 1 Comparison of patients with atypical lesions and patients with classic lesions of American tegumentary leishmaniasis (ATL)

| | Classic ATL ($n = 1361$) | Atypical ATL ($n = 19$) | P-value | OR (95% CI) |
|-----------------------------------|----------------------------|---------------------------|---------------------|-----------------|
| Males (%) | 64.6 | 84.2 | 0.091 | |
| Age (mean \pm SD) (years) | 27 \pm 17.6 | 33 \pm 11.7 | 0.098 ^a | |
| DTH (mean) (mm) | 17 \pm 5.8 | 15 \pm 5.3 | 0.167 ^a | |
| DTH negative (%) | 3.9 | 11.7 | 0.147 ^b | 2.4 (0.0–13.6) |
| Lesion on face (%) | 11 | 68 | <0.001 ^b | 17.5 (6.1–52.4) |
| Lesions above the waist (%) | 27 | 79 | <0.001 ^b | 10 (3.3–30.4) |
| Antimony failure (%) ^c | 5.2 | 94.7 | <0.001 ^b | 327 (45–6668) |

OR: odds ratio; DTH: delayed-type hypersensitivity.

^a Student's *t*-test.

^b Fisher's exact test.

^c Failure of three or more courses of meglumine antimony.



Figure 1 (A) Patient with verrucous lesions, without clear borders, positive delayed-type hypersensitivity (DTH) and culture isolate of *Leishmania (Viannia) braziliensis*. (B) Patient with exophytic, verrucous and crusted lesion on the nose, positive DTH and positive culture. (C) Patient with lupoid lesion on the face, positive DTH and positive culture.

observations in our routine work in this health post. In all eight patients in whom parasites were isolated, they were identified as *L. braziliensis*. In the group with atypical ATL, only three patients healed their lesions with antimony alone, although two of these required three courses of meglumine antimony to achieve cure. Two patients healed when treated with meglumine antimony and pentoxifylline, which was introduced following treatment failure with three courses of antimony. Thirteen cases healed with amphotericin B, introduced after failure of three or more courses of antimony. One patient was treated initially with amphotericin B owing to an allergy to antimony. The histopathology of atypical ATL lesions showed an infiltration of lymphocytes and plasma cells with few or absence of parasites. In all 19 patients, laboratory studies did not show any kidney dysfunction, liver enzyme alterations or abnormalities in the blood glucose level. All serologies for HIV, HTLV, HBV and HCV were negative.

The clinical data of the 1361 cases of classic ATL were compared with the 19 atypical patients (Table 1). The age of atypical patients ranged from 14 years to 55 years (mean \pm SD 33 ± 11.7 years) and did not differ from the patients with classic ATL (27 ± 17.6 years). The atypical patient group had a male:female ratio of 5.3:1 compared with 1.8:1 ($P=0.091$) in the classic ATL group. Lesions above the waist were much more frequent in the atypical group ($P<0.001$). Moreover, whilst 68% of the atypical ATL patients had lesions on the face, only 11% of those with classic ATL had facial lesions [$P<0.001$; odds ratio (OR)=17.5, 95% CI 6.1–52.4]. Three patients (15.8%) in the atypical group developed mucosal lesions concomitantly that were contiguous with the skin lesions. No differences were seen between the two groups regarding the response to the skin test. Additionally, in five atypical patients IFN γ levels (mean \pm SD $10\,434 \pm 6886$ pg/ml) were measured in the supernatant of lymphocyte cultures stimulated with *L. braziliensis* antigens and concentrations of this cytokine were similar to those observed in patients with classic CL (5174 ± 6582 pg/ml) ($P=0.19$). There was also no difference in TNF α levels ($P=0.15$). Patients with atypical ATL had a higher rate of treatment failure with antimonials ($P<0.001$; OR=327, 95% CI 45–6668) compared with patients with classic lesions treated in the same period.

4. Discussion

Reports of atypical forms of ATL have been described sporadically.^{9–12} Here we describe the frequency of atypical forms of ATL in a large cohort and compared clinical and immunological characteristics of 19 patients with atypical ATL with cases of classic ATL diagnosed in the study period.

Classic CL is characterised by a well defined ulcer with raised borders. DL presents with more than 10 multiple acneiform and small papular lesions in different areas of the body that eventually ulcerate.⁵ The atypical ATL patients had a clinical presentation quite distinct from CL and DL. The majority of patients had a small number of lesions, but their characteristics were different from the ulcerated lesion observed in CL. Although two atypical ATL patients had a very high number of lesions similar to the DL cases, in these patients the disease was concentrated in only a few areas of the body where lesions became confluent.

The host immunological response plays a pivotal role in the outcome of *Leishmania* infection. In the absence of a protective type 1 immune response, patients infected with *L. amazonensis* develop diffuse cutaneous leishmaniasis characterised by nodular lesions with abundant macrophages containing *Leishmania*.¹³ In contrast, patients with a dominant type 1 immune response present with localised ulcerated lesions.^{14,15} The histopathology of atypical lesions was similar to that observed in classic CL. Moreover, the majority of these patients had a positive *Leishmania* DTH test and in all cases in whom an in vitro immunological study was performed there was a dominant type 1 immune response. Thus, we conclude that there were no data indicating that the clinical manifestations presented by these patients were associated with a specific impairment in the host immunological response.

Vectors and parasite factors may influence the outcome of *Leishmania* infection. The observation that atypical ATL occurs predominantly in adult males suggests a potential role for environmental factors. In such cases, intraspecies differences among *Leishmania*^{16,17} and the influence of vector species in disease outcome should also be considered. We did not find a cluster of atypical cases in a specific geographical location within the endemic area, but the small numbers of cases limited the power of the study to evaluate

this variable. In a recent report of atypical forms of leishmaniasis in Pakistan, a cluster of cases in a geographically restricted area was observed.¹⁸

The major finding of this study was the identification of a distinct and emerging form of leishmaniasis characterised by atypical lesions. For clinicians and infectious diseases specialists, the clinical description of atypical leishmaniasis is even more relevant, as such presentations are not depicted in textbooks. Furthermore, it is critical to diagnose atypical ATL accurately and to treat these patients appropriately. Since patients with atypical ATL had a poor response to antimonial therapy, we recommend that amphotericin B be considered as the drug of choice for all patients diagnosed with atypical ATL.

Authors' contributions: LHG and EMC designed the study protocol; LHG, PRLM, DJM and ELL carried out the clinical assessments; OB carried out the immunoassays and cytokine determination; EMC and AS analysed and interpreted the data; LHG, ELL, DJM and OB drafted the manuscript; PRLM, AS and EMC revised the manuscript. All authors read and approved the final version. EMC and LHG are guarantors of the paper.

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Conflicts of interest: None declared.

Ethical approval: This study was approved by the Ethical Committee of the Professor Edgard Santos University Hospital, Federal University of Bahia, Salvador, Brazil, which is responsible for evaluating and approving scientific studies performed in the clinic of Corte de Pedra.

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