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BRIEF REPORT

Clinical Profile and Diagnosis of Recurrent Cutaneous Leishmaniasis

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This case-control study compared the clinical profile, parasite load, polymerase chain reaction positivity, and response to therapy in patients with recurrent cutaneous leishmaniasis (RCL) with primary cutaneous leishmaniasis (CL). The RCL patients had milder diseases with lower parasite loads, a lower number of lesions, and more self-healing diseases than primary CL patients.

Keywords. clinical manifestation; *Leishmania braziliensis*; recurrent leishmaniasis; treatment.

Cutaneous leishmaniasis (CL) caused by *Leishmania braziliensis* is the most common form of American tegumentary leishmaniasis (ATL) [1]. Recurrent CL (RCL) has been detected in both the Old and New World [2, 3]. In an area of *L braziliensis* transmission in the northeast region of Brazil, RCL was found in 4% of the cases, usually more than 5 years after the treatment and cure of primary CL [1]. Others have found that although the parasite load was similar in lesions from RCL patients infected with *L braziliensis* and with primary CL (PCL), it was higher in the blood of RCL patients compared with PCL patients [4]. In CL caused by *Leishmania major*, recurrence was associated with female gender, number of lesions, and longer duration of therapy of PCL, but it is not known which factors influence the occurrence of RCL in areas of *L braziliensis* transmission. From January 2020 to December of 2021, we observed an increase in the number of RCL cases in our endemic area and decided to perform a case-control study, comparing clinical parameters, diagnosis, and response to therapy in RCL and PCL patients.

Received 31 March 2023; editorial decision 17 July 2023; accepted 19 July 2023; published online 22 July 2023

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https://doi.org/10.1093/ofid/ofad387

METHODS

Study Design

This study was conducted in the Health Post of Corte de Pedra, municipality of Tancredo Neves, an ATL-endemic area in the southeastern region of Bahia, Brazil. Participants were patients with PCL (n = 41) or RCL (n = 20), diagnosed between 2020 and 2021 and for whom histopathologic analysis of the skin ulcer was performed. Primary CL was defined as individuals who had CL for the first time. Recurrent CL was defined as subjects who developed CL after their initial episode of the disease after adequate treatment and cure. Demographic, clinical features and size induration of the leishmanial skin test (LST) were recorded. Diagnosis was confirmed by identification of amastigotes in the skin biopsies by immunohistochemistry and by detection of *L braziliensis* by polymerase chain reaction (PCR). Patients were treated with meglumine antimoniate (MA) in doses of 20 mg/kg per day for 20 days, or with conventional Amphotericin B, with total dosage ranging from 25 to 40 mg/kg. Clinical cure was defined as complete healing of the lesion with re-epithelization of the skin including the absence of raised borders, 90 days after initiation of treatment. Failure was defined by presence of an active ulcer or healing of the lesion but with raised borders.

Patient Consent Statement

All patients agree to participate in the study and a written consent was obtained. This study was approved by the ethical committee of the Federal University of Bahia Medical School.

Diagnosis and Leishmanial Skin Test

All participants of this study had 1 or more cutaneous ulcers, as well as a negative human immunodeficiency virus test. None had immunosuppressive factors. Diagnosis was performed through the detection of amastigotes by immunochemistry and the identification of *L braziliensis* deoxyribonucleic acid (DNA). Skin biopsies were performed in the border of the ulcers with a 4-mm punch, following local anesthesia. Biopsied tissue was fixed in 4% paraformaldehyde, dehydrated in an alcohol gradient, and embedded in paraffin. Tissue sections were stained in hematoxylin-eosin and in periodic acid-Schiff, as well as Grocott-Gomori silver methenamine. The histopathological analysis included a description of the presence of chronic inflammation, granuloma, and necrosis foci. For amastigote detection by immunohistochemistry, electrically charged slides containing 4-µm tissue sections were subjected to immunohistochemistry [5], using polyclonal mouse anti-*Leishmania* serum at 1:2000 dilution. Reactions were developed with MACH 1 Universal HRP-Polymer Detection (M1U539;

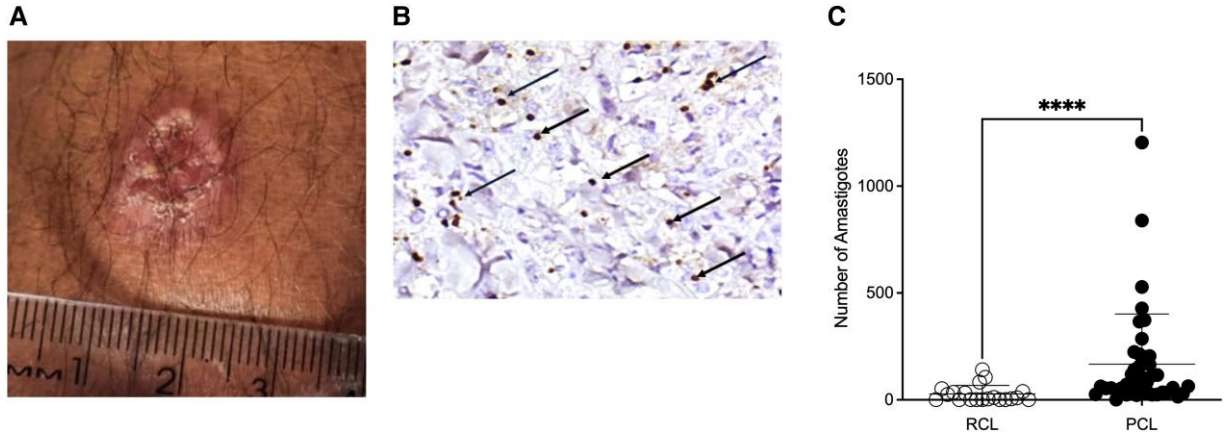

Figure 1. Recurrent cutaneous leishmaniasis (RCL) caused by *Leishmania braziliensis*. (*A*) Representative ulcer observed in RCL patient. (*B*) Immunolabeling of *L braziliensis* amastigotes in RCL lesion tissue. Bound primary antibodies were visualized using MACH 1 Universal HRP-Polymer Detection. Arrows indicate amastigotes. (*C*) Number of amastigotes in patients with RCL and primary CL. Data are shown for individual patients.

Biocare Medical). Tissue sections were examined at 40× normal strength under a Nikon Eclipse E microscope. Biopsied tissue was also submitted to PCR, as described [6]. The delayed type hypersensitivity reaction to leishmania (LST) was performed with a soluble leishmania antigen [7], and an induration of ≥5 mm was considered a positive test.

RESULTS

Sixty-one patients were included in this study (RCL = 20, PCL = 41). Among the 20 RCL patients, 11 had the first CL episode earlier than 5 years before and 9 had the first CL episode later than 5 years, with a mean time of 10 ± 8.1 years of previous history. Comparing the 2 groups, no significant difference was observed in age or gender. Lesions from RCL patients were more superficial, borders were less infiltrated, and size was also smaller (Figure 1*A*). Patients with recurrent CL had longer ($P = .04$) illness duration (mean time of 90.5 ± 95.9 days compared to 50.8 ± 56.7 days in PCL patients), fewer lesions (1.1 ± 0.3 versus 1.9 ± 1.7; $P = .04$), and higher LST induration (mean of 209.2 ± 109.3 mm^2 compared to 126.6 ± 109.9 mm^2 in PCL patients; $P = .01$). In both groups, histopathological analysis was characterized by infiltration of macrophages and lymphocytes. Figure 1*B* shows the presence of amastigotes by immunochemistry in 1 representative ulcer from a RCL patient. Finally, PCR detection of *L braziliensis* DNA was negative in 82.9% of RCL cases compared to 20% in PCL cases ($P = .0001$). The number of amastigotes, detected by immunohistochemistry, was lower in RCL patients (27.5 ± 8.89) compared to PCL (167.2 ± 36.63) ($P = .01$) (Figure 1*C*).

Four RCL patients (20%) presented self-healing lesions and 16 were treated with MA. Of the 41 PCL patients, 1 died of septicemia before treatment initiation and 6 were treated with Amphotericin B. Of the 34 treated with MA, 1 (2.9%) self-healed the ulcers. Taking in account only patients who self-healed or were cured at day 90 with MA, the cure rate was 75% in RCL and 44% in PCL ($P = .14$).

DISCUSSION

In this study, we compare the clinical characteristics, diagnostic tests, parasite load, and response to treatment in patients presenting a secondary episode of CL (RCL) versus those with PCL caused by *L braziliensis*. We found that RCL patients had longer illness duration, a lower number of lesions, a more superficial presentation of the ulcers, and a milder disease compared to PCL patients. Of note, the distinctive satellite lymphadenopathy observed in PCL, early in the infection, was not documented in RCL patients [8]. Moreover, RCL lesions were very small and did not resemble classic CL. The lack of these clinical features may explain the delay in seeking medical attention. The lower number of amastigotes observed in RCL patients may be related to the milder disease, because there is an association between parasite load and severity of CL [9].

The immune response to leishmania infection was examined in household contacts of CL patients. Protection against leishmaniasis caused by *L tropica* or *L braziliensis* was associated with a positive LST, evidence of interferon-γ production, and greater ability of monocytes to kill *L braziliensis* [10, 11]. In this study, LST induration was higher in RCL patients compared to PCL patients, indicating a greater ability to respond to leishmanial antigen and control parasite replication, without precluding lesion development. This highlights the need to investigate how different cell populations sustain immunity to secondary infections.

Cutaneous leishmaniasis diagnosis is frequently challenging due to lesion similarity with other infectious diseases or

ischemic ulcers frequently presented in older patients. In this study, PCR positivity was lower in RCL cases, and these patients also presented a significantly lower number of amastigotes in lesion tissue. We suggest that the lower number of parasites and, hence, target DNA may have impacted the outcome of PCR testing in RCL. In these cases, immunohistochemistry displayed excellent sensitivity for amastigote detection and should be used for RCL diagnosis.

We are aware of the limitations of this study, including the low number of RCL patients, as well as lack of immunologic data. In addition, we did not have access to parasites or leishmanial DNA obtained from the primary CL in RCL patients. However, the data show that the cure of a primary CL caused by *L braziliensis* does not confer resistance against a secondary CL, despite the finding that RCL patients have a strong LST response, associated with lower number of tissue amastigotes and an overall milder and self-healing disease. In addition, the cure rate with MA was higher in RCL patients compared to that usually observed in Corte de Pedra (50%) [12]. These data open the possibility of performing further studies to elucidate risks factors for RCL, including differences in *L braziliensis* strains, as well differences in the local and systemic immune response in RCL patients.

CONCLUSIONS

Recurrent CL cases had longer illness duration, fewer lesions, lower number of amastigotes, and higher LST induration compared to PCL patients, which was likely due to the lower parasite load. Detection of leishmanial DNA by PCR was very low in RCL cases, whereas immunochemistry showed high sensitivity and should be used for diagnosis of RCL caused by *L braziliensis*. Clinically, RCL lesions were more superficial with less infiltrated borders and a smaller size. Overall, these findings indicate that patients with RCL have a better ability to control parasite replication, suggesting that they mount an immune response to *L braziliensis* antigens that can inhibit parasite replication, resulting in a milder disease and a better response to therapy.

Acknowledgments

We thank Dr. Hiro Goto from the Institute of Tropical Medicine (Universidade de São Paulo) for providing anti-*Leishmania braziliensis* polyclonal mouse serum.

Financial support. This work was supported by National Institutes of Health Grant Number AI136032 (Tropical Medicine Research Center; to EMC), Brazilian Ministry of Science, Technology, and Innovation, Instituto Nacional de Ciência e Tecnologia de Doenças Tropicais (INCT-DT) Grant Number 465229/2014-0—CNPq (to EMC), Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) INC 0003/2019 (to EMC), and PROEP- IGM-Fiocruz Bahia (to CIdO).

Potential conflicts of interest. All authors: No reported conflicts of interest.

References

1. Jirmanus L, Glesby MJ, Guimarães LH, et al. Epidemiological and clinical changes in American tegumentary leishmaniasis in an area of Leishmania (viannia) braziliensis transmission over a 20-year period. Am J Trop Med Hyg **2012**; 86:426–33.
2. Bamorovat M Sharifi I, Dabiri S, et al. Major risk factors and histopathological profile of treatment failure, relapse and chronic patients with anthroponotic cutaneous leishmaniasis: a prospective case-control study on treatment outcome and their medical importance. PLoS Negl Trop Dis **2021**; 15:e0009089.
3. Khattak FA, Khan TA, Hussain M, et al. Analysis of associated risk factors among recurrent cutaneous leishmaniasis patients: a cross-sectional study in Khyber Pakhtunkhwa, Pakistan. J Infect Public Health **2022**; 15:1175–9.
4. de Carvalho BC, Vital T, Osiro J, et al. Multiparametric analysis of host and parasite elements in new world tegumentary leishmaniasis. Front Cell Infect Microbiol **2022**; 12:956112.
5. Saldanha MG, Pagliari C, Queiroz A, et al. Tissue damage in human cutaneous leishmaniasis: correlations between inflammatory cells and molecule expression. Front Cell Infect Microbiol **2020**; 10:355.
6. Weirather JL, Jerônimo SMB, Gautam S, et al. Serial quantitative PCR assay for detection, species discrimination, and quantification of Leishmania spp. in human samples. J Clin Microbiol **2011**; 49:3892–904.
7. Reed SG, Badaró R, Masur H, et al. Selection of a skin test antigen for American visceral leishmaniasis. Am J Trop Med Hyg **1986**; 35:79–85.
8. Machado PRL, Araújo C, Silva AT, et al. Failure of early treatment of cutaneous leishmaniasis in preventing the development of an ulcer. Clin Infect Dis **2002**; 34: E69–73.
9. Amorim CF, Novais FO, Nguyen BT, et al. Variable gene expression and parasite load predict treatment outcome in cutaneous leishmaniasis. Sci Transl Med **2019**; 11:eaax4204.
10. Ben Salah A, Louzir H, Chlif S, et al. The predictive validity of naturally acquired delayed-type hypersensitivity to leishmaniasis in resistance to Leishmania major-associated cutaneous leishmaniasis. J Infect Dis **2005**; 192:1981–7.
11. Muniz AC, Bacellar O, Lago EL, et al. Immunologic markers of protection in Leishmania (Viannia) braziliensis infection: a 5-year cohort study. J Infect Dis **2016**; 214:570–6.
12. Prates FV, Dourado ME, Silva SC, et al. Fluconazole in the treatment of cutaneous leishmaniasis caused by Leishmania braziliensis: a randomized controlled trial. Clin Infect Dis **2017**; 64:67–71.