Revista da Sociedade Brasileira de Medicina Tropical
Journal of the Brazilian Society of Tropical Medicine
Vol.:52:e20180323: 2019
doi: 10.1590/0037-8682-0323-2018

Case Report

First report of treatment failure in a patient with cutaneous leishmaniasis infected by *Leishmania (Viannia) naiffi* carrying *Leishmania* RNA virus: a fortuitous combination?

***Ricardo Vieira-Gonçalves*[1]#, *Giselle Aparecida Fagundes-Silva*[1]#, *Júlia Furtado Heringer*[1], *Maria Fantinatti*[1], *Alda Maria Da-Cruz*[1], *Manoel Paes Oliveira-Neto*[2], *Jorge Augusto Oliveira Guerra*[3] and *Adriano Gomes-Silva*[1],[4]**

[1]. Laboratório Interdisciplinar de Pesquisas Médicas, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, RJ, Brasil.
[2]. Instituto Nacional de Infectologia Evandro Chagas, FIOCRUZ, Rio de Janeiro, RJ, Brasil.
[3]. Gerência de Leishmaniose, Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Manaus, AM, Brasil.
[4]. Laboratório de Pesquisa Clínica em Micobacterioses, Instituto Nacional de Infectologia Evandro Chagas, FIOCRUZ, Rio de Janeiro, RJ, Brasil.

Abstract

We report the case of a 32-year-old man from Rio de Janeiro, who was infected in the Amazon region of Brazil by *Leishmania (Viannia) naiffi*. Generally, patients with *L. naiffi* cutaneous leishmaniasis exhibit a good therapeutic response to either pentavalent antimonials or pentamidine. However, after pentamidine treatment, this patient's infection evolved to therapeutic failure. To understand this clinical outcome, we investigated the presence of the *Leishmania* RNA virus (LRV) in parasites isolated from the cutaneous lesion; herein, we discuss the possible association between a poor response to pentamidine therapy and the presence of the LRV.

Keywords: *Leishmania (Viannia) naiffi.* Therapeutic failure. *Leishmania* RNA virus.

INTRODUCTION

Leishmania (Viannia) naiffi was first isolated in the armadillo (*Dasypus novemcinctus*) in 1989 by Lainson and Shaw in the Brazilian Amazon[1]. Soon after, this species was detected in a patient with human cutaneous leishmaniasis (CL). Other cases were reported in Latin America[2], most of which involved military personnel or tourists[3]. However, *L. naiffi* is still considered an unusual parasite species. In Brazil, these cases occur mainly in the Amazon region, where frequencies of 4%, 20%, or even 26.7%[4] have been reported.

L. naiffi CL lesions are usually ulcerated, unique, and small, located on the hands, arms, or legs. They usually develop with a favorable prognosis and even spontaneous healing[3], and mucosal leishmaniasis has not yet been reported. Commonly, patients with *L. naiffi* CL exhibit a good therapeutic response to either pentavalent antimonials or pentamidine[2]. In addition, experimental studies have shown that *L. naiffi* causes discrete or even unapparent infections on the skin of hamsters[1].

We describe an unusual *L. naiffi* CL case that evolved with pentamidine resistance, and we also discuss the possible association between poor therapy response and the presence of the *Leishmania* RNA virus (LRV).

CASE REPORT

A 32-year-old man from Rio de Janeiro state, Brazil, was infected in September 2009 during his military training in the Amazon region (Manaus, AM). A papular lesion approximately 0.5 cm in diameter was noted on his left hand 30 days later. No lymphadenopathy was detected. The patient was treated with a three-day course of pentamidine (4 mg/kg). The treatment was unsuccessful, and an active lesion persisted (**Figure 1A**). In March 2010, the patient underwent surgical removal of the entire lesion. Pseudo-epitheliomatous squamous hyperplasia and chronic granulomatous dermatitis were observed on

# Both authors contributed equally to this work.
Corresponding author: Dr. Adriano Gomes-Silva.
e-mail: adriano.gomes@ini.fiocruz.br
Orcid: 0000-0003-0623-3860
Received 16 August 2018
Accepted 17 December 2018

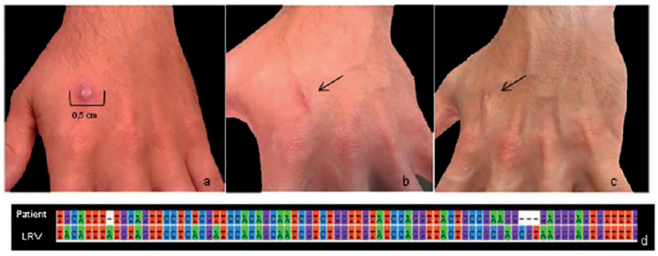

FIGURE 1: *Leishmania (Viannia) naiffi* cutaneous leishmaniasis lesion on the patient's hand after pentamidine therapy failure **(A)** and progressive healing after its surgical removal (scar at one "**B"** and three years "**C"** after surgical removal). (**D)** The alignment of *Leishmania* RNA virus (LRV) sequences from *L. naiffi* isolated from CL patient with the LRV1 sequences from GenBank showed 91% identicality. LRV cDNA was amplified using previously described primers[6], and the PCR products were sequenced using the ABI Prism™ BigDye Terminator Cycle Sequencing Kit and run on an ABI 3730 automatic DNA sequencer (Applied Biosystems, Foster City, CA). The electropherograms were analyzed using Chromas software and the consensus DNA sequence compared was generated by the CAP3 program. The contig was aligned with known LRV sequences obtained from Genbank using the ClustalW algorithm in MEGA version 4.0.

histopathological analysis. No amastigotes were found. *Leishmania* spp. was isolated and characterized as *L. naiffi* based on isoenzyme electrophoresis. One month after the surgical procedure and without undergoing another round of therapy, no signs of active disease were detected. The scar remained completely healed until three years after surgery (**Figure 1B and 1C**), at which time clinical follow-up was discontinued.

Anti-*Leishmania* immunoglobulin G (IgG), IgG1, and IgG3 were measured using the enzyme-linked immunosorbent assay method. Despite therapeutic failure, immunoglobulin levels remained negative up to two years after treatment. The LRV was detected in the sample using a sequencing technique (**Figure 1D**). The LRV from *L. naiffi* showed 91% identicality with LRV1 sequences from GenBank.

DISCUSSION

This case involved therapeutic pentamidine failure in CL caused by *L. naiffi*, and it is intriguing that complete cure was achieved after surgical removal of the lesion. Two possible hypotheses to explain this therapeutic failure occurrence are as follows: 1) therapeutic failure in *L. naiffi* CL is a more common phenomenon than previously reported and 2) the presence of the LRV contributed to increased parasite virulence.

The frequency of human *L. naiffi* infection is likely underreported due to the failure of health systems to isolate and identify *Leishmania* species. Recent reports suggest that transmission of *L. naiffi* in the Amazon region may be more common than expected[4]. Furthermore, we described another two of eight cases of *L. naiffi* infection that evolved with therapeutic failure post-pentamidine or -antimony treatment[4]. However, the mechanisms associated with *L. naiffi* resistance to these drugs are unknown.

The LRV has primarily been detected in two *Viannia* subgenera *(L. braziliensis* and *L. guyanensis* species), and its role as a virulence factor remains controversial. Indeed, studies have suggested that the LRV reduces sensitivity to oxidative stress, which may contribute to the decreased effectiveness of drugs[5]. Furthermore, this double-stranded RNA virus is known to increase the transcription of proinflammatory cytokines[6], which in turn promotes tissue damage and poor prognosis. Remarkably, we first evidenced the presence of the LRV in *L. naiffi* species, raising the possibility that the presence of this virus could increase *Leishmania* spp. virulence and thereby influence therapeutic failure. Case studies of CL caused by *L. braziliensis*[7] or *L. guyanensis*[8] revealed that treatment failure with antimonial therapy or pentamidine, respectively, was significantly higher when an isolated parasite presents with the LRV infection. Further studies are needed to confirm its role in the dynamics of *Leishmania* spp. infection.

This study reinforces the importance of defining the true epidemiological importance of *L. naiffi* as an etiological agent of CL in South America, especially in the Amazon region. Thus, the identification of possible factors associated with treatment failure should allow for better clinical monitoring of patients.

Acknowledgments: The authors thank the technical team from *"Gerência de Leishmaniose – Fundação de Medicina Tropical Heitor Vieira Dourado."* We also thank the *"Laboratório de Referência Nacional para Tipagem de Leishmania"* (IOC), FIOCRUZ, and the sequencing platform (IOC).

Conflict of Interest: The authors declare that there is no conflict of interest.

Financial Support: This work was funded by *Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro* (FAPERJ-APQ1) (grant number E-26/110.497/2012) (www.faperj.br), and *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) (number 458858/2014-5). AMDC is a CNPq and FAPERJ (CNE) research fellow. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

1. Lainson R, Shaw JJ. *Leishmania (Viannia) naiffi sp.* n., a parasite of the armadillo, *Dasypus novemcinctus (L.)* in Amazonian Brazil. Ann Parasitol Hum Comp. 1989;64(1):3-9.
2. Pratlong F, Deniau M, Darie H, Eichenlaub S, Pröll S, Garrabe E, et al. Human cutaneous leishmaniasis caused by *Leishmania naiffi* is wide-spread in South America. Ann Trop Med Parasitol. 2002;96(8):781-5.
3. van der Snoek EM, Lammers AM, Kortbeek LM, Roelfsema JH, Bart A, Jaspers CA. Spontaneous cure of American cutaneous leishmaniasis due to *Leishmania naiffi* in two Dutch infantry soldiers. Clin Exp Dermatol. 2009;34(8):e889-91.
4. Fagundes-Silva GA, Romero GA, Cupolillo E, Yamashita EP, Gomes-Silva A, Guerra JA. *Leishmania (Viannia) naiffi*: rare enough to be neglected? Mem Inst Oswaldo Cruz. 2015;110(6): 797-800.
5. Hartley MA, Ronet C, Zangger H, Beverley SM, Fasel N. *Leishmania* RNA virus: when the host pays the toll. Front Cell Infect Microbiol. 2012;2:99.
6. Ives A, Ronet C, Prevel F, Ruzzante G, Fuertes-Marraco S, Schutz F, et al. *Leishmania* RNA virus controls the severity of mucocutaneous leishmaniasis. Science. 2011;11(6018):775-8.

7. Adaui V, Lye LF, Akopyants NS, Zimic M, Llanos-Cuentas A, Garcia L, et al. Association of the endobiont double-stranded RNA virus LRV1 with treatment failure for human leishmaniasis caused by *Leishmania braziliensis* in Peru and Bolivia. J Infect Dis. 2016;213(1):112-21.

8. Bourreau E, Ginouves M, Prévot G, Hartley MA, Gangneux JP, Robert-Gangneux F, et al. Presence of Leishmania RNA virus 1 in *Leishmania guyanensis* increases the risk of first-line treatment failure and symptomatic relapse. J Infect Dis. 2016;213(1):105-11.