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FAMILIAL AGGREGATION OF MUCOSAL LEISHMANIASIS IN NORTHEAST BRAZIL

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Abstract. To evaluate whether familial clustering occurs in mucosal leishmaniasis (ML), patients with ML (index cases) were randomly selected from medical records at a health post in an endemic area for *Leishmania braziliensis* infection. Control individuals (index controls) matched by age, gender, and place of residence to index cases were selected. Family members of index cases and controls were compared with respect to environmental factors and the incidence of cutaneous leishmaniasis (CL) and ML. Delayed type hypersensitivity test (DTH) to *Leishmania* antigen was tested in selected families. Among 289 members of 46 families enrolled, significant differences were found in the frequencies of CL (37% versus 20%) and ML (5% versus 0) in case versus control families, respectively. Families with 2 cases of ML had a higher frequency (29.6%) of DTH-positive individuals than control families (9.4%). These data demonstrate familial clustering of CL, ML, and positive DTH skin tests in an area endemic for *L. braziliensis* infection.

INTRODUCTION

Leishmaniasis is a group of parasitic diseases transmitted by a sand fly vector and affecting 15 million people worldwide. Different species of *Leishmania* cause a spectrum of diseases.[1] *Leishmania braziliensis* is a cause of cutaneous leishmaniasis (CL). Mucosal leishmaniasis (ML) occurs in less than 5% of patients with CL and is characterized by involvement of the nasal, oral, pharyngeal, and/or laryngeal mucosa. ML can progress to severe destructive lesions of the involved mucosal surfaces.[2]

The combined effect of parasite factors, host immune or genetic factors, and environmental factors may influence the outcome of leishmaniasis.[3–5] Previous studies have described familial clustering of visceral leishmaniasis (VL) and CL.[3,6,7] ML is associated with a vigorous inflammatory response to parasite antigens.[8] The current study addresses the hypothesis that familial clustering of ML will also occur. To test this hypothesis, we conducted a family study to determine whether there is familial aggregation of ML in a region with a high prevalence of *L. braziliensis* infection.

MATERIALS AND METHODS

Study area. The study area surrounds the village of Corte de Pedra, located in an Atlantic forest region 280 km from Salvador, the capital of Bahia, Brazil. Agriculture is the basis of the local economy. The region has the highest incidence of CL of the state (316.3 per 100,000 annually; State Health Department, 1998). The population is stable with little migration out of the area. A health post established in Corte de Pedra in 1989 is a reference center for the diagnosis and treatment of leishmaniasis and serves 29 municipalities. Chagas disease and visceral leishmaniasis (*Leishmania chagasi*) have not been diagnosed in the area (Ministry of Health and health post records). More than 90% of the isolated parasites from lesions are *L. braziliensis*. *Leishmania amazonensis* constitutes most of the remainder.[9] There are two sand fly species in this area: *Lutzomia intermedia* and *Lutzomia withmani*.

Study design. The study was a reconstructed cohort, a hybrid between a case control and a retrospective cohort study, which consists of selection of index cases and controls, and accesses the history of exposure factors and disease in all their family members, confirming the history of disease by clinical examination (presence of scar or medical records). Medical records from 1992 to 2001 from the health post of Corte de Pedra were reviewed, and 260 confirmed cases of ML were identified. The case definition of ML is a characteristic mucosal lesion with either parasitological confirmation or two of the three following criteria: positive delayed-type hypersensitivity test (DTH), positive leishmania serology, and a histopathology suggestive of leishmaniasis. All cases in the current study also responded to antileishmanial therapy.

Informed consent was obtained from the patients or parents or guardians of minors. Guidelines for human experimentation and clinical research at the authors' institution were followed. The project was approved by the ethical committee of Hospital Universitário Professor Edgard Santos in Salvador, Brazil, and the Institutional Review Boards of the Weill Medical College of Cornell University and the University of Iowa. The ethical committee of Hospital Universitário Professor Edgard Santos is registered at the National Institutes of Health USA.

Selection of case and control families. Thirty ML and 30 neighborhood control families were selected. Cases were selected randomly from 260 confirmed ML cases in clinic records in which a patient address could be identified. For each index case, an "index control" individual was identified by asking the family of the ML case about their nearest neighbors. The index control was matched by age (± 5 years if < 20 years, ± 10 years if ≥ 20 years), gender, place of residence (1 m to 2 km distance from the case house), and approximately similar family size (± 3). Index control individuals were included regardless of whether they had a history of CL or whether they were disease-free. In instances when the index control had no history of CL, at least one member of his/her family was required to have had CL to ensure that there was

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a reasonable chance of exposure to leishmania infection. The exclusion criteria for the index control were a history of ML or belonging to the same family as the ML case. First-degree blood relatives (parents, siblings, and children) of index cases ("case family cohort") were compared with those of index controls ("control family cohort"). The same degree of effort was made to interview case and control family members. Family sizes in the study averaged 7 members. Seven case families were excluded either because they refused to participate, the majority of family members was not accessible to be examined, or no control family could be found.

Case definition and evaluation of family cohorts. All participants were interviewed using a questionnaire. Information was gathered regarding the years living in the same house as the index case or control individuals, main occupation, and environmental exposures related to the risk of sand fly contact. A medical doctor examined all subjects for past or present CL and/or ML. An otolaryngology specialist confirmed all suspected cases of ML by clinical examination, nasal aspirate for *Leishmania* culture, and biopsy for histopathological exam. Past CL was defined as history of an ulcerated lesion of more than 3 months duration, a clinical response to therapy specific for leishmaniasis, and a typical scar confirmed by a dermatologist. If patients had been treated at the Corte de Pedra health post, the diagnosis was confirmed through medical records. Individuals without a typical history and scar were considered disease-free. Parasite cultures were performed on all new cases of CL and ML by aspiration and biopsy at the leading edge of the lesion. Parasites were cultured in blood-agar media (NNN media) enriched by Schneider's liquid media.

To compare the exposure rates of the case and control family cohorts, we selected 15 families for DTH skin testing to *Leishmania* antigen. Five families with 2 cases of ML, 5 families with only 1 case of ML, and 5 control families without mucosal disease were randomly selected. *Leishmania* skin testing was performed on these family members with 25 μg of soluble *L. amazonensis* antigen (Brazilian Ministry of Health standard antigen) in 0.1 mL administered intradermally in the forearm. The DTH was considered positive if there was an induration > 5 mm at the site of inoculation 48 hours after intradermal inoculation.

Statistical analyses. Analyses were performed using EpiInfo 6.04c (CDC, Atlanta, GA) and SAS 6.12 (SAS Institute Inc., Cary, NC). Univariate logistic regression analysis was used to compare dichotomous variables using Fisher exact test and Student's *t* test to compare the continuous variables between the case and control cohorts. To calculate incidence densities for CL and ML, person-years of risk was defined as the sum of "at risk" years (years living in the endemic area at disease onset or total years living in the area at interview if disease-free). Differences in the size of the DTH reaction between the subjects with disease (CL and ML) were calculated by Mann-Whitney test. In individuals without history of disease, odds ratios and exact 95% confidence intervals were calculated to evaluate the association between positive DTH and belonging to a family with 2 cases of ML, 1 case of ML, or a control family.

RESULTS

Description of case and control family cohorts. A total of 46 families were enrolled in this study: 23 case families and 23 control families. The demography, frequency of environmental variables, and history of leishmaniasis in each cohort group are shown in Table 1. The case family and control family cohorts had similar demographics, including age, number of siblings and children, duration of residence in the house of the index case or control individuals, and the main occupation. The environmental exposures surveyed were also similar between the two family cohorts.

Frequency of past or present ML and CL in the case and control family cohorts. There were significant differences between the frequencies of CL and ML in each group. Among the 150 people in the case family cohort, there were 54 individuals with confirmed CL (36%) and 7 individuals with confirmed ML (5%), using the case definitions defined above. Among the 139 individuals in the control family cohort, there were 27 with confirmed CL (19%) and no individuals with confirmed ML. Of the 7 individuals discovered to have ML through this study, 3 had scars and were confirmed by medical records but were not previously on the list of the 260 ML cases. The other 4 cases had active lesions confirmed by an otolaryngologist, a positive DTH skin test, and a lesion biopsy positive for *Leishmania*. One of the 4 new ML lesions cultured was positive for *Leishmania*.

The case family cohort had significantly increased risk of acquiring ML compared with the control family cohort [risk ratio = 1.87; 95% confidence interval (1.15, 3.12), $P = 0.016$, Fisher exact test], as well as significantly increased risk of acquiring CL [risk ratio = 1.96; 95% confidence interval (1.24, 3.11), $P = 0.015$, Fisher exact test].

DTH skin test reactivity to *Leishmania* antigen in selected families. DTH skin tests were positive in all patients with past CL ($N = 45$) or ML ($N = 15$), with no significant differences in the size of reaction between ML and CL patients (mean ± SD, 25 ± 12.3 mm versus 23 ± 9.04 mm, respectively, $P > 0.05$, Mann-Whitney test). To compare the exposure rates of case and control family cohorts, cases of CL and ML were excluded from analysis of DTH reactivity. The frequency of positive DTH was compared in 92 subjects without a history of leishmaniasis across the three family cohorts (Table 2). Fifteen of the 92 individuals had positive DTH skin tests. The families with 2 cases of ML had a higher frequency of DTH-positive individuals (30%) than either the families with 1 case of ML (12%) or control families (9.4%). Disease-free individuals that were members of families with 2 ML cases were more likely to have positive DTH responses than those from control families (OR 4.07; $P = 0.048$).

The family distribution of ML, CL, and DTH-positive individuals across the families with more than 1 ML case shows a vertical distribution of ML (from parent to child), although the data is too preliminary to draw any conclusion. Figure 1 exemplifies the pedigrees of two families.

DISCUSSION

We documented a familial aggregation of CL and ML in a region where *L. braziliensis* is endemic. This could be due to shared environmental and/or genetic factors influencing the clinical outcome of *L. braziliensis* infection. In addition, a higher frequency of DTH-positive skin test was observed in family members of ML cases without evidence of disease according to history or clinical examination. This could reflect a

TABLE 1
Comparison of case family cohort with control family cohort: demograhics, environmental factors, and disease history

	Case family cohort	Control family cohort	
	Number and/or percentage of total subjects		*P* value*
Number of families	23	23	NS
Number of persons	150	139	NS
Mean age (years) ± SD	26.2 ± 16.7	25.8 ± 16.5	NS
Male gender	54% (81)	44% (61)	NS
Relationship to index case or control individual			
Parent	13% (20)	12% (17)	NS
Sibling	43% (65)	45% (63)	NS
Child	44% (66)	42% (58)	NS
Years lived in the same house as the index case/control (mean ± SD)	22.6 ± 14.7	21.1 ± 15.7	NS
Electricity in the home	44% (66)	50% (70)	NS
Occupation			
Farm	45% (68)	40% (56)	NS
Domestic	10% (15)	7% (10)	NS
Student/child	38% (57)	42% (58)	NS
Other	6% (9)	11% (15)	NS
Risk exposures			
Farm exposure	92% (138)	89% (124)	NS
Hunting at night	27% (41)	17% (24)	NS
Animals in home environment	90% (135)	86% (120)	NS
Mosquito net use	16% (24)	10% (14)	NS
Chicken coop in the household environment	52% (78)	50% (70)	NS
Pigsty near in the household environment	18% (27)	15% (21)	NS
Stable in the household environment	15% (23)	10% (14)	NS
Farm in the household environment	88% (132)	90% (125)	NS
Forest at the border of household	62% (93)	60% (83)	NS
Disease history			
Confirmed CL	36% (54)	19% (27)	0.005†
Confirmed ML	5% (7)	0% (0)	0.016†

NS, not statistically significant; CL, cutaneous leishmaniasis; ML, mucosal leishmaniasis.
* Univariate logistic regression analysis was used to compare demographic characteristics and potential exposure factors between the case and control family cohorts.
† To calculate incidence densities for CL and ML, person-years of risk was defined as the sum of "at risk" years in the endemic area (years in the endemic area at disease onset or total years living in the area at the time of interview if disease-tree). Incidence densities were compared and Fisher exact 2-tailed *P* values are represented.

higher rate of exposure to infection in family members of index cases and/or a propensity to develop or retain a positive skin test in these individuals. More recently, experimental data suggest that *Leishmania* persistence in the host influences the maintenance of immunologic response.[10,11] Then, it is possible that an aggregation of ML and DTH-positive individuals in families might represent a propensity to maintein the infection that could predispors to a future development of ML.

TABLE 2
Comparison of the frequency of positive DTH skin test reactions in 92 individuals without a history of cutaneous or mucosal leishmaniasis in a subset of families from the case and control family cohorts

Subgroups	No. of DTH positive skin test /total individuals tested* (%)	OR†	95% Confidence intervals‡
Case families with 2 ML cases	8/27 (30)	4.07	1.34–13.48§
Case families with 1 ML case	4/33 (13)	1.33	0.20–9.88¶
Control families with no ML cases	3/32 (9)		

OTH, delayed-type hypersensitivity; OR, odds ratio; ML, mucosal leishmaniasis.
* DTH skin test reactivity was evaluated in 3 groups of 5 families selected from case families with 2 members and 1 member with past ML and control families. Sixty patients with past CL ($N = 45$) or ML ($N = 15$) had positive DTH skin tests and were excluded from the analysis. The frequency of positive skin test was compared in 92 subjects without a history of CL or ML.
† Compares case families to control families.
‡ Exact 95% confidence intervals are represented.
§ $P = 0.048$, Mantel-Haenszel χ^2 test.
¶ $P = 0.52$, Fisher's exact test.

A number of factors could influence the outcome of human leishmaniasis. Environmental factors include the rate of exposure to sand flies, the number of parasites inoculated by infected sand flies, preexisting immune responses to sand fly saliva products, and variation between isolates of *L. braziliensis*.[12,13] Although not studied in humans, the outcome of infection in BALB/c mice differs when different inocula of *Leishmania major* are introduced.[14,15] In addition, the immunomodulatory effects of a preexisting immune response to sand fly salivary components influence infection in mice.[13] Our group has previously demonstrated different biological characteristics of different *L. amazonensis* isolates during infection of both humans and BALB/c mice.[16] Furthermore, polymorphisms within the *L. braziliensis* species have been demonstrated, including in the endemic area of Corte de Pedra, and these likely influence human disease outcome.[17,18] Finally, host genetic factors influencing the immune response and clinical outcome of leishmaniasis have been documented in mice and humans.[5,7] *L. major* causes a progressive disease with a predominant type 2 immune response in BALB/c mice, whereas the same isolate causes self-healing infection and type 1 immune response in C57BL/6.[19] Peripheral blood mononuclear cells from ML patients produce higher levels of IFN-γ and TNF-α than CL patients in response to *Leishmania* antigen.[8,20,21] Additionally, stronger DTH reaction and high levels of TNF-α in sera are observed *in vivo* in ML patients during active disease.[4]

The familial clustering of CL, ML, and DTH observed in the current study could reflect a common environmental ex-

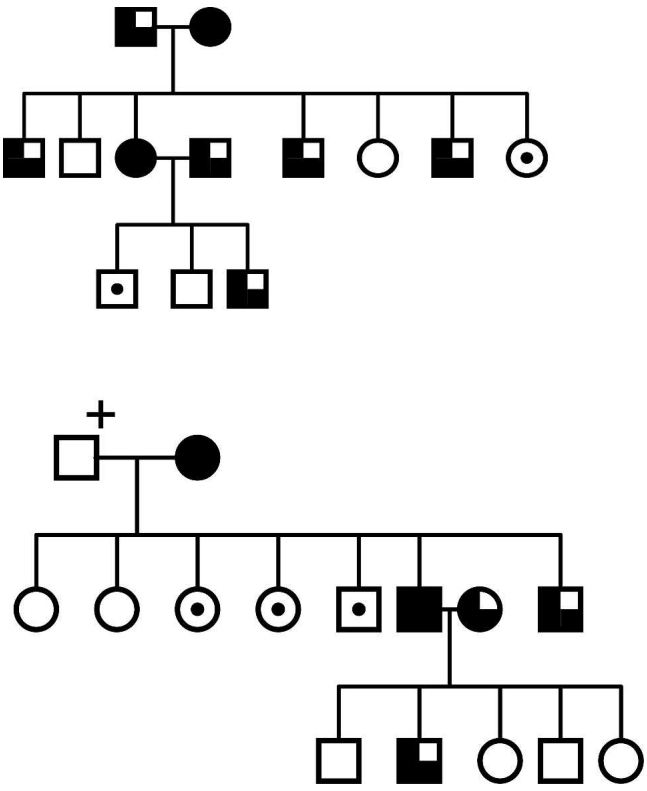

FIGURE 1. Two representative pedigrees of families with more than 1 ML case showing ML patients (■), CL patients (◪) and DTH-positive individuals without history of disease (⊡). All patients with past ML and CL also presented positive DTH skin tests.

posure rate. However, although both environmental and parasite factors might participate in the occurrence of CL and ML, differences in the environmental factors that we evaluated, including the years lived in the same house of the index case or control individuals, were not detected between ML and neighborhood control families. Because ML and DTH reaction both reflect a type 1 cellular infiltrate, it is also possible that familial clustering reflects a stronger reactivity to parasite antigens or parasite persistence in cases compared with control family members, manifested clinically as a higher frequency of both ML and positive DTH response. Recent data from experimental models of leishmaniasis suggest that DTH reactivity reflects parasite persistence.[11,22] Moreover, genetic polymorphisms have been associated with human susceptibility to cutaneous, mucosal, and visceral leishmaniasis in other endemic areas.[5,7,23] Whether the genetic backgrounds of some individuals from this region also predisposes them to develop vigorous immune responses to *Leishmania* antigen awaits further study of host genetic polymorphism. The distribution of CL and especially ML cases occurs from parents to siblings. However, the sample size is too small to draw any conclusions. We are accumulating more multicase families for future studies.

In conclusion, in this study we observed familial aggregation of ML and CL through a study of families of ML index cases and matched controls. It remains unclear whether the clustering is due to environmental factors influencing the manifestations of infection or to human genetic factors.

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