Dermatological Findings in 3 Generations of a Family with a High Prevalence of Human T Cell Lymphotropic Virus Type 1 Infection in Brazil

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Background. Dermatologic manifestations are quite common in patients with adult T cell leukemia and lymphoma and patients with myelopathy and/or tropical spastic paraparesis associated with human T cell lymphotropic virus type 1 (HTLV-1). The aim of this study was to investigate dermatological findings presented by 30 members of a Brazilian family, half of whom are infected with HTLV-1 (as confirmed by enzyme-linked immunosorbent assay and Western blot).

Methods. The subjects underwent dermatologic examination and laboratory assessment, which included the search for the HTLV-1 genome in peripheral blood mononuclear cells (PBMCs) by qualitative and semiquantitative polymerase chain reaction (PCR) and in skin samples by nested qualitative PCR and immunofluorescence assay.

Results. We found that cases of xerotic dermatological alterations, including 3 cases of acquired ichthyosis, were more frequent among the infected patients (7 cases vs. none among the uninfected individuals; \( P = .0063 \)). Other lesions observed in this group included impetigo, scabies, epidermal nevus, herpes zoster scar, rosacea, and juvenile acne. One HTLV-1–infected individual presented with concurrently acquired ichthyosis, impetigo, scabies, dermatophytosis, and seborrheic dermatitis. The PCR performed on PBMCs and skin samples from 24 patients confirmed the serological results in all cases. Additionally, the HTLV-1 proviral load was higher in patients with >1 skin lesion. Finally, HTLV-1 could be identified in the skin by immunofluorescence assay, which, by use of PCR as the gold standard, showed a sensitivity and specificity of 61.5% and 100%, respectively.

Conclusions. Altogether, these clinical and laboratory findings point to an HTLV-1 tropism toward the skin, even in HTLV-1 carriers without adult T cell leukemia/lymphoma or HTLV-1–associated myelopathy and/or tropical spastic paraparesis.

Human T cell lymphotropic virus type 1 (HTLV-1) infection was described in the early 1980s and currently affects several million individuals worldwide. The virus is endemic in Japan, the Caribbean, Melanesia, Africa, and South America [1]. HTLV-1 and -2 are transmitted vertically from mother to child (mainly by breast-feeding), by the sharing of syringes, sexually, and by blood transfusion [1]. Soon after its description, HTLV-1 was regarded as the etiological agent of adult T cell leukemia and lymphoma (ATLL) [2–4], HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP) [5–7], and HTLV-1–associated uveitis [8]. Additionally, reports in the early 1990s showed a strong association in Jamaica between HTLV-1 and severe childhood eczema, known as “infective dermatitis” [9]. Infective dermatitis was also reported in other countries, including Brazil, Peru, and Japan [10–12]. Some additional conditions associated with HTLV-1 infection are chronic arthritis, recurrent and/or severe strongyloidiasis, reduction of late skin reaction, and severe scabies [1, 13].

Dermatologic alterations other than infective dermatitis have been described in patients infected with HTLV-1, notably in those with ATLL or HAM/TSP [14–16]. In patients with ATLL, there is a predominance of maculopapular and nodular lesions, whereas xeroder-
mia and acquired ichthyosis are the main dermatological findings associated with HAM/TSP [17]. The frequency of skin lesions in HTLV-1 carriers is unknown, although some cases have been described [13, 15, 18]. Additionally, some reports suggest that HTLV-1–infected subjects with skin lesions have a higher risk of developing ATLL or HAM/TSP than do those without skin lesions [19, 20]. The aim of this study was to investigate the epidemiological, dermatological, and laboratory findings of 30 subjects in a Brazilian family with a high prevalence of HTLV-1 infection.

SUBJECTS AND METHODS

Subjects. This cross-sectional study included 30 subjects in 3 generations of a family residing in the southeast region of Brazil whose index patient was a man with HAM/TSP. All 30 patients underwent serological tests for HTLV (EIA [anti-HTLV-1/2; OrthoClinical Diagnostics] and Western blot [GLD HTLV BLOT 2.4; Genelabs Diagnostics]), and 15 (50%) were found to be seropositive. The epidemiological and clinical parameters obtained were sex, age at the time of study, possible risk factors for acquired HTLV infection, and dermatological assessment. The Ethics Committee on Human Research at the Federal University of Minas Gerais approved the study protocol, and written informed consent was obtained from all participants.

Dermatological assessment. A complete dermatological examination was performed for all subjects, without knowledge of HTLV-1 serological status. When suspected, dematophytes or Sarcoptes scabiei species were searched for in skin scrapings of the elevated margins of erythematous-desquamative lesions. In short, the specimens were placed on a glass slide and were examined with an optical microscope after application of a 10% KOH solution for 30 min. The presence of septated hyphae confirmed the diagnosis of tinea. The identification of preserved eggs and/or adult forms of S. scabiei was used to diagnose scabies.

Skin biopsies. Skin biopsies were performed in the interscapula vertebral area from 24 subjects. The biopsy procedure was as follows: (1) local asepsis, (2) placement of a fenestrated surgical drape over the biopsy site, (3) local anesthesia, (4) biopsy by use of a 5-mm-round disposable knife, and (5) dressing and orientation. Each skin sample was minced into 3 smaller fragments, which were used for conventional histology, PCR, and immunofluorescence (IF) assay. Then, the specimens were prepared in accordance with standard recommendations. The specimens for anatomopathological studies were included; they were fixed in 10% tamponade formalin and then were stored at room temperature. Afterward, they were stained with hematoxylin-eosin before being analyzed with an optic microscope. The specimens for molecular biology and antibody-based studies were stored in the freezer at −70°C.

Nested PCR specific to HTLV-1 in PBMCs and skin biopsy specimens. Nested PCR specific to the HTLV-1 env gene was used to investigate the presence of the HTLV proviral genome and to confirm the virus type in PBMCs and in skin biopsy specimens. Blood and skin samples were obtained from 24 subjects, including 13 who were found to be seronegative for HTLV-1. PBMCs were isolated by density-gradient centrifugation, and DNA was extracted using DNAzol Reagent (Invitrogen) in accordance with the manufacturer’s recommendations. DNA from skin biopsy specimens was obtained using overnight digestion with proteinase K, as described elsewhere [21].

One microgram of genomic DNA from PBMCs or skin was used as the target in a reaction of 20 μL containing 20 mmol/L Tris-HCl (pH 8.0), 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 200 mmol/L of each deoxyribonucleoside triphosphate, 0.5 U of Taq polymerase (Invitrogen), and 10 pmol of primers SK248 (5′-CTAGTGCAGCTCCAGGATATGACC-3′) and SK249 (5′-CAGACCCGACCGTACCGTCGCG-3′). PCR was done using a thermal cycler (PTC-100; MJ Research) with 35 cycles for 45 s at 95°C, 45 s at 58°C, and 1 min at 72°C and a final extension period of 7 min at 72°C. Two microliters of this first PCR product were used as the target for a second round of amplification, under the same reagent conditions and with primers ENV-IF (5′-TCCCTAATACCGAACCAGCAACTG-3′) and ENV-IR (5′-GGTCAAAGCAGTGGGTCCAGTTAAAT-3′). The thermal conditions were 30 cycles for 45 s at 95°C, 45 s at 60°C, and 1 min at 72°C, with a final extension period of 7 min at 72°C. The amplified product (311 bp) was submitted to electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized with UV light.

Measurement of HTLV-1 proviral load in PBMCs. The HTLV-1 proviral load in PBMCs was semiquantified by densitometry analysis. The HTLV-1 proviral copy number and cell number were measured using PCR specific to the HTLV-1 env region and the human β-actin gene, respectively. We considered that 1 μg of DNA corresponds to 1.5 × 10⁶ PBMCs, so DNA was diluted to contain 1 × 10⁴ cells in a 3-μL volume, which was used to amplify HTLV-1 env and the β-actin gene in different reaction tubes. PCR env amplification was done using 3 μL of DNA in a 30-μL volume containing 20 mmol/L Tris-HCl (pH 8.4), 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 200 μmol/L deoxynucleoside triphosphates, 0.75 U Taq DNA polymerase (Invitrogen), and 10 pmol of primers SK248 and SK249. Forty cycles for 30 s at 95°C, 45 s at 58°C, and 1 min at 72°C were performed in a thermal cycler (PTC-100; MJ Research). PCR β-actin amplification was done using 3 μL of DNA in a 20-μL volume containing 20 mmol/L Tris-HCl (pH 8.4), 50 mmol/L KCl, 3.5 mmol/L MgCl₂, 200 μmol/L deoxynucleoside triphosphates, 0.5 U Taq DNA polymerase (Invitrogen), and 10 pmol of the primers actin-1 (5′-TCACCCACACTTGTCGCCAT-
Two-sided tests were used, and was considered to be statistically significant. Calculations were made using the EpiInfo 2000 statistical program (Centers for Disease Control and Prevention).

CTACGA-3′; nucleotide positions, 2141–2165) and actin-2 (5′-CAGCGGAACGCTATTGCCAATGG-3′; nucleotide positions, 2435–2411). Twenty-five cycles for 30 s at 95°C, 30 s at 60°C, and 30 s at 72°C were performed in a thermal cycler (PTC-100; MJ Research). An aliquot of 12 μL of β-actin (295 bp) and 20 μL of env (469 bp) amplified products was submitted to electrophoresis on a 2% agarose gel stained with ethidium bromide, and the image visualized with UV light was captured by ImageMaster VDS (Amersham). Analysis of densitometry was done using TotalLab software version 2.00 (Amersham). Standard curve to proviral copies was generated using serial dilutions of DNA from MT2, an HTLV-1–infected cell line, to represent 2 × 10^4, 8 × 10^4, 2 × 10^5, 2 × 10^6, and 10^7 copies of HTLV DNA. To cell number standard curve were used dilutions represented 2 × 10^4, 10^4, 10^5, 10^6, and 10 cells. We assumed that each MT2 cell contains 2 copies of env and that each PBMC has 2 copies of the β-actin gene.

HTLV-1 identification by use of anti-p19 fluorescent monoclonal antibody. Each skin specimen was pressed between a pair of glass slides to obtain an imprinting for the IF assay by use of anti-p19 monoclonal antibody (monoclonal antibody 8817; containing conjugated AP 124F, fluorescein isothiocyanate, and goat anti-mouse IgG). The technical procedures applied in the IF assay were published elsewhere [22]. Importantly, the interpretation of the findings of this examination was done without knowledge of the serological results. MT2 cells [23] and skin cells from an HTLV-1–negative individual unrelated to the studied family were used as positive and negative controls, respectively.

Statistical analysis. All data were recorded in a database designed especially for this study. Analyses were performed by χ² test, Fisher’s exact test, and Student’s t test, as appropriate. Two-sided tests were used, and P < .05 was considered to be statistically significant. Calculations were made using the EpilInfo 2000 statistical program (Centers for Disease Control and Prevention).

RESULTS

HTLV-1 transmission among members of family. Of 30 individuals evaluated, 15 (50%) were HTLV-1 seropositive; 12 were female, and 3 were male (figure 1). This sex distribution suggested significant correlation between HTLV-1 infection and female sex (12 [73.3%] of 15 vs. 3 [13.3%] of 15; P = .028) (table 1). Epidemiological variables, such as age, and different risk factors for acquired HTLV-1 infection showed no statistical differences between HTLV-1 carriers and seronegative individuals (table 1). Breast-feeding was the most common mode of HTLV-1 transmission among the family members, occurring in 77.7% from generation I to II and 36.6% from generation II to III (figure 2). Interestingly, all cases of HTLV-1 infection observed in members of the third generation occurred in female subjects (figure 2). Despite presenting a high proviral charge with HAM/TSP, the index case (II-9) had not transmitted the virus to his wife.

Dermatological findings. More than one skin lesion was observed in some individuals, a finding which was unrelated to their serological status. Nevertheless, dermatological alterations related to xerosis were noticeably more frequent in the HTLV-1–infected group; 7 infected patients presented significant skin xerosis, which was characterized as acquired ichthyosis in 3 patients. One of these patients had concomitant diagnoses of ichthyosis, classic scabies, impetigo, tinea manus, and seborrheic dermatitis. Among the 3 patients with ichthyosis, 2 had moderate and 1 had severe skin harm (figure 1). Regarding the patients with cutaneous xerosis, besides the clear diagnosis they received after the physical examination, they reported the need to use moisturizing products daily, independent of environmental conditions (e.g., temperature). None of the patients in the uninfected group presented with xerotic skin conditions (P = .0063). Other relevant lesions found among the HTLV-1–infected individuals were tinea pedis (2 patients),

Table 1. Epidemiological data from 30 family members, with comparison of infected and uninfected subjects.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HTLV-1–positive subjects</th>
<th>HTLV-1–negative subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio of male to female subjects</td>
<td>1:4</td>
<td>2.7:1</td>
</tr>
<tr>
<td>Age, mean years ± SD</td>
<td>28.8 ± 16.6</td>
<td>28.6 ± 15.7</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sexual contact</td>
<td>67</td>
<td>73</td>
</tr>
<tr>
<td>Breast-feeding</td>
<td>100</td>
<td>88</td>
</tr>
<tr>
<td>Injection drug use</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total no. of members</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

**NOTE.** Data are percentage of subjects, unless otherwise indicated. HTLV-1, human T cell lymphotropic virus type 1.

* P < .05.
interdigital candidiasis (1 patient), herpes zoster scar (2 patients), rosacea (1 patient), and scabies (1 patient). Dermatological alterations were also seen in uninfected individuals, including tinea pedis (4 subjects), pityriasis versicolor, neurotic excoriations, axillary’s trichomycosis, scabies, acne conglobata, and plantar hyperkeratosis. Overall, 6 of 10 individuals without any dermatological lesion tested negative for HTLV-1 antibodies, but this observation did not reach statistical significance (P = .69).

**HTLV-1 proviral load.** Nested PCR for the HTLV-1 env gene performed in PBMCs from 24 subjects confirmed the results of the serological tests and the type of virus. HTLV-1 proviral load from 13 subjects showed values in a range from 1.23% to 16.06% (table 2). The mean proviral load was 6.14%, and the median was 3.28%. A higher proviral load corresponded to more abnormal findings from the dermatological examination, and as expected, to HAM/TSP occurrence (individuals 9 and 13 in table 2). Individual 12 (table 2), who presented a proviral load of 14.86% at the time of sample collection (March 2002), had normal results of dermatological and neurological assessments. However, she developed HAM/TSP 2 years later.

**Histological findings in skin samples.** PCR for the HTLV-1 env gene was performed in 26 skin samples from 24 patients, 12 (50%) of whom had reactive serological results for HTLV-1. As observed in PBMCs, the nested PCR for the env gene in skin samples confirmed the serological findings in all tested specimens.

Twenty-six skin fragments from 24 patients were analyzed by conventional histologic examination (12 patients were seropositive, and 12 were seronegative). The histological findings were predominantly unremarkable. Only 4 specimens—3 from HTLV-1–infected individuals—showed chronic dermatitis (P = .58). The microscopic findings in the 2 samples obtained from areas with ichthyosis confirmed this diagnosis.

**IF assay of skin samples.** To confirm the presence of HTLV-1 through expression of viral proteins in skin, 22 skin samples from 20 subjects were used in the IF assays with anti-p19 monoclonal antibody. The test was able to identify HTLV-1 in some samples, and given PCR for the HTLV-1 env gene in the skin as the gold standard, the sensitivity and specificity of the indirect IF assay were 61.5% (5 false-negative results from 13 samples) and 100% (no positive results from 9 samples), respectively.

**DISCUSSION**

In this cross-sectional study, 30 relatives were found to have a high prevalence of HTLV-1 infection (50% seropositivity) and were investigated for epidemiological, dermatological, and laboratory parameters. The core findings from this study were that (1) HTLV-1–infected subjects presented a significantly higher number of xerotic dermatological lesions, including 3 cases of acquired ichthyosis; (2) the nested PCR for the HTLV-1 env gene performed in PBMCs and in skin samples confirmed the serological results in the 24 subjects who underwent this procedure; and (3) the search for HTLV-1 by use of the IF assay with anti-p19 monoclonal antibody in 22 skin samples showed a sensitivity and specificity of 61.5% and 100%, respectively.

The high prevalence of HTLV-1 infection (50%) found in this Brazilian family is noteworthy. For example, in a study of Jamaican subjects, La Grenade et al. [24] found a prevalence of 17% among 19 relatives of 2 patients with ATLL. In another publication [25], the same group showed an HTLV-1 intrafamilial prevalence of 30%. Breast-feeding practices [26], genetic factors, and intrinsic viral characteristics could be implicated in these discrepancies. The predominance of females among infected individuals has been shown in the literature and is probably linked to a genetic predisposition and to the higher sexual risk observed in women [27, 28].
Table 2. Human T cell lymphotropic virus type 1 (HTLV-1) proviral load in PBMCs and clinical findings for 13 family members.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Proviral load, %</th>
<th>Dermatological assessment</th>
<th>HAM/TSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.23</td>
<td>Tinea interdigitalis</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>1.34</td>
<td>Unchanged</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>1.37</td>
<td>Juvenile acne</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>1.59</td>
<td>Xerosis</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>2.65</td>
<td>Unchanged</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>3.14</td>
<td>Xerosis</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>3.28</td>
<td>Herpes zoster scar, digital intertrigus, tinea pedis</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>5.44</td>
<td>Acquired ichthyosis</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>7.91</td>
<td>Acquired ichthyosis, epidermic nevus, herpes zoster scar</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>9.12</td>
<td>Acquired ichthyosis, impetigus, scabies, tinea manus, seborrheic dermatitis</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>12.81</td>
<td>Rosacea, scabies</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>14.86</td>
<td>Unchanged</td>
<td>No</td>
</tr>
<tr>
<td>13*</td>
<td>16.06</td>
<td>Onicomicosis, xerosis, tinea cruris</td>
<td>Yes</td>
</tr>
</tbody>
</table>

NOTE. HAM/TSP: HTLV-1–associated myelopathy/tropical spastic paraparesis.
\* Index patient.

The absence of other risk factors for HTLV-1 infection, in conjunction with breast-feeding for 100% of case patients, allowed us to conclude that breast-feeding was the most common mode of viral acquisition among participating individuals. Surprisingly, all infected children in generation III were girls. Early observations in Taiwan showed that mother-to-child transmission is responsible for the majority of cases of HTLV-1 infection occurring among relatives [29], but the prevalence of intrafamiliar infection observed in the present study is higher than that found in other studies. In one report, Kajiyama et al. [30] showed that this mode of transmission accounted for 28% of intrafamiliar transmissions. Finally, in ~6% of cases, the fetal infection may be acquired transplacentally or during delivery [31–33].

Several dermatological lesions were found among the subjects, especially among those infected with HTLV-1. Some relevant differences were seen between infected and uninfected individuals. First and most importantly, xerotic lesions were found only in HTLV-1 carriers. Overall, 7 individuals showed this dermatological alteration, including 3 patients with acquired ichthyosis. Furthermore, 1 patient had 5 simultaneous dermatological conditions. Taken as a whole, these findings may suggest that HTLV-1 is able to invade the skin. Additionally, it reinforces the probable relationship between HTLV-1 infection and dermatopathy, even in asymptomatic carriers [15]. Acquired ichthyosis may be related to several clinical conditions, such as lymphoproliferative disorders, hypothyroidism, sarcoidosis, and leprosy, and to consumption of drugs that influence lipidic metabolism [34]. None of these findings were observed among participating individuals.

As mentioned above, skin lesions other than infective dermatitis may be associated with HTLV-1 infection, mostly in patients with HAM/TSP or ATLL [16]. Recently, Setoyama et al. [35] demonstrated that HTLV-1 can infect cells of sweat glands. However, this virus also may infect keratinocytes [36]. It was demonstrated that patients with HAM/TSP and ichthyosis have their keratinocytes activated by cytokines released by HTLV-1–infected lymphocytes [37]. This mechanism resembles that described in other HTLV-1–associated conditions, such as HAM/TSP and uveitis [17].

Molecular biology assays. Approximately 10%–15% of PBMCs are infected with HTLV-1 in patients with HAM/TSP [38, 39]. This percentage is even higher among patients with ATLL but is lower in viral carriers without symptoms [40]. Nested PCR for the HTLV-1 env gene performed in PBMCs from 24 subjects confirmed the serological tests and the virus type. As mentioned, a patient with high proviral load received a diagnosis of HAM/TSP 2 years after having an unremarkable clinical evaluation (individual 12 in table 2). Patients with a high viral load may have a worse clinical outcome, thus requiring close observation.

Finally, PCR for the env gene performed on the skin samples from 12 patients with reactive serologic tests revealed positive findings in all cases. Recently, Gonçalves et al. [15] demonstrated that the frequency of PCR results positive for HTLV-1 is significantly higher in histologically abnormal skin samples.

IF assay. The IF assay results positive for HTLV-1 identification. A remarkable finding was that the morphology of all HTLV-1–positive cells resembled that of lymphocytes,
even though the precise histological localization in the skin could not be defined. As mentioned above, the increased presence of lymphocytes could partially explain the cutaneous injury associated with HTLV-1 infection. Nevertheless, additional studies, such as analyses of cytokine-releasing tests, are necessary to confirm this assumption [35].

The data presented here point to a cutaneous tropism of HTLV-1. Undoubtedly, this study has some limitations. First and foremost is the lack of a randomized design. However, the dermatological assessment and most laboratory tests were performed without knowledge of HTLV-1 infection status. Furthermore, we could not perform the laboratory examinations on all included subjects, because 4 patients declined to submit a second blood smear specimen (for PCR) and, for 2 patients, the collected material was insufficient. Finally, 6 patients living in one rural area declined to undergo skin biopsy. On the other hand, several advantages related to a familial study may be mentioned—notably, the homogeneity of genetic and environmental factors for the studied individuals. Also, in this study, we could estimate the dynamic of viral intrafamilial transmission and its consequences.

In conclusion, our findings reinforce the relationship between HTLV-1 infection and dermatological lesions, mostly xerotic disturbances of skin. Additionally, these lesions seem to occur even in asymptomatic carriers of this virus. Further studies are needed to investigate the physiopathologic mechanism of these findings.

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