DERMATOLOGIC LESIONS IN ASYMPTOMATIC BLOOD DONORS SEROPOSITIVE FOR HUMAN T CELL LYMPHOTROPIC VIRUS TYPE-1

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Abstract. Dermatologic manifestations are quite common in patients with adult T cell leukemia/lymphoma and myelopathy/tropical spastic paraparesis associated with infection with human T cell lymphotropic virus type-1 (HTLV-1). In this study, we evaluated the dermatologic lesions of eligible blood donors in the state of Minas Gerais in Brazil who were seropositive but asymptomatic for infection with HTLV-1. The study population was composed of 128 HTLV-1-seropositive individuals and 108 seronegative controls. All individuals underwent a dermatologic evaluation. Biopsy specimens were obtained from abnormal and normal skin samples of seropositive individuals in an attempt to detect HTLV-1 in tissue samples by a polymerase chain reaction. Dermatologic alterations were observed more frequently in the seropositive group (adjusted odds ratio [OR] = 8.77, 95% confidence interval [CI] = 4.11–18.71). The most common skin diseases were dermatophytoses (adjusted OR = 3.32, 95% CI = 1.50–7.35), seborrheic dermatitis (OR = 3.53, 95% CI = 0.67–24.66), and acquired ichthyosis (P = 0.001). Virus was detected more frequently in abnormal skin samples. Dermatologic lesions probably related to HTLV-1 infection were diagnosed in eligible blood donors who were infected with this virus, who were previously considered to be asymptomatic carriers of HTLV-1.

INTRODUCTION

The human T cell lymphotropic virus type-1 (HTLV-1) is associated with adult T cell leukemia/lymphoma (ATL) and other disorders, including an inflammatory demyelinating chronic progressive myelopathy known as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP).1,2 Dermatologic manifestations are quite common in both diseases.3,4 Patients with HAM/TSP may have skin manifestations such as xerosis and erythema.3 Infective dermatitis of childhood, a severe, exudative eczema involving infection with Staphylococcus aureus or β-hemolytic Streptococcus, is the only well-described outcome of childhood HTLV-1 infection. Anecdotal data suggest that it may be a risk factor for development of ATL in Jamaica.4

Infection with HTLV-1 is endemic in southern Japan,5 the Caribbean region, and intertropical Africa. The prevalence of this virus in Brazil has been estimated to be approximately 0.41%, ranging from 0.1% in the blood donor population in the state of Minas Gerais (southeastern region) to 1.4% in the state of Bahia in the northeastern part of the country.

In this study, we investigated the association of HTLV-1 with skin diseases among blood donors and whether HTLV-1 is found more often in skin lesions of infected persons compared with their normal skin.

MATERIALS AND METHODS

Patients and controls. Blood donors at the Minas Gerais Blood Center in Belo Horizonte, Brazil who were seropositive for infection with HTLV-1 were selected for participation in the study. After filling out a routine pre-donation questionnaire and undergoing and passing a clinical examination, individuals considered eligible for blood donation (those between 18 and 60 years of age who were in good general health, had no risk behavior for retrovirus infections [e.g., illegal injectable drug use, unsafe sex, and tattooing], and who had not received blood [or blood product] transfusions) were tested for blood-transmitted infections, namely human immunodeficiency viruses-1/2 (HIV-1/2), hepatitis B virus (HBV), HCV, Trypanosoma cruzi, and Treponema pallidum. In Brazil, all blood donors are volunteers and any reimbursement for blood donation is illegal. The seronegative control group was randomly selected from qualified blood donors during the same period. One of every three individuals on the list of clinically and serologically approved blood donors was chosen to participate in the study. Two-hundred thirty-six subjects, 128 who were seropositive for HTLV-1 and 108 who were seronegative for HTLV-1, were enrolled in the study between March 1997 and April 1999. The study was reviewed and approved by the ethics committee of the Faculty of Medicine of the Federal University of Minas Gerais (Belo Horizonte, Brazil). Informed consent was obtained from each participant in the study.

Dermatologic evaluation. Skin examinations were performed by a dermatologist from the Faculty of Medicine of the Federal University of Minas Gerais. Dermatologic diagnoses were defined by clinical and histologic criteria for each disease; staining with potassium hydroxide was used in cases of cutaneous mycosis. Biopsies of normal skin midway along the left interscapulo-vertebral area were also performed. The specimens obtained were examined by light microscopy using routine stains, and the presence of HTLV-1 was investigated by a nested polymerase chain reaction (PCR). One dermatopathologist was responsible for the histologic analysis. Examiners did not know the serologic status of the individuals/specimens during clinical and histologic evaluations.

Seropositivity for HTLV-1. Seropositivity for infection with HTLV-1 was defined as repeated reactivity in blood samples tested by an enzyme-linked immunosorbent assay (Abbott Laboratories, Abbott Park, IL). This was confirmed by Western blotting (HTLV-1/2, Cambridge Biotech, Cambridge, MA) and an HTLV-1 nested PCR with peripheral blood mononuclear cells.

Nested polymerase chain reaction (PCR). The same PCR procedure was used for testing of blood and skin samples.
DNA was isolated from peripheral blood mononuclear cells using the DNAzol kit (Gibco-BRL, Gaithersburg, MD) according to manufacturer's instructions. DNA was isolated from skin samples by the mammalian DNA isolation procedure.6

Three-millimeter punch biopsy specimens were obtained, placed in 500 µL of 20 mM Tris-HCl, 1 mM EDTA, pH 8.0, and frozen. The DNA was isolated from skin samples6 and 2 µL was subjected to PCR amplification using a series of temperature-dependent cycles in an automated thermal cycler (PTC-100; MJ Research, Waltham, MA).7 Amplification was performed with a pair of synthesized primers: SK110-pol (4757-4778, 5'-CCCTACAAATCCAACCGTCAGC-3') and SK44-tax (7536-7496, 5'-GAGCCGATAACCGGTCTC CATCG-3'). Thirty-five cycles of amplification were performed with denaturation (94°C for one minute), annealing (58°C for one minute), and extension (72°C for two minutes). Two microliters of the first PCR product was amplified for 30 cycles using the same conditions with two other primers: 248 envelope (env) (5669-5693, 5'-CTAGTGCAGCTC CAGGATATGACC-3') and 249-env (6137-6113, 5'-CAG ACGCCACCGTACCGTCGCG-3'). The final product, a 469-basepair HTLV-1 env gene sequence, was analyzed by electrophoresis on a 1.8% agarose gel and photographed. All samples were subjected to PCR amplification of the HLA-DQB gene as a quality control.8 One HTLV-1-negative skin sample from a blood donor was subjected to the HTLV-1 PCR as a negative control of the procedure.

Statistical analysis. All data were recorded in a database especially designed for this study. Statistical analysis included the chi-square test, Fisher’s exact test, and McNemar’s test for matched analysis. The adjustment for the effects of age, race, and sex as possible confounding factors was done using unconditional logistic regression. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to determine and quantify associations. Statistical significance was based on an α level of 0.05. Calculations were made using the Epi-Info 2000 statistical program (Centers for Disease Control and Prevention, Atlanta, GA).

RESULTS

For the two-year period in which this study was conducted, the overall seroprevalence of HTLV-1 among eligible blood donors in Minas Gerais, Brazil was 0.3%. The age range of the study population was 19–59 years (mean = 34.2) and 139 (58.8%) of the participants were men. The mean ± SD age was 38 ± 15 years in the seropositive group and 31 ± 12 years in the seronegative group. Analysis of the age intervals showed a significant trend of increasing HTLV-1 prevalence with age (Table 1). Skin color and sex of the study group are listed in Table 1.

Dermatologic alterations were more frequently observed in the seropositive group (adjusted OR = 8.77, 95% CI = 4.11–18.71). The most common skin diseases were dermatophytes (adjusted OR = 3.32, 95% CI = 1.50–7.35), seborrheic dermatitis (OR = 3.53, 95% CI = 0.67–24.66), and acquired ichthyosis (P = 0.001). Dermatologic lesions and data for other diseases are shown in Table 2.

Skin biopsy specimens were obtained from 73 HTLV-1-infected individuals. Based on clinical examinations, HTLV-1-infected persons who provided skin biopsy specimens were divided into two groups. Group I consisted of 20 persons with abnormal skin and skin diseases that were consistent with known HTLV-1-associated- or HIV-associated-skin disease, or diseases of unknown etiology. Group II consisted of 55 HTLV-1-infected persons with normal skin.

Twenty patients in group I had dermatologic lesions. The PCR analysis of the skin of one patient with acquired ichthyosis was not performed because of an insufficient sample. Thus, 19 skin specimens were examined and the results of the nested PCR were negative in three of them. The normal matched skin specimens from these three patients were also negative for virus (Table 3). In the matched analysis, HTLV-1 was detected more frequently in abnormal skin than in the normal skin of the same subject (P = 0.004).

Virus was detected by the PCR in the normal skin of the interscapulo-vertebral area of seven of 20 individuals (35%) who were seropositive for HTLV-1 and had skin lesions in other areas (group I). The results of the HTLV-1 PCR were positive in the normal skin of the interscapulo-vertebral area of 23 of 53 individuals (43%) who were seropositive for HTLV-1, but did not have skin lesions in other areas (group

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**Table 1**

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>HTLV-1 positive</th>
<th>HTLV-1 negative</th>
<th>Odds ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19–28</td>
<td>26 (20)</td>
<td>37 (34)</td>
<td>1.00</td>
</tr>
<tr>
<td>29–38</td>
<td>30 (24)</td>
<td>45 (42)</td>
<td>0.95 (0.45–1.99)</td>
</tr>
<tr>
<td>39–48</td>
<td>41 (32)</td>
<td>15 (14)</td>
<td>3.89 (1.68–9.14)</td>
</tr>
<tr>
<td>49–58</td>
<td>31 (24)</td>
<td>11 (10)</td>
<td>4.01 (1.59–10.32)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>70 (55)</td>
<td>69 (64)</td>
<td>1.00</td>
</tr>
<tr>
<td>Female</td>
<td>58 (45)</td>
<td>39 (37)</td>
<td>1.35 (0.78–2.36)</td>
</tr>
<tr>
<td>Skin color</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>70 (55)</td>
<td>88 (81)</td>
<td>1.00</td>
</tr>
<tr>
<td>Black or Mulatto</td>
<td>58 (45)</td>
<td>20 (19)</td>
<td>3.65 (1.93–6.94)</td>
</tr>
</tbody>
</table>

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**Table 2**

<table>
<thead>
<tr>
<th>Dermatologic examination result</th>
<th>HTLV-1 positive</th>
<th>HTLV-1 negative</th>
<th>Crude odds ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal</td>
<td>63 (49.2)</td>
<td>13 (12.0)</td>
<td>7.08 (3.45–14.77)</td>
</tr>
<tr>
<td>Dermatophytosis*</td>
<td>44 (34.4)</td>
<td>16 (14.8)</td>
<td>3.32 (1.78–6.23)</td>
</tr>
<tr>
<td>Seborrheic dermatitis</td>
<td>8 (6.3)</td>
<td>2 (1.9)</td>
<td>3.53 (0.67–24.66)</td>
</tr>
<tr>
<td>Acquired ichthyosis</td>
<td>9 (7.0)</td>
<td>0</td>
<td>Undefined</td>
</tr>
<tr>
<td>Contact dermatitis</td>
<td>4 (3.1)</td>
<td>2 (1.9)</td>
<td>1.71 (0.26–13.73)</td>
</tr>
<tr>
<td>Vitiligo</td>
<td>5 (2.3)</td>
<td>0</td>
<td>Undefined</td>
</tr>
<tr>
<td>Herpes labialis</td>
<td>2 (1.6)</td>
<td>0</td>
<td>Undefined</td>
</tr>
<tr>
<td>Pityriasis versicolor</td>
<td>2 (1.6)</td>
<td>2 (1.9)</td>
<td>0.86 (0.08–8.67)</td>
</tr>
<tr>
<td>Scabies</td>
<td>2 (1.6)</td>
<td>0</td>
<td>Undefined</td>
</tr>
<tr>
<td>Acanthosis nigricans</td>
<td>1 (0.8)</td>
<td>0</td>
<td>Undefined</td>
</tr>
<tr>
<td>Asteatotic dermatitis</td>
<td>1 (0.8)</td>
<td>0</td>
<td>Undefined</td>
</tr>
<tr>
<td>Lichen planus</td>
<td>1 (0.8)</td>
<td>0</td>
<td>Undefined</td>
</tr>
<tr>
<td>Nummular dermatitis</td>
<td>1 (0.8)</td>
<td>0</td>
<td>Undefined</td>
</tr>
<tr>
<td>Xerosis</td>
<td>1 (1.8)</td>
<td>0</td>
<td>Undefined</td>
</tr>
</tbody>
</table>

* Tinea pedis, T. unguium, T. corporis, and T. cruris
infected by this virus. This may also explain why the PCR
with ATL. Thus, skin cells, in addition to lymphocytes, can be
involved in the pathogenesis of the skin lesions or present in
the skin because inflammatory cells containing virus migrated
to the lesion needs to be evaluated. Setoyama and others,\textsuperscript{14} in
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discussed virus more frequently in abnormal skin than in the
normal skin of the same subject in the present study.

It would be of interest to know whether the identification
of HTLV-1 in the skin can predict the development of cuta-
aneous T cell lymphoma. Analysis of the type of HTLV-1 in-
tegration in the cell would indicate its role in pathogenesis,
taking into account that it is generally random or polyclonal
in asymptomatic carriers and monoclonal in leukemic cells.\textsuperscript{3} An
intermediate state of HTLV-1 infection has been pro-
posed as a clinical condition that occurs between the healthy
carrier state and smoldering ATL.\textsuperscript{15} Monoclonal, but nonma-
nignant, proliferation of HTLV-1-infected cells may occur in
carriers, indicating an increased risk for development of
ATL.\textsuperscript{16}

The nested PCR used in this study did not evaluate clonal-
ity. However, the detection of expression of proteins involved
in HTLV-1 pathogenesis and the use of specific molecular
and taxonomic techniques using viral mRNA should also pro-
vide additional information on this subject. Thus, acquired
ichthyosis and dermatophytosis may be associated with infec-
tion with HTLV-1. Dermatologic evaluation may be of value
in blood centers in endemic areas for screening of HTLV-1
infection.

\textbf{TABLE 3}

<table>
<thead>
<tr>
<th>Skin lesion</th>
<th>Normal skin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive PCR</td>
<td>Negative PCR</td>
</tr>
<tr>
<td>Positive PCR</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Negative PCR</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>13</td>
</tr>
</tbody>
</table>

\textsuperscript{*} HTLV-1 = human T cell lymphotropic virus type-1; PCR = polymerase chain reaction; \(P = 0.004\).

\textbf{DISCUSSION}

Consistent with the epidemiology of HTLV-1 in other en-
demic areas, the prevalence of HTLV-1 increased with age
and was higher among female blood donors.\textsuperscript{9,10} The associa-
tion of HTLV-1 and acquired ichthyosis demonstrated in this
study has been reported in patients with HAM/TSP, and it
has been related, in some cases, to hypohidrosis caused by
involvement of the autonomic nervous system by inflamma-
tory cells.\textsuperscript{3} The ichthyosiform dermatoses are characterized
by an excess accumulation of cutaneous scale, whose severity
varies from mild to life threatening. In the cases presented
herein, the skin disease was asymptomatic and more pro-
nounced in the lower legs, as is expected in mild cases of
acquired ichthyosis. Dermatophytosis, which was shown in
this study to be more prevalent in the HTLV-1-positive popu-
lation, is caused by fungi that thrive only in nonviable kera-
tinized tissue of the skin (stratum corneum, nails, hair). It was
reported in pre-adult ATL,\textsuperscript{10} and it has been considered an
indication of immunosuppression.\textsuperscript{11} Other dermatologic le-
sions have been related to HTLV-1,\textsuperscript{12,13} Therefore, the
HTLV-1 asymptomatic carriers deserve systemic dermato-
logic investigation of possible cutaneous manifestations that
can disclose evolution to HTLV-1-related diseases.

HTLV-1 has been demonstrated in the skin of patients with
cutaneous T cell lymphoma, but it had not been reported in
the normal skin of people infected with HTLV-1.\textsuperscript{3} Since there
was no significant difference related to the presence of
HTLV-1 in the normal skin of individuals with (group I) and
without (group II) skin lesions in other areas, the identifica-
tion of HTLV-1 by PCR in 43% of the normal skin specimens
of seropositive individuals suggests that lymphocytes carrying
the retrovirus are commonly found in the skin. Virus was
found more frequently in abnormal skin than in normal skin
of the same individual. However, whether HTLV-1 is in-
volved in the pathogenesis of the skin lesions or is present in
the skin because inflammatory cells containing virus migrated
to the lesion needs to be evaluated. Setoyama and others,\textsuperscript{14} in
a recent study that used PCR \textit{in situ} hybridization, reported
that HTLV-1 DNA was present in the nuclei of sweat gland
epithelial cells and in vascular endothelial cells of patients
with ATL. Thus, skin cells, in addition to lymphocytes, can be
infected by this virus. This may also explain why the PCR

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