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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 samples[6] 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′-CCCTACAATCCAACCAGCTCAG-3′) and SK44-*tax* (7536-7496, 5′-GAGCCGATAACGCGTC 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′–CTAGTCGACGCTC CAGGATATGACC-3′) and 249–env (6137-6113, 5′-CAG ACCGCCACCGGTACCGCTCGGC-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-DQα 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 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). 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

TABLE 1

Frequency distribution of 128 human T cell lymphotropic virus type-1 (HTLV-1) seropositive and 108 seronegative subjects according to age, sex, and skin color (March 1997 to April 1999, Brazil)

Demographic characteristics	HTLV-1 positive n = 128 (%)	HTLV-1 negative n = 108 (%)	Odds ratio (95% confidence interval)
Age, years			
19–28	26 (20)	37 (34)	1.00
29–38	30 (24)	45 (42)	0.95 (0.45–1.99)
39–48	41 (32)	15 (14)	3.89 (1.68–9.14)
49–58	31 (24)	11 (10)	4.01 (1.59–10.32)
Sex			
Male	70 (55)	69 (64)	1.00
Female	58 (45)	39 (37)	1.35 (0.78–2.36)
Skin color			
White	70 (55)	88 (81)	1.00
Black or Mulatto	58 (45)	20 (19)	3.65 (1.93–6.94)

TABLE 2

Frequency distribution of 128 human T cell lymphotropic virus type-1 (HTLV-1) seropositive and 108 HTLV-1 seronegative participants according to dermatologic examination result (March 1994 to April 1999, Brazil)

Dermatologic examination result	HTLV-1 positive n = 128 (%)	HTLV-1 negative n = 108 (%)	Crude odds ratio (95% confidence interval)
Abnormal	63 (49.2)	13 (12.0)	7.08 (3.45–14.77)
Dermatophytosis*	44 (34.4)	16 (14.8)	3.32 (1.78–6.23)
Seborrheic dermatitis	8 (6.3)	2 (1.9)	3.53 (0.67–24.66)
Acquired ichthyosis	9 (7.0)	0	Undefined
Contact dermatitis	4 (3.1)	2 (1.9)	1.71 (0.26–13.73)
Vitiligo	3 (2.3)	0	Undefined
Herpes labialis	2 (1.6)	0	Undefined
Pityriasis versicolor	2 (1.6)	2 (1.9)	0.86 (0.08–8.67)
Scabiosis	2 (1.6)	0	Undefined
Acantthosis nigricans	1 (0.8)	0	Undefined
Asteatotic dermatitis	1 (0.8)	0	Undefined
Lichen planus	1 (0.8)	0	Undefined
Nummular dermatitis	1 (0.8)	0	Undefined
Xerosis	1 (1.8)	0	Undefined

* *Tinea pedis, T. ungium, T. corporis,* and *T. cruris*

TABLE 3
Matched comparison of the HTLV-1 PCR result from the individuals with dermatologic lesions and their normal skin (March 1997 to April 1999, Brazil)*

	Normal skin		
Skin lesion	Positive PCR	Negative PCR	Total
Positive PCR	6	10	16
Negative PCR	0	3	3
Total	6	13	19

* HTLV-1 = human T cell lymphotropic virus type-1; PCR = polymerase chain reaction; $P = 0.004$.

II). No difference related to the presence of HTLV-1 was found between both groups ($P = 0.644$). The associations found in the present study were not influenced by age, race, and sex when adjustments were made for these variables as possible confounding factors.

DISCUSSION

Consistent with the epidemiology of HTLV-1 in other endemic areas, the prevalence of HTLV-1 increased with age and was higher among female blood donors.[9,10] The association of HTLV-1 and acquired icthyosis 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 inflammatory cells.[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 pronounced 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 population, is caused by fungi that thrive only in nonviable keratinized tissue of the skin (stratum corneum, nails, hair). It was reported in pre-adult ATL,[10] and it has been considered an indication of immunosuppression.[11] Other dermatologic lesions have been related to HTLV-1.[12,13] Therefore, the HTLV-I asymptomatic carriers deserve systematic dermatologic investigation of possible cutaneous manifestations that may 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.[5] 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 identification 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 involved 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,[14] in a recent study that used PCR *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 detected 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 cutaneous T cell lymphoma**.** Analysis of the type of HTLV-1 integration 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.[1] An intermediate state of HTLV-1 infection has been proposed as a clinical condition that occurs between the healthy carrier state and smoldering ATL.[15] Monoclonal, but nonmalignant, proliferation of HTLV-1-infected cells may occur in carriers, indicating an increased risk for development of ATL.[16]

The nested PCR used in this study did not evaluate clonality. 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 provide additional information on this subject. Thus, acquired ichthyosis and dermatophytosis may be associated with infection with HTLV-1. Dermatologic evaluation may be of value in blood centers in endemic areas for screening of HTLV-1 infection.

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