Immunologic Markers, Uveitis, and Keratoconjunctivitis Sicca Associated with Human T-Cell Lymphotropic Virus Type 1

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• PURPOSE: To verify the occurrence of keratoconjunctivitis sicca (KCS) and human T-cell lymphotropic virus type 1 (HTLV-1) associated uveitis (HAU) and to evaluate the immunologic status related to HTLV-1.
• DESIGN: Cross-sectional study.
• METHODS: Ophthalmic examination (both eyes) and immunophenotyping of peripheral blood lymphocytes were performed in 207 infected asymptomatic blood donors (AS), 55 controls (NI), and 55 patients with HTLV-1 associated myelopathy (HAM/TSP). Examiner was masked to patient’s serologic status.
• RESULTS: KCS was more frequent in HAM/TSP (30/55, 54.5%) than in NI and AS (7/55, 12.7% and 42/207, 20.3%, respectively). Presence of lacrimal hyposecretion in KCS individuals was higher in the HAM/TSP group ($P < .001$) as compared with NI and AS. HAU was found in 1/55 (1.82%) of HAM/TSP patients and 4/207 (1.93%) of HTLV-1 seropositive donors. Higher levels of activated CD4$^+$ and CD8$^+$ T cells were observed in HAM/TSP. Patients with HAU displayed higher percentage of both CD4$^+$ HLA-DR$^+$ and CD8$^+$ HLA-DR$^+$ when compared with NI and AS without HAU.
• CONCLUSIONS: Patients with HAM/TSP manifested more ophthalmologic symptoms than asymptomatic HTLV-1–infected individuals, with significantly higher KCS and immunologic alterations. Levels of activated CD8$^+$ T cells could be used as a prognosis marker of inflammatory disease manifestation to follow-up AS individuals. (Am J Ophthalmol 2006;142:811–815. © 2006 by Elsevier Inc. All rights reserved.)

HUMAN T-CELL LYMPOTHROPIC VIRUS TYPE 1 (HTLV-1) is associated with adult T-cell leukemia/lymphoma (ATLL), a neurologic disorder (HTLV-1–associated myelopathy/tropical spastic paraparesis [HAM/TSP]) and with uveitis (HTLV-1–associated uveitis [HAU]). Associations with many other diseases in humans are suspected, as with other ocular manifestations, varied skin disease, Sjögren’s syndrome, and arthropathies, among others. Apparently only 5% to 10% develop any disease during their lives, perhaps depending on unknown cofactors (genetic, demographic, environmental, and others), which could vary according to geographic localization.1

Ophthalmologic disturbances related to HTLV-1 infection include malignant infiltrates of the eye in patients with T-cell leukemia/lymphoma,2 vasculitis and exudation in peripheric retinæ,3 retinal degeneration,4 keratoconjunctivitis sicca,5 uveitis,6–9 corneal damage,9 and uveitis associated with tubulointerstitial nephritis.10 HAU may be found in association with patients with HAM/TSP11–14 or as an isolated manifestation in the virus infection, without other accompanying symptoms.8,11,12,15

The epidemiologic pattern of HTLV-1 infection is characterized by clusters of the infection in defined geographical areas over the world, spatial variation of seroprevalence rates, within high-prevalence areas; increasing prevalence with age, and higher prevalence rates in women, which is accentuated after 40 years of age.16 It is endemic in Japan, Caribbean, Africa, and South America, with variable prevalence in the general population. In
Brazil, it is present in all regions and the prevalence varies from 0.08% to 1.35% in blood donors.\(^7\) In Belo Horizonte, the mean prevalence in blood donors dropped from 0.32% at the initial evaluation in 1992 to 0.1% currently;\(^7\) in the general population, it is estimated to be 1.0%.\(^18\) Although ATLL and HAM/TSP are frequently reported in Brazil,\(^19\)\(^20\) there are few reports of ocular manifestations of HTLV-1 infection in the country.\(^15\)

An open prevalent cohort of HTLV-infected individuals was initiated in 1997 to better study the natural history of this infection in our region, including the occurrence of HAU and other possible ocular manifestations of HTLV-1 infection, which composed asymptomatic individuals with positive, indeterminate, and negative (controls) results for HTLV-1 and patients with HAM/TSP.

### METHODS

GIPH (INTERDISCIPLINARY HTLV RESEARCH GROUP) IS AN open prevalent cohort of HTLV-infected individuals that have been followed since 1997 to better understand the epidemiologic, clinical, and laboratory aspects of this infection, with focus on the natural history and predictive factors. GIPH’s participating institutions include a blood center (Hemominas Foundation) and a rehabilitation hospital (Rede Sarah). This study was performed as a cross-sectional evaluation of blood donors (n = 262) from the Hemominas Foundation followed in the cohort and patients with HAM/TSP (n = 55) who were referred from Rede Sarah.

Eye exam was included in the GIPH protocol and performed by an ophthalmologist who was masked to the serologic status of the individuals. The study protocol was approved by the Ethics Committee of Research in Human Subjects, according to Brazilian federal regulation. Examination was performed after informed consent by the patients.

HTLV-1 diagnosis was done with commercial screening tests (EIA, Ortho-Raritan, New Jersey, USA) and confirmed with Western blot (Cambridge Biotech or Genelabs Diagnostics, Worcester, Massachusetts, USA) and polymerase chain reaction (PCR).

The ophthalmic exam was as follows: measure of visual acuity by Snellen table, with optical correction; optical motility, application tonometry, biomicroscopy, and binocular indirect ophthalmoscopy with or without depression, in both eyes. The evaluation of lacrimal film was performed with 0.1% solution Rose Bengal staining, Schirmer I test, and break-up time. Rose Bengal test was considered pathologic when its total score was higher than three points (Van-Bijsterveld score).\(^21\) Scores of 5 mm for Schirmer I test were defined as abnormal. The final diagnosis of keratoconjunctivitis sicca (KCS) was defined based on the presence of symptoms and alterations of the lacrimal film (alterations in at least two of the three tests used). In all patients, tests were performed to exclude other possible diseases associated with the ophthalmic disturbances found.

The statistical analysis and Chi-square calculations were performed by Student t-test. Significance was considered at P < .05.

Flow cytometric immunophenotyping analysis of peripheral blood leukocytes was performed as described by Brito-Melo and associates.\(^22\) Cell phenotypes were analyzed with an immunofluorescence procedure recommended by Becton Dickinson (USA), using undiluted monoclonal antibodies specific for several cell surface markers (anti-CD4 clone 13B8.2, and anti-CD8 clone B9.11 labeled with fluorescein isothiocyanate; and anti-CD3 clone UCTH-1, anti-CD16 clone 3G8, anti-CD19 clone J4.119, and anti-HLA-DR clone TU36 labeled with phycoerythrin). Cytofluorimetric data acquisition was performed with a Becton Dickinson FAC-Scalibur instrument (USA). Cell phenotype analysis within gated lymphocyte populations and subpopulations was performed by using Cell-Quest software. Lymphocytes were first identified based on their morphometric features followed by the analysis throughout their fluorescence properties. Data were expressed as percentages of cells positive for a given cell marker by dual-color dot plot graphics.

Flow cytometric data were first analyzed to evaluate the independence, normality, and variance. Because all data sets were found to display nonparametric distribution, they were analyzed by the Kruskal-Wallis test, followed by the Dunn test. Additional analysis was also performed by Chi-square test. Significance was defined in all analyses at P < .05.

### RESULTS

MEAN AGE WAS 31.9 YEARS (RANGE, 17 TO 53 YEARS) FOR HTLV-1 seronegative blood donors (NI), 38.6 years (range, 18 to 61 years) for HTLV-1 asymptomatic seropositive patients (AS), and 54.2 years (range, 26 to 87 years) in the HAM/TSP group. Of NI, 34/55 (61.8%) were males and 21/55 (38.2%) were females. Of the AS, 96/207 were males and 111/207 (53.6%) were females. In the HAM/TSP group, 21/55 (38.2%) were males and 34/55 (61.8%) were females (Table 1).

Dry eye symptoms were significantly higher (P < .001) in 23/55 (41.8%) patients from HAM/TSP group, when compared with 5/55 (9.1%) in NI and 36/207 (17.4%) in AS. Complaints were burning, eye redness, itching, foreign body sensation, and visual blurring. KCS was significantly higher (P < .001) in HAM/TSP (30/55, 54.5%) when compared with NI (7/55, 12.7%) and AS (42/207, 20.3%) (Table 1). Lacrimal hyposcretion in KCS individuals was significantly higher in HAM/TSP group (P < .001) when compared...
TABLE 1. Keratoconjunctivitis Sicca and Intermediate Uveitis in HTLV-I Seropositive Asymptomatic Carriers (n = 207) and HTLV-1–Associated Myelopathy/Tropical Spastic Paraparesis Patients (n = 55) as Compared with Seronegative Individuals (n = 55) from Belo Horizonte, Brazil, 1997–2003*

<table>
<thead>
<tr>
<th>Group</th>
<th>Origin</th>
<th>Number</th>
<th>Male/ Female</th>
<th>Age Range (Mean)</th>
<th>KCS (%)</th>
<th>HAU (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NI</td>
<td>HEMOMINAS</td>
<td>55</td>
<td>34/21</td>
<td>17–53 (31.9)</td>
<td>07/55 (12.7)</td>
<td>0/55 (0)</td>
</tr>
<tr>
<td>AS</td>
<td>Transfusion center</td>
<td>207</td>
<td>96/111</td>
<td>18–61 (38.6)</td>
<td>42/207 (20.3)</td>
<td>4/207 (1.93)</td>
</tr>
<tr>
<td>HAM/TSP</td>
<td>Sarah Hospital</td>
<td>55</td>
<td>21/34</td>
<td>26–87 (54.2)</td>
<td>30/55 (54.5)</td>
<td>1/55 (1.82)</td>
</tr>
</tbody>
</table>

NI = HTLV-1 seronegative individuals; AS = asymptomatic HTLV-1 seropositive patients; HAM/TSP = HTLV-1–associated myelopathy/tropical spastic paraparesis; KCS = keratoconjunctivitis sicca; HAU = intermediate uveitis.

The letters “a” and “b” represent statistical significant differences at P < .001 in comparison with NI and AS, respectively.

*Data are expressed as number of individuals, except for age range expressed in years.

TABLE 2. Lacrimal Film Features of Keratoconjunctivitis Sicca Individuals, Including HTLV-I Asymptomatic Carriers and HTLV-1–Associated Myelopathy/Tropical Spastic Paraparesis Patients as Compared With Seronegative Controls from Belo Horizonte, Brazil, 1997–2003*

<table>
<thead>
<tr>
<th>Group</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tear Break-up Time</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>NI</td>
<td>4 (57.1%)</td>
</tr>
<tr>
<td>AS</td>
<td>25 (59.3%)</td>
</tr>
<tr>
<td>HAM/TSP</td>
<td>05 (16.7%)</td>
</tr>
</tbody>
</table>

NI = HTLV-1 seronegative individuals; AS = asymptomatic HTLV-1 seropositive patients; HAM/TSP = HTLV-1–associated myelopathy/tropical spastic paraparesis.

The letters “a”, “b,” and “c” represent statistical significant differences at P < .05, as compared with NI, AS, and H/T, respectively.

*Data are expressed as number of individuals and percentage in parenthesis.

with NI and AS. Lacrimal film features of KCS individuals also revealed consistent lower quality in HAM/TSP patients as compared with NI and AS (P < .05) (Table 2). Visual acuity and eye pressure were normal in all groups studied.

Intermediate uveitis (HAU) was found in 4/207 (1.93%) individuals in the AS group and in 1/55 (1.82%) in HAM/TSP individuals. They composed two females and three men, with age range of 18 to 46 years. None of the HTLV-1 seronegative blood donors had HAU. One of the HAU patients had vitreous opacities and localized retinal vasculitis. The remaining four HAU patients had only vitreous opacities (“snowball” type). The inflammation was controlled with topical corticosteroids. All four blood donors also presented skin lesions characterized as seborrheic dermatitis, pityriasis versicolor, onychomycosis, hair loss, and acanthosis nigricans-like lesion.

Immunophenotyping analysis of major of peripheral blood lymphocytes subsets is presented in Table 3. Data analysis demonstrated that independently of HAU, HAM/TSP subgroups showed higher levels of T cells from increased levels of CD4+ T cells. Lower levels of B cells leading to higher T/B-cell ratio was also documented for both HAM/TSP subgroups (with and without HAU).

Lower frequency of CD16+ cells was the hallmark of the AS group, even in the presence of HAU. Additional phenotypic analysis was carried out to evaluate the activation status of T-cell subsets. Despite the presence of HAU, higher levels of activated CD4+ and CD8+ T cells were observed in HAM/TSP subgroups in comparison to NI. Interestingly, we have noticed that AS patients presenting HAU showed higher percentage of both CD4+ HLA-DR+ and CD8+ HLA-DR+ in comparison with NI and AS without HAU.

DISCUSSION

THE PRESENT COHORT STUDY ALLOWED OBSERVATION OF a large number of HTLV-1 seropositive individuals, including HAM/TSP patients. KCS is a frequent occurrence and is present in a high percentage of the population. Our study demonstrated that the frequency of KCS in HAM/TSP patients was almost three times higher than that of asymptomatic HTLV-1–infected individuals and more than four times that of seronegative ones, and were associated with both qualitative and quantitative defects in the lacrimal film.
Yamamoto and associates\(^{15}\) reported 21.0% of qualitative alterations in asymptomatic HTLV-1 infected individuals, similar to our results in the equivalent population, but did not study HAM/TSP individuals. Hajjar and colleagues\(^ {23}\) observed qualitative lacrimal film defects in 83.0% of HTLV-1–infected individuals, but also reported lacrimal hyposcretion in 79.0% of the cases, associated with Sjögren’s syndrome. Although we also found quantitative disturbances, Sjögren’s syndrome was not observed in our study.

In contrast to the Japanese literature\(^ {4,11,12}\) HAU is reported in Brazil in low numbers\(^ {15,16}\) and in the present study was equally found in HAM/TSP patients and in asymptomatic HTLV-1–infected individuals. None were found in the seronegative control group. A multicenter study in Japan\(^ {24}\) showed that vitreal opacities were associated with vasculitis and that a presenting feature of HAU was visual blurring, which was also reported by all cases in our group.

The association of HTLV-1 and cutaneous disorders was reported previously\(^ {25}\) and is frequently observed in patients infected by this virus, such as infective dermatitis, psoriasis, seborrheic dermatitis, ichthyosis, acanthosis nigricans-like, erythroderma, and prurigo. All patients with HAU in the present study showed skin lesions in addition to the ophthalmic symptoms.

Studies reporting the role of the immune system in the chronic process of HTLV infection\(^ {26,27}\) have demonstrated that some parameters may be associated with the presence of HAM/TSP, such as elevated proviral load, high in vitro spontaneous lymphocytic proliferation, high numbers of HTLV-1–specific T-CD8\(^ {–}\)–activated lymphocytes, and presence of HTLV-1 in the spinal fluid.\(^ {31}\) It has been previously demonstrated that HAM/TSP is accompanied by high levels of activated T-lymphocytes, both CD4\(^ {+}\) and CD8\(^ {+}\) cells.\(^ {28}\) The present study has confirmed a significant elevation of CD4\(^ {+}\) HLA-DR\(^ +\) and CD8\(^ +\) HLA-DR\(^ +\) cells in HAM/TSP patients when compared with NI. Moreover, HAM/TSP patients presenting ophthalmic disease (HAU) did not show further increase on their circulating activated T-cell subsets. On the other hand, asymptomatic HTLV-1 carriers with HAU also presented immunologic disturbances, with increased levels of both CD4\(^ {+}\) and CD8\(^ {+}\) cells. This may suggest that HAU could be an additional feature in HAM/TSP patients because ophthalmologic alteration is not followed by further modification of T-cell activation status. However, as in AS, HAU appears as an exclusive and particular inflammatory event, accompanied by significant changes on the patients’ immunologic profile. We believe that the abnormal immunologic features observed in this study, especially in HTLV-1 asymptomatic carriers presenting HAU, strongly suggest a possible link between this clinical entity and the immune status of the patient. However, because this was not a longitudinal investigation, the hypothesis that these immunologic findings could explain the inflammatory response and the ophthalmic symptoms needs confirmation, and additional investiga-

### TABLE 3. Frequency of Major Peripheral Blood Lymphocyte Subpopulations in HTLV-I–Seropositive Asymptomatic Carriers and HAM/TSP Patients, Categorized by the Absence (−) and Presence of HTLV–Associated Uveitis as Compared With Seronegative Controls (NI) from Belo Horizonte, Brazil, 1997–2003*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cell Phenotypes</th>
<th>CD3(^ {+})</th>
<th>CD19(^ {+})</th>
<th>CD16(^ {+})</th>
<th>CD4(^ {+})</th>
<th>CD8(^ {+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>NI</td>
<td>69.0 ± 6.4</td>
<td>13.3 ± 3.9</td>
<td>9.2 ± 6.4</td>
<td>43.7 ± 7.5</td>
<td>24.0 ± 5.3</td>
<td></td>
</tr>
<tr>
<td>AS</td>
<td>69.7 ± 5.9</td>
<td>12.7 ± 5.1</td>
<td>12.2 ± 4.9</td>
<td>41.3 ± 7.6</td>
<td>26.9 ± 5.4</td>
<td></td>
</tr>
<tr>
<td>HAU</td>
<td>63.8 ± 1.1</td>
<td>14.5 ± 0.1</td>
<td>11.6 ± 0.3</td>
<td>31.1 ± 6.8</td>
<td>29.6 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>HAM/TSP</td>
<td>77.2 ± 6.8(^ {a,b,c})</td>
<td>7.7 ± 2.5(^ {a,b,c})</td>
<td>8.0 ± 5.7</td>
<td>52.3 ± 8.5(^ {a,b,c})</td>
<td>23.6 ± 7.9</td>
<td></td>
</tr>
<tr>
<td>HAU</td>
<td>89.1 ± 3.3(^ {a,b,c})</td>
<td>5.5 ± 0.8(^ {a,b,c})</td>
<td>2.9 ± 2.3</td>
<td>50.5 ± 2.1(^ {a,b,c})</td>
<td>40.3 ± 6.7</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cell Ratio</th>
<th>CD3(^ {+})/CD19(^ {+})</th>
<th>CD4(^ {+})/CD8(^ {+})</th>
<th>CD4(^ {+})HLA-DR(^ +)/CD4(^ {+})</th>
<th>CD8(^ +)HLA-DR(^ +)/CD8(^ +)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NI</td>
<td>5.8 ± 2.4</td>
<td>1.9 ± 0.5</td>
<td>12.1 ± 4.9</td>
<td>39.9 ± 15.0</td>
<td></td>
</tr>
<tr>
<td>AS</td>
<td>6.6 ± 3.5</td>
<td>1.6 ± 0.5</td>
<td>13.2 ± 3.3</td>
<td>47.7 ± 15.0</td>
<td></td>
</tr>
<tr>
<td>HAU</td>
<td>4.5 ± 0.1</td>
<td>1.1 ± 0.3</td>
<td>54.9 ± 6.3(^ {a,b})</td>
<td>64.1 ± 5.1(^ {a,b})</td>
<td></td>
</tr>
<tr>
<td>HAM/TSP</td>
<td>10.7 ± 3.5(^ {a,b,c})</td>
<td>2.4 ± 1.2</td>
<td>35.3 ± 16.2(^ {b})</td>
<td>65.8 ± 13.4(^ {b})</td>
<td></td>
</tr>
<tr>
<td>HAU</td>
<td>16.4 ± 2.9(^ {a,b,c})</td>
<td>1.2 ± 0.2</td>
<td>42.3 ± 5.2(^ {a,b})</td>
<td>71.4 ± 6.1(^ {a,b})</td>
<td></td>
</tr>
</tbody>
</table>

NI = HTLV-1–seronegative individuals (n = 16); AS = asymptomatic (n = 22); AS HAU = asymptomatic with uveitis (n = 2); HAM/TSP = HAM/TSP (n = 12); HAM/TSP HAU = HAM/TSP with uveitis (n = 2); CD3\(^ {+}\) = T lymphocytes; CD19\(^ {+}\) = B lymphocytes; CD4\(^ {+}\) = T-helper cells; CD8\(^ {+}\) = T citotoxic cells; CD16\(^ {+}\) = NK cells.

Letters “a”, “b”, “c”, “d”, and “e” represent significant differences at P < .05 in comparison with NI, AS, AS HAU, HAM/TSP−, and HAM/TSP HAU, respectively.

*Data are expressed as mean percentage, except for CD3 “CD19\(^ {+}\)” and CD4\(^ {+}\)/CD8\(^ {+}\) ratio.
tions are required to verify whether the immune response triggered by chronic HTLV-1 infection has any causative effect on the HAU.

Our group had previously reported that phenotypic features outside the reference ranges observed in HAM/TSP patients (including percentage of B cells, T/B-cell ratio, and percentage of CD8+ HAL-DR+ T cells) could be used as important immunologic indicators that could help clinicians monitor HTLV-1 infection and differentiate the HAM/TSP group from the asymptomatic group.24 Herein we have further addressed this issue and pointed out that among these immunologic indicators, the frequency of activated CD8+ T-cells could be used as a prognosis indicator of inflammatory disease manifestation in AS individuals.

REFERENCES


Biosketch

Sonia Regina A. A. Pinheiro, MD, PhD, was born in Brazil and finished her Medical Degree at the Federal University of Minas Gerais Medical School, Belo Horizonte, Minas Gerais, Brazil where she also did her medical residence in ophthalmology. Dr Pinheiro obtained her PhD at Federal University of São Paulo Brazil and was trained in epidemiology at the Johns Hopkins University, Baltimore, Maryland. Dr Pinheiro is a member of GIPH (Interdisciplinary HTLV-1/2 Research Group) and her research interests are related to ophthalmological manifestations of HTLV infection.