Unique evidence of atypical lymphocytes and flower cells in indigenous Xikrin do Bacajá people infected with HTLV-2

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Abstract

The presence of lymphocytic alterations in the blood of indigenous people infected with human T-lymphotropic virus 2 (HTLV-2) was investigated in peripheral blood smears from 25 indigenous people of the Xikrin do Bacajá ethnicity (11 men and 14 women) diagnosed with HTLV-2 infection by Western blot and qPCR. Smears were stained using the Leishman method and examined for lymphocyte morphology under an optical microscope. A group of 25 individuals seronegative for HTLV-1/2 infection were used as controls. Among the HTLV-2-infected group, six slides (24%) showed atypical lymphocytes (05 with atypical lymphocytes and 01 with petal-shaped nuclei usually referred to as flower cells). The finding was significantly different from that observed among the noninfected group, in which no lymphocytic alteration was observed (p=0.0223). This unique finding is reported for the first time among indigenous peoples and suggests that HTLV-2 infection may be associated with lymphocytic alterations, reinforcing the need for a larger and more detailed clinical study of people living with HTLV-2, particularly the indigenous populations of the Amazon in which the infection is hyperendemic.

Full Text

Human T-lymphotropic virus 1 (HTLV-1) was the first described human retrovirus, isolated from a patient with a severe form of cutaneous T-cell lymphoma resembling mycosis fungoides [1]. HTLV-1 was classified into the family *Retroviridae*, subfamily *Orthoretrovirinae*, and genus *Deltaretrovirus* [2]. Two years later, a new type named human T-lymphotropic virus 2 (HTLV-2) was isolated from a patient with hairy cell leukemia [3], but its etiology with leukemia is still controversial, and there is no consensus that HTLV-2 can induce lymphocytic alterations [4]. Both viruses share molecular properties, including the evolutionary aspect of viral and cell nucleic acid integration, silent and lifelong persistence, and transmission pathways [5-7].

While HTLV-1 is widely associated with a large array of lymphoproliferative and inflammatory diseases, as well as with high morbidity and mortality [8,9], a definition of the etiological association of HTLV-2 infection with diseases in humans remains unclear despite case reports of neurological diseases similar to HTLV-1-associated myelopathy (HAM) [7,10]. Considering that the prevalence of HTLV-2 is frequently lower than that of HTLV-1 among urban populations throughout the world, evidence of the association of HTLV-2 with diseases will only be possible if clinical and laboratory follow-up studies are performed in those populations where the virus is endemic or hyperendemic, as observed among the indigenous communities in the Brazilian Amazon region.

The present study aimed to investigate the occurrence of atypical lymphocytes in samples of indigenous people infected with HTLV-2 in the Xikrin do Bacajá (Kayapó) indigenous community where the virus is hyperendemic [11,12].

In 2020, a health support expedition was directed to Xikrin do Bacajá village located in the state of Pará to provide clinical and laboratory evaluations, including the investigation of HTLV-1/2 infections. The
A new visit occurred in 2021, when blood smear slides were collected from 25 indigenous individuals (11 men and 14 women) formerly diagnosed with HTLV-2 infection by Western blot and qPCR [12]. The slides were stained using the Leishman method and then analyzed for lymphocyte morphology under an optical microscope (100x). To compare lymphocyte profiles, a control group of blood smears was collected from 25 Xikrin individuals who were seronegative for HTLV-1/2 infection (11 men and 14 women). The slides were examined independently by two hematologists in a double-blind analysis. This study was approved by the National Research Ethics Committee (CONEP) (CAAE: 27290619.2.0000.0018).

Among the 25 HTLV-2-infected individuals, six slides (24%) showed atypical lymphocyte counts ranging from 4% to 10%; in one individual presenting 4% atypical lymphocytes, 1% of atypical lymphocytes had petal-shaped nuclei usually referred to as flower cells. Among the noninfected individuals, no case of atypical lymphocytes was observed (p=0.0223).

The Virus Laboratory has kept the Kayapó villages under close surveillance for HTLV-2 infection and disease since the 1990s [11-14]. A major investigation was recently performed, and the seroepidemiological results of this visit, together with those from 11 other indigenous villages, were reported by Abreu et al [12]. The endemic presence of HTLV-2 in communities comprising the Kayapó ethnic group has been described since 1992 [15]; however, neither clinical nor hematological laboratory alterations could ever be attributed to HTLV-2 infection.

Diseases associated with HTLV-1 infection usually cause disease in approximately 2-5% of persons living with HTLV (PLHTLV) [9], although there is evidence of a much higher frequency considering all the different manifestations associated with HTLV-1 infection [16,17]. In contrast, diseases associated with HTLV-2 seem to be a rare event and can only be better evaluated in prospective studies of populations where HTLV-2 is endemic, such as the indigenous populations in the Amazon. However, traveling to villages is not an easy task, as there are no appropriate roads to reach the villages or small plane airfields; health personnel for clinical and laboratory work are not always immediately available; and establishing a well-equipped laboratory \textit{in situ} entails several obstacles, including the risk in transporting sophisticated equipment and the lack of a stable electrical energy source for their operation, among other difficulties.

Finding these atypical lymphocytes and flower cells would also require a detailed immune-hematological study to define the phenotype of the cells, but flow cytometry \textit{in loco} is almost impossible to consider. Furthermore, traveling back to the laboratory sometimes requires several days, which is another problem
in traveling with biological samples that require viability before testing for specific assays, including immunophenotyping. Examination of stained blood smears is an attempt to circumvent such limitations and difficulties in developing laboratory work in villages. Despite all the difficulties, this has been a constant investigation over the years, and to the best of our knowledge, this is the first description of lymphocyte atypia with the presence of flower cells in the blood smears of persons living with HTLV-2.

The clinical evaluation of the five individuals did not show altered conditions that could indicate signs or symptoms associated with any hematological disease. A strategy has been defined to start a long-term clinical and laboratory follow-up study and to implement hematological surveillance among other Kayapó villages. It is important to reinforce the need for a more detailed study of persons living with HTLV-2 among the indigenous populations of the Amazon region of Brazil in which the virus is hyperendemic and the need to provide better health care for these vulnerable groups.

**Abbreviations**

HTLV: Human T-lymphotropic virus; HAM: HTLV-1-associated myelopathy; CESIPT: State Coordination of Indigenous Health and Traditional Peoples; CIIR: Integrated Center for Inclusion and Rehabilitation; SESPA: Health Secretariat of Pará; DSEIs: Special Indigenous Health Districts; SESAI: Special Secretariat for Indigenous Health; CONEP: National Research Ethics Committee.

**Declarations**

**Acknowledgments**

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**Authors’ contributions**

ACRV, JFG and RI wrote the manuscript. AMAS and JSSG examined the blood smears. IMVCV, JSSG, INA, VOF, CNCL, BSB, ERPS, AMAS and JFG participated in data collection and manuscript revision.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the National Research Ethics Committee (CONEP) (CAAE: 27290619.2.0000.0018).

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

References


**Table**

**Table 1** – Distribution of lymphocyte atypia according to the presence or absence of HTLV-2 infection among the Xikrin do Bacajá people, Brazil.

<table>
<thead>
<tr>
<th>HTLV-2</th>
<th>Atypical lymphocytes</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Presence, n (%)</td>
<td>Absence, n (%)</td>
</tr>
<tr>
<td>Positive</td>
<td>6 (24)</td>
<td>19 (76)</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>25 (100)</td>
</tr>
</tbody>
</table>

* Fisher’s exact test
Atypical lymphocytes (A and B) observed in blood smears from HTLV-2-infected individuals from Xikrin do Bacajá. Presence of flower cells in sections C and D. Optical microscope (100x).