



Short communication

Flower cells in patients with infective dermatitis associated with HTLV-1

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ABSTRACT

Background: Infective dermatitis associated with HTLV-1 (IDH) is a severe childhood form of eczema that may progress to adult T-cell leukemia/lymphoma (ATL).

Objective: In this study, the presence of clinical and laboratory parameters suggestive of ATL was evaluated in a cohort of 30 patients with IDH.

Study design: Over a period of 33 months, the patients were submitted to three-monthly clinical evaluations, routine laboratory exams, full blood count and blood smears, and to six-monthly blood sampling for HTLV-1 proviral load determination. HTLV-1 proviral load was quantified using real-time TaqMan PCR assay.

Results: Abnormal cells (Ably) were found in the peripheral blood smears of nine patients (30%), flower cells being detected in five of these cases (16.6%). The presence of Ably and flower cells was not associated with a higher proviral load in those patients.

Conclusions: This is the first report on the presence of flower cells in HTLV-1-infected children and adolescents. Furthermore, these cells have not previously been reported in IDH patients. The cases with flower cells probably represent precursory ATL cases, these patients being at a greater risk of developing ATL.

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1. Background

Many clinical disorders have been associated with HTLV-1 infection and a small percentage of HTLV-1-infected subjects may develop severe diseases including adult T-cell leukemia/lymphoma (ATL), HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) and HTLV-1-associated infective dermatitis (IDH).¹ IDH occurs in vertically-infected children.² It is a severe, recurrent and infected form of eczema that usually begins at 18 months of age and generally disappears in adolescence. It presents as an exudative eruption involving mainly the scalp, external ear and neck. The disease responds to antibiotic therapy but relapses immediately once treatment is withdrawn.^{2,3}

ATL is an aggressive T-cell malignancy that generally occurs fifty years after vertically acquired HTLV-1 infection.⁴ It has been classified into five clinical types: acute, chronic, lymphomatous,

smoldering and primary cutaneous tumoral.^{4,5} The acute and chronic types present lymphocytosis, which is not observed in the other types of ATL.⁵ In acute ATL and occasionally in the chronic and smoldering types of ATL, lymphocytes showing markedly polylobulated nuclei with homogeneous and condensed chromatin, small or absent nucleoli and basophilic cytoplasm, referred to as flower cells, may appear in peripheral blood.⁵ In addition to these cells, abnormal lymphocytes (Ably) with other morphologies are also observed.⁶

The relationship between IDH and ATL appears to be close, at least in the population living in the Brazilian state of Bahia, where 37.5% of patients with ATL affecting the skin were found to have had a history of severe eczema in childhood with characteristics similar to those of IDH.⁷ Moreover, two cases of ATL in adolescence were diagnosed in Bahia and in both cases the patients had had IDH in childhood.^{1,8}

2. Objectives

The objective of this study was to evaluate the presence of clinical and laboratory parameters suggestive of ATL in a cohort of 30 patients with IDH.

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3. Study design

The cohort consisted of 19 girls and 11 boys with ages ranging from 2 to 18 years (mean 12.73 ± 4.96 years), followed-up at the dermatology and pediatric neurology outpatient clinics of the *Professor Edgard Santos* Teaching Hospital, Federal University of Bahia, Brazil. All patients were HTLV-1 positive (as shown by ELISA and confirmed by Western Blot) and HIV-negative. Ten patients had associated HAM/TSP. Diagnosis of IDH and HAM/TSP was performed according to previously established criteria.^{3,9} Between June 2006 and March 2009, the patients were submitted to three-monthly clinical evaluations, routine laboratory exams and blood sampling for full blood count and blood smears. In cases in which flower cells were present in blood smears, blood levels of lactate dehydrogenase (LDH) and calcium were investigated. Chest X-rays and abdominal ultrasonography scans were also performed. Evaluation of lymphocytosis was based on age-specific normal values using previously established criteria.¹⁰ Mild lymphocytosis detected at one single test was not taken into consideration. The percentage of Ably was determined in 100 lymphocytes by microscopic analysis of a Wright's stained peripheral blood smear. Ably were identified using previously established criteria.^{5,6} Proviral load analysis was performed in 81 blood samples (a mean of 2.7 samples per patient) and was quantified using real-time TaqMan polymerase chain reaction (PCR) assay.¹¹ To calculate each patient's viral load, the mean of all the measurements taken was calculated and this value was then used to obtain the mean viral load for each group. The GraphPad Prism software program, version 4.03 (San Diego, CA, USA) was used throughout the statistical analysis. The Mann–Whitney test was used to compare data. *p*-Values <0.05 were considered statistically significant.

The study was approved by Ethical Committee of the *Professor Edgard Santos* Teaching Hospital, Federal University of Bahia.

4. Results

A total of 156 blood samples were taken for hematological analysis, a mean of 5.2 samples per patient. No lymphocytosis was observed in the cohort when findings were compared with established normal ranges. In two patients, mild lymphocytosis was detected in a single exam only and was not therefore considered to be due to the HTLV-1 infection. Ably were found in the peripheral blood smears of nine patients (30%) (Table 1). In three patients, 5% of Ably were found in one single sample from each patient. The following types of Ably were found: small prototype, prolymphocytoid, cells with vacuolated morphology, intermediated cells, prototype cells (typical flower cells) being detected in five of these cases (16.6%) (Fig. 1).

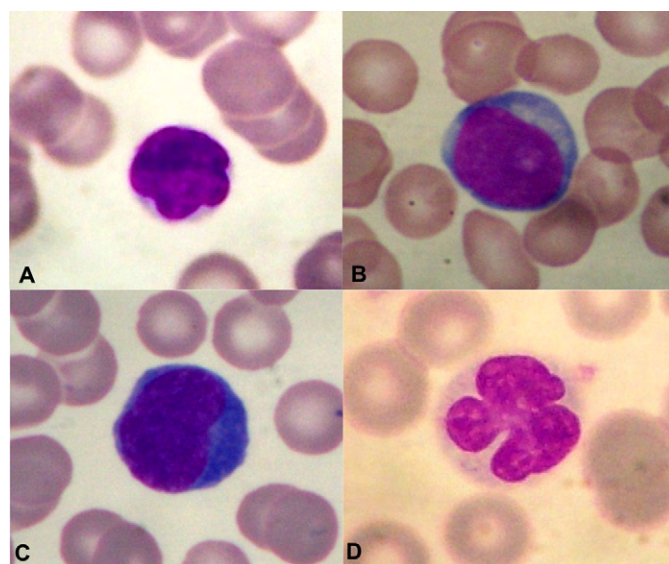


Fig. 1. Peripheral blood smears with abnormal cells. (A) Small flower cell (prototype); (B) prolymphocytoid cell; (C) intermediate cell; (D) lymphocyte with condensed chromatin and markedly polylobulated nuclei, flower cell (Wright, $\times 1000$).

The age at which flower cells appeared following onset of the disease ranged from 2 to 15 years, including a 29-month old child with a severe form of the disease. In the cases in which flower cells were present, blood levels of LDH and calcium were normal and no abnormalities were found at chest X-ray or abdominal ultrasonography.

HTLV-1 proviral was highest in the patients with flower cells ($1,540,283.68 \pm 2,984,409.34$ copies/ 10^6 PBMCs) followed by the patients with Ably ($941,779.39 \pm 2,231,480.4$ copies/ 10^6 PBMCs), then the patients in whom Ably were not found ($344,840.04 \pm 520,069.92$ copies/ 10^6 PBMCs). However, these differences in proviral loads between the groups were not statistically significant ($p = 0.70$ for flower cell patients compared to patients without Ably and $p = 0.74$ for patients with Ably compared to the patients without Ably).

One patient with flower cells died during the study, autopsy results revealing rheumatic pancarditis and no evidence of lymphoma.

In the 10 children with associated HAM/TSP, Ably was documented in five cases (50%), while in the 20 cases of IDH without HAM/TSP these cells were found in only four cases (20%) ($p = 0.11$).

Table 1

Data from nine patients with abnormal lymphocytes (Ably).

Patients	Association with HAM/TSP	Age at first sampling (years)	Samples with Ably compared to total number of samples	Ably (%) ^a	Flower cells (%) ^b
1	+	15	4/10	5	0–100
2 ^c	+	12	2/7	2	0–100
3	+	8	7/10	5	0–100
4	–	9	2/10	2	100
5	–	2.5	3/4	5	0–100
6	+	11	4/10	3	0
7	–	14	2/8	2	0
8	–	7	1/10	2	0
9	+	13	1/10	1	0

Ably – abnormal lymphocytes.

^a The highest percentage of Ably in different samples.

^b Variations in the percentages of flower cells in Ably.

^c Death.

5. Discussion

The finding of flower cells in HTLV-1-infected children and adolescents is reported here for the first time. In fact, flower cells were reported for the first time in IDH. As previously mentioned flower cells are commonly found in the acute type of ATL and occasionally in the chronic and smoldering types. The presence of Ably without reference to flower cells has been reported in symptomless HTLV-1 carrier children.¹²

Ably have been found in frequencies ranging from 10% to 43% in the peripheral blood of adult HTLV-1 carriers considered to be at a high risk of developing ATL^{13–15} In addition, flower cells have been found in 7% of adult HTLV-1 carriers.¹⁶ These findings are quite similar to those observed in the present study.

The presence of flower cells in children and adolescents with IDH, a disease with a high proviral load,¹⁷ may be considered indicative of a greater risk for the development of ATL compared to the occurrence of these cells in asymptomatic and adult carriers. Furthermore, in the cases included in this study the period of infection was short, including a child of less than 3 years of age.

According to Shimoyama et al.,⁵ cases in which 5% or more of Ably are found in the peripheral blood of an HTLV-1-infected individual, even when no other manifestations are present, should be considered leukemic smoldering ATL. However, in the present study the cases in which 5% of Ably were found were not considered leukemic smoldering ATL since this percentage of Ably was detected on one single occasion.

Ably have been found more frequently in HTLV-1 adult carriers with a high proviral load than in carriers with a low proviral load.^{13,18} In the present study, proviral load was higher in the group of IDH patients with Ably and in the group with only flower cells compared to those in whom these lymphocyte abnormalities were not found. Although these differences were not statistically significant, failure to reach significance may have been due to the small number of cases studied. Nevertheless, other studies with a larger number of IDH patients and a group of asymptomatic child/adolescent carriers will be required in order to establish the relationship between the presence of flower cells and proviral load.

Although IDH may progress to ATL,¹ these cases cannot be considered as constituting ATL at this stage, since no other clinical or laboratory signs of leukemia or lymphoma were present. Further studies over longer periods of time are required.

Conflict of interest

Funding: The authors declare that there are no competing interests.

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