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Evolution of HTLV-1 proviral load in patients from Salvador, Brazil

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ABSTRACT

Introduction: Variations in human T cell lymphotropic virus type 1 (HTLV-1) proviral load (PVL) in infected individuals over time are not well understood.
Objective: To evaluate the evolution of proviral load in asymptomatic individuals and HAM/TSP patients in order to help determine periodicity for measuring proviral load.
Methods: A group of 104 HTLV-1 infected patients, followed at the HTLV reference center in Salvador, Brazil, were included in the study (70 asymptomatic and 34 HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients). HTLV-1 PVL was measured using real-time polymerase chain reaction (PCR) at baseline and again at another point, either $\leq$ 12 months, between 12-24 months, or $\geq$ 24 months.
Results: HAM/TSP patients had higher PVL (ranging from 11,041 to 317,009 copies/10^6 PBMC) when compared to asymptomatic individuals (ranging from 0 to 68,228 copies/10^6 PBMC). No statistically significant differences were observed in the medians of PVL in HAM/TSP patients or asymptomatic individuals over time. However, in asymptomatic individuals with a PVL below 50,000 copies/10^6 PBMC, a statistically significant two-fold increase was observed over time.
Conclusion: HTLV-1-PVL remained stable in both asymptomatic individuals and HAM/TSP patients over time. Frequent monitoring of asymptomatic individuals with low PVLs is recommended and further studies should be conducted to assess the course of PVL in these patients over extended periods of time.

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Introduction

Human T cell lymphotropic virus type 1 (HTLV-1) infects a variable number of individuals according to the geographical area. The highest prevalence is found in Southern Japan, Central and West Africa, the Caribbean Islands, and Central and South America.[1] Brazil appears to have the highest absolute number of HTLV-1-infected individuals in the world, and it is estimated that 1.7% of the general population of the city of Salvador, in the state of Bahia, is infected by the virus.[2] HTLV-1 is the etiological agent of

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HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), adult T-cell leukemia (ATL), uveitis,[3–5] and keratoconjuntivitis sicca (KCS).[6] The infection is also associated with an increasing occurrence of infectious diseases, such as infective dermatitis in children,[7] tuberculosis,[8,9] disseminated strongyloidiasis,[10,11] and scabies.[12] The majority of infected individuals remain asymptomatic over their lifetime, and less than 5% will develop an HTVL-1-associated disease. The HTLV-1-PVL may represent a biological marker of disease development, especially HAM/TSP. A higher HTLV-1 proviral load (PVL) is observed in HAM/TSP patients compared to asymptomatic carriers.[13–15] However, the progression of the HTLV-1-PVL in infected individuals remains unclear. This study evaluated the course of HLTV-1-PVL among asymptomatic and HAM/TSP patients at baseline at intervals of less than 12 months, 12 to 24 months, and more than 24 months. The authors were interested in evaluating the evolution of proviral load in asymptomatic individuals and HAM/TSP patients in order to help determining an ideal periodicity for measuring proviral load. The results suggest that the HTLV-1-PVLs of patients have a stable course in the evaluated periods of time.

Methods

This is a retrospective study with a non-probability sampling. The patients selected for the study were followed at the Escola Bahiana de Medicina e Saúde Pública, Centro de Referência de HTLV, in Salvador, Northeast Brazil, from 2005 to 2009. This center is a free outpatient clinic that since 2002 has provided comprehensive care for more than 1,400 HTLV-1 infected individuals. Approximately 500 patients regularly attend the clinic twice a year. All patients with at least two HTLV-1-PVL measurements in intervals of up to 12 months, 12 to 24 months, and more than 24 months, and one neurological evaluation were included in the study. Data were obtained from medical charts. The revised criteria proposed by the World Health Organization in 1989 were used in order to diagnosis HAM/TSP. All asymptomatic individuals were evaluated by a neurologist and did not present any neurological signs. Patients co-infected with HIV and/or HCV were excluded from the study. This study was approved by the Institutional Review Board of the Fundação Oswaldo Cruz. Peripheral blood mononuclear cells (PBMCs) were obtained from ethylenediaminetetraacetic acid (EDTA) blood by density gradient centrifugation, and cryopreserved until use. DNA was extracted using a spin column DNA extraction system (QUIAGEN, Germany). HTLV-1-PVL was measured using a real-time TaqMan PCR method, in accordance with Dehéee et al. Albumin DNA was used as an endogenous reference. The value of HTLV-1-PVL was reported as the [(HTLV-1 average copy number)/(albumin average copy number)] x 2 x 10^6 and expressed as the number of HTLV-1 copies per 10^6 PBMCs. All HTLV-1-PVL measurements were performed in duplicates. The analysis was repeated when there was a variation greater than 30% between duplicate values of HTLV-I or albumin DNA copy numbers. Median values of HTLV-1-PVL were calculated for the different groups of patients. Wilcoxon matched pairs test was used for comparison of medians. The level of statistical significance of the type-1 error was set at less than 0.05. Graphpad (5.0) was used for statistical analyses.

Results

Out of a total of 104 HTLV-infected patients, 69 were females (66.3%). The median age of the individuals was 49.5 years old (IQR: 40-57). Seventy patients were asymptomatic and 34 had HAM/TSP. The median of HTLV-1-PVL of HAM/TSP patients was higher (ranging between 11,041 and 317,009 copies/10^6 PBMC) when compared to asymptomatic individuals (ranging between undetectable and 68,228 copies/10^6 PBMC) at all time points evaluated. There was no significant difference between the medians of HTLV-1-PVL of asymptomatic carriers and HAM/TSP patients at baseline and in a second measurement after an intervals of up to 12 months [median 7 (IQR: 2-11)], 12 to 24 months [median 18 (IQR: 13-24)], and more than 24 months [median 29 (IQR: 25-43)] (Table 1). When asymptomatic individuals were stratified according to HTLV-1- PVL (above or below 50,000 copies/10^6 PBMC), a statistically significant two-fold increase in HTLV-1-PVL was observed in individuals with low HTLV-1-PVLs over time, specifically when the second measurement was taken $\geq$ 12 months after baseline (Table 2).

Discussion

The present study demonstrated that the HTLV-1-PVLs of both asymptomatic and HAM/TSP patients remain stable for a period of more than two years. Moreover, the medians of HTLV-1-PVL were higher in the HAM/TSP patients than in asymptomatic carriers, as demonstrated in previous studies.[13–16] Several studies indicated that HTLV-1-PVLs remain stable for long periods of time in both asymptomatic and HAM/TSP patients. Taylor et al. showed that proviral load remain stable over many months (maximum 64 months).[17] However, these studies were heterogeneous regarding their methodology, sample size, and the period of time assessed. HTLV-1-PVL could remain relatively constant over a follow-up period from 24 months to 10.4 years.[17–19] Another study demonstrated that the HTLV-1-PVL of HAM/TSP patients may present a four- to ten-fold fluctuation in a one-three-year follow-up period, with variations in the clinical course of HAM/TSP disease.[20] Recently, a HTLV-1-PVL above 50,000 copies/10^6 PBMC was identified as the best cutoff value to distinguish asymptomatic individuals from HAM/TSP patients.[15]

In the present study, a two-fold increase was observed in asymptomatic individuals with proviral loads below 50,000 copies/10^6 PBMC (which corresponded to 5% of HTLV-1-infected PBMC) over a period of 12 months to > 24 months, yet proviral loads remained below the cutoff level. No difference was observed in HTLV-1-PVLs from HAM/TSP patients over time. Recently, Furtado et al.,[21] using a cutoff value of 1% HTLV-infected PBMC found that the HTLV-1-PVL was more stable in asymptomatic carriers than in HAM/TSP patients, but this difference was not significant. The different methodologies used to measure viral load in both studies could explain the discrepancy between the established cutoff values. While Furtado et al.[21] used whole blood as the source of DNA samples, in this study DNA was extracted from 1x10^6 PBMC. Similarly

Table 1 – Proviral load variations at baseline and in intervals of 12 months, 12 to 24 months, and more than 24 months in asymptomatic carriers and HAM/TSP patients.

Interval (months)	n	Age (years)	Median time (months)	Gender (% female)	HTLV-1 proviral load[a] (baseline)	HTLV-1 proviral load[b] (2nd point)	p- value[c]
Asymptomatic							
< 12	27	45 (34-49)	5 (0.4-12)	66	9,157 (1,015-37,410)	6,780 (150-42,368)	0.6
≥ 12 to < 24	31	51 (37-59.5)	18 (13-23)	77	5,075 (436-35,389)	3,422 (0-68,228)	0.8
≥ 24	12	41 (23-49)	29 (25-43)	75	12,334 (1,590-43,712)	15,205 (148-52,091)	0.6
HAM/TSP							
< 12	11	52 (39.5-59.5)	7 (2-11)	71	86,560 (31,762-186,633)	88,889 (41,557-149,008)	0.9
≥ 12 and < 24	13	54 (45.5-62)	18 (13-24)	69	118,842 (15,392-317,009)	85,916 (11,041-205,807)	0.4
≥ 24	10	49.5 (40-64)	30 (25-34)	50	73,911 (19,384-90,380)	73,885 (37,690-109,860)	0.7

[a] Variables expressed as median (25^{th} percentile, 75^{th} percentile).
[b] Copies/10^6 PBMC.
[c] Wilcoxon test was used to compare the medians of proviral load.

Table 2 – Proviral load variations at baseline and in intervals of 12 months, 12 to 24 months, and more than 24 months in asymptomatic individuals with low proviral load (< 50,000copies/10^6PBMC).

Interval (months)	n	Median time (months)	HTLV-1 proviral load[a] (baseline)	HTLV-1 proviral load[b] (2nd point)	p-value[c]
< 12	21	5 (3-9)	3,305 (97-26,481)	3,451 (282-12,040)	0,6
≥ 12 and < 24	29	18 (14-19)	2,676 (8-27,145)	4,549 (133-53,541)	0,01
≥ 24	11	30 (26-32)	6,114 (265-28,665)	11,530 (109-57,142)	0,02

[a] Variables expressed as median (25^{th} percentile, 75^{th} percentile).
[b] Copies/10^6 PBMC.
[c] The cut-off point were 50.000 copies/10^6 PBMC; Wilcoxon test was used to compare the medians of proviral load.

to Furtado et al., no correlation could be found between HTLV-1-PVL and severity of HAM/TSP disease, age at disease onset, or duration of illness (data not shown).

The HTLV-1 -PVL has been described as a marker of disease development, especially HAM/TSP.[13–16] An increased HTLV-1-PVL is also present in patients with other HTLV-1-associated diseases, such as infective dermatitis,[22] ATL,[23] KCS,[6] as well as in HTLV-1-infected patients with rheumatoid arthritis or other connective tissue diseases.[24] PVL represents the amount of virus integrated into the genome. It is maintained mainly through clonal expansion of infected CD4 + T-lymphocytes.[25] However, their levels remain stable over time probably because specific HTLV-1 cytotoxic response of CD8 T-lymphocytes eliminates part of these infected cells.[26] This study is limited by the small sample size analyzed and by the short period of time evaluated. Moreover, it was not possible to evaluate patients continuously at the intervals of 12 months, 12 to 24 months, and more than 24 months. Further studies, involving large number of individuals with a serial HTLV PVL could complete these results.

In summary, the results obtained indicated that HTLV-1-PVL of HTLV-1 infected individuals remains stable for 24 months, in both asymptomatic and HAM/TSP patients. The authors recommend the annual measurement of PVL in asymptomatic individuals, as well as the monitoring of patient disease progression.

Conflict of interest

All authors declare to have no conflict of interest.

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