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Low DNA HTLV-2 proviral load among women in São Paulo City

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Abstract

Background: HTLV-2 infections are almost always asymptomatic, and diseases associated with the infection are rarely reported. Little information is available on the relationship between HTLV-2 proviral load and gender or expression of disease, especially among patients with HIV-1 co-infection.
Methods: We studied 77 HTLV-2-infected subjects followed in our clinic for the last 9 years; 53 (69%) of them were co-infected with HIV-1. HTLV-2 DNA proviral load (PVL) was measured by real time PCR, a test with a sensitivity of 10 in 10^4 PBMCs.
Results: Six of 53 HTLV-2/HIV-1 cases had a myelopathy (all of them had undetectable PVL of HTLV-2). Only 3 of 35 women (2 out of 3 co-infected with HIV) had a detectable PVL, whereas 10 of 42 men had a detectable PVL. Regardless of their HIV status women had significantly lower PVL than men (10 vs. 43 copies/10^4 PBMCs, $p < 0.05$).
Conclusions: We noticed the occurrence of myelopathy in HTLV-2/HIV-1 co-infected patients, with undetectable HTLV-2 viral load. There was a sex difference in viral load for HTLV-2, what may be the result in mode of transmission or acquisition of the virus.

Keywords: HTLV-2; IDU; HIV-1-; Transmission; DNA proviral load

1. Introduction

Human T-lymphotropic virus type 1 (HTLV-1) and type 2 (HTLV-2) were the first two known human retroviruses, discovered in 1980 and 1982, respectively (Poiesz et al., 1980; Kalyanaraman et al., 1982). HTLV infections are thought to have existed in humans for 10,000 years (Cann, 1990; Slattery et al., 1999), and CD4 and CD8 T lymphocytes are their main cells' targets, respectively (Franchini, 1995; Hall et al., 1996). HTLV-2 infection is prevalent among injecting drug users (IDUs) (Lee et al., 1991; Hall et al., 1990) and blood donors in the United States, Europe, Asia (Hern, 1996) and South America (Perez et al., 1993; Switzer et al., 1995; Ferreira Junior et al., 1995). HTLV-1 infection is associated with spontaneous proliferation of T cells, mediated by Tax protein (Green and Chen, 1990; Jacobson et al., 1990) which, in association and synergy with host and environmental factors, may result in adult T-cell leukemia (ATL) or tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM) in 1–5% of carriers (Kaplan et al., 1990). HTLV-2 infections are usually asymptomatic, but in urban areas there have been reports of increased risks for pneumonia, minor fungal infections and kidney infections among HTLV-2 infected persons (Modahl et al., 1997; Hall et al., 1996; Murphy, 1996). In contrast, there are only two cases of neurodegenerative syndrome in North American Indian women with HTLV-2, despite the fact that those populations have been infected by this virus for thousands of years (Salemi et al., 1999). HTLV-2-infected IDUs may have high viral load when co-infected with HIV-1, and it is possible that concomitant HIV-1 and HTLV-2 infections can increase HTLV-2 proviral load (Machuca and Soriano, 2000).

HTLV-1/2 RNA is undetectable in human serum, although viral RNA can sometimes be detected within peripheral blood

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mononuclear cells of carriers of the virus. The virus spreads by lymphocyte clonal expansion when integrated into host DNA (Wattel et al., 1995) and by transference of viral RNA via direct contact between infected and uninfected cells (Igakura et al., 2003). In this study, we evaluated the relationship between levels of HTLV-2 DNA proviral load and the following variables: (1) gender, (2) mode of transmission, (3) HIV-1 co-infection status, (4) immunologic parameters, and (5) expression of disease.

2. Subjects and methods

All patients included in the study were followed at Institute of Infectious Diseases "Emílio Ribas" (IIER). The serologic diagnosis of HLTV infection was made by ELISA (Ortho Diagnostics, USA) and Western Blot 2.4 HTLV tests (DBL, Singapore), using standard protocols. All patients whose serum samples were reactive by either test were submitted to nested-PCR utilizing generic primers HTLV-1/2 and amplified products digested with restriction enzymes (Novoa et al., 2007).

The HTLV specialized unit is made up of a multidisciplinary team including infectious diseases specialists, neurologists, a physical therapist and dentists. The following neurological scales were applied by neurologists at each patient visit: After giving informed consent, patients underwent a neurological assessment by a neurologist blinded to their HTLV status. Patients with at least two pyramidal signs, such as paresis, spasticity, hyperreflexia, clonus, diminished or absent superficial reflexes, or the presence of pathologic reflexes (e.g., Babinski sign), were defined as having myelopathy. Other causes of myelopathy, such as vitamin B12 deficiency, spinal cord compression, hypothyroidism and neurosyphilis, were excluded. All participants signed an informed consent that was approved by the local Ethical Board at the Institute of Infectious Diseases "Emílio Ribas".

Blood samples were collected in acid–citrate–dextrose solution and PBMC were separated by Ficoll density gradient centrifugation (Pharmacia, Uppsala, Sweden). The cells were washed with saline solution and cell number adjusted to 2×10^6 cells were stored at −70 °C. DNA was extracted using a commercial kit (GFX, Pharmacia, Uppsala, Sweden).

3. Real time PCR

The forward and reverse primers used for HTLV-2 DNA quantification were selected using the Oligo (version 4, National Biosciences, Plymouth, MN) and Primer Express (PerkinElmer Applied Biosystems) software programs and checked by a search on the GenBank. The probe carried a 5′ reporter dye FAM (6-carboxy fluorescein) and a 3′ quencher dye TAMRA (6-carboxy tetramethyl rhodamine). For quantitation of the human albumin gene, the primers Alb-S (5′-GCTGTCATCTCTTGTGGGCTGT-3′) and Alb-AS (5′-AAACTCATGGGAGCTGCT GGTT-3′) and the Alb TaqMan probe (5′-FAM-CCTGTCATGCCCACACAAATCTC TCC-TAMRA-3′) were used, as described previously (Dehee et al., 2002). Albumin DNA quantification was performed in parallel on all samples in order to determine the amount of cellular DNA present and was used as an endogenous reference to normalize variations due to differences in PBMC count or DNA extraction. The protocol was done in accordance to previous published data, and the sensitivity was 10 copies/10^4 PBMC (Montanheiro et al., 2005).

3.1. Statistical analysis

Differences in patient's characteristics or laboratory values from the groups were compared either by two-way Mann–Whitney's test, chi-square test or Fisher's exact test, when appropriate.

4. Results

A total of 538 individuals were referred to our outpatient clinic at the Institute of Infectious Disease Emilio Ribas (IIER)

Table 1
Demographical, epidemiological and immunological characteristics of the 77 HTLV-2-infected subjects

Variable	HIV-1/HTLV-2 ($n = 53$)			HTLV-2 ($n = 24$)		
	Women (n 16)	Men (n 37)	All subjects (n 53)	Women (n 19)	Men (n 05)	All subjects (n 24)
Age, mean years ± S.D.	37 ± 5	37 ± 7	37 ± 6	42 ± 10	37 ± 13	41 ± 11
HCV infection, n (%)	08 (50)	27 (73)	35 (66)	3 (16)	1 (20)	4 (17)
IDU, number (%)	7 (44)	29 (78)	36 (68)	3 (16)	1 (20)	4 (17)
Myelopathy diagnosis, number (%)	1 (6)	5 (13)	6 (17)	–	–	–
HTLV-2 proviral load, subjects with > 10 copies/10^4 PBMC (%)	2 (12)	9 (17)	11 (21)	1 (5)	1 (20)	2 (8)
HTLV-2 proviral load, mean ± S.D. copies/10^4 PBMC	10 copies/10^4 PBMCs	43 ± 158[a]	31 ± 132	10 ± 38	181 ± 356[a]	41 ± 154
CD4+ T cells count, median (interquartile range)	596 (308–773)	267 (113–494)	362 (147–570)	1035 (789–1166)	758 (635–913)	927 (728–1156)
CD8+ T cells count, median (interquartile range)	1074 (523–1524)	886 (607–1686)	902 (607–1584)	545 (383–585)	439 (296–862)	500 (379–692)

Notes: IDU, intravenous drug users; HCV, Hepatitis C virus.
[a] $p < 0.05$, using non-parametric two-tailed Mann–Whitney test, chi-square test, or Fisher's exact test, when appropriate.

for HTLV investigation from June 1997 to December 2006. A total of 77 HTLV-2 infected subjects were analyzed in this study (35 women). Among females, sixteen were HIV-1 co-infected, 11 had HCV co-infection, and one patient had a myelopathy (Table 1). The mean age was 42 (ranging from 32 to 59 years). Among males 37 were HIV-1 co-infected patients, 27 were co-infected with HCV, and five had a myelopathy diagnosis. The mean age was 40 years (27–54). The median T CD4 counts were 596 for females and 267 cells/mm^3 for males, and the median T CD8 counts were 1074 and 886 cells/mm^3 for women and men, respectively. These differences were not statistically significant (Table 1).

Among women, 3 of the 35 had detectable proviral loads (PVL), (mean, 10 copies/10^4 PBMC for the singly HTLV-2-infected, $p = 0.14$). All of them were over 40 years of age, were former IDU, and 2 of them were co-infected with HIV-1. In contrast, men had a mean number of 43 copies/10^4 PBMC and 181 copies/10^4 PBMC, for the co-infected and singly, respectively, $p < 0.05$). Co-infected men were more likely to be IDU than women ($p = 0.03$), but singly infected men for HTLV-2 were not more likely to be IDU ($p = 1.0$).

Results of two-way Fisher's tests for the proportion of men and women whose viral loads were higher than 10 copies/10^4 PBMC were not significant for both co-infected and singly infected subjects ($p = 0.47$ and $p = 0.38$, respectively).

All six patients (five men) diagnosed with myelopathy disease yielded undetectable HTLV-2 viral load and were also co-infected with HIV-1.

5. Discussion

In this study, women had lower HTLV-2 PVL than men, and only three women had detectable levels. We believe this lower PVL may be due to the route of infection. Similarly, Murphy et al. (2004) showed that higher HTLV-2 proviral loads in males were independently associated with subtype B and history of blood transfusion in this population. In contrast, another study showed no gender differences in the PVL (Hisada et al., 2005). Furthermore, it seems that HTLV-2 replication rate is higher among older patients, what could explain the higher risk for sexual transmission from male to female observed in this population (Kaplan et al., 1996).

It is unlikely that our findings could be explained by HTLV-2 subtypes since subtype A predominates in this cohort, where only one case of subtype B was found (Novoa et al., 2007]. In another study with IDU, a greater variation was observed in the number of copies of HTLV-2 proviral sequences in the PBMCs of Italian drug abusers, ranging from 5–10 to 16,239 copies/10^5 cells (Cimarelli et al., 1995). There was not a clear-cut correlation between proviral load, CD8 count, stage of HIV-1 co-infection, or therapy. A considerable variation in HTLV-2 proviral load was also observed in PBMCs of Amerindians and Central Africans without any correlation between the amount of HTLV-2 proviral copies and the geographic origin and/or ethnicity of the infected individuals (Mahieux et al., 2000).

In our study, HTLV-2 infected males concomitantly co-infected with HIV-1 and HCV displayed higher PVL (16–123 copies/10^4). HCV co-infection is highly prevalent among HTLV-2-infected subjects, probably due to the similar transmission route (Toro et al., 2005]. It might be that the presence of other viral co-morbidities could also influence the HTLV-2 replication rate. Further studies have been proposed in our laboratory to determine if this viral interaction may cause differences in HCV outcome, similarly to what happens in HTLV-1 infection (Boschi-Pinto et al., 2000). The impact of each viral species replication in this co-infection should also be investigated in the future. Among all subjects enrolled in this study, only HIV-1/HTLV-2 co-infected subjects presented with a myelopathy; three of them were also HCV-infected. It is more likely that the HIV-1 infection is responsible for the neurological symptoms, as nobody so far has been able to etiologically associate HTLV-2 infection with a known disease (Posada-Vergara et al., 2006), although some neurological diseases have already been ascribed to it (Zehender et al., 1995; Beilke et al., 2004).

These findings about HTLV-2 proviral load are important for an understanding of the cause of the low pathogenicity of this infection. Other factors must be analyzed, such as the longitudinal study design and larger sample size. However, we could speculate that because of the low replication rate and poorly pathogenic infectious virus (Kwaan et al., 2006), especially among women, the HTLV-2 has the potential to be in a process of adaptive evolution. Finally, the importance of this unique cohort and its findings point out to the need for further studies in this area.

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