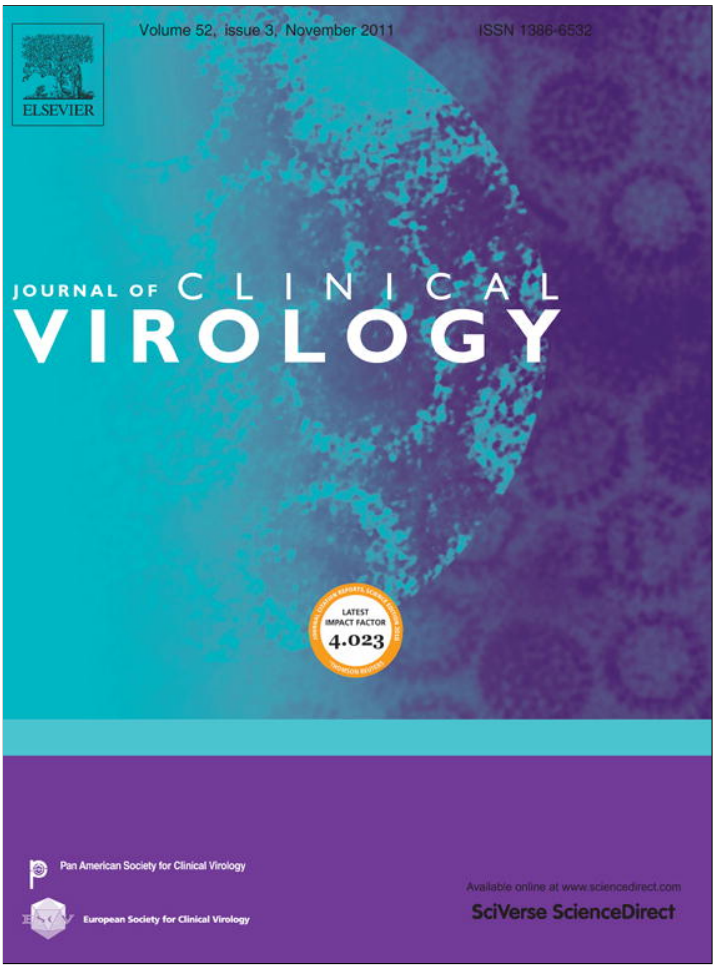

Journal of Clinical Virology 52 (2011) 177–180

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Keratoconjunctivitis sicca of human T cell lymphotropic virus type 1 (HTLV-1) infected individuals is associated with high levels of HTLV-1 proviral load

Cristina Castro-Lima Vargens[a,*], Maria Fernanda Rios Grassi[b], Ney Boa-Sorte[b], Regina Helena Rathsam-Pinheiro[a,b], Viviana Nilla Olavarria[b,c], Ramon de Almeida Kruschewsky[a], Bernardo Galvão-Castro[b,c]

[a] Instituto Brasileiro de Oftalmologia e Prevenção da Cegueira (IBOPC), Salvador, BA, Brazil
[b] Centro Integrativo e Multidisciplinar de HTLV, Escola Bahiana de Medicina e Saúde Pública (EBMSP), Salvador, BA, Brazil
[c] Laboratório Avançado de Saúde Pública, Centro de Pesquisas Gonçalo Moniz, Rua Waldemar Falcão, 121, Candeal, CEP: 40296-710, Salvador, BA, Brazil

ARTICLE INFO

Article history:
Received 31 May 2011
Received in revised form 15 July 2011
Accepted 20 July 2011

Keywords:
HTLV-1
Ocular lesions
Keratoconjuntivitis sicca
Proviral load

ABSTRACT

Background: A high HTLV-1 proviral load is found in HTLV-1-associated diseases, mainly HAM/TSP. However, the association between proviral load and keratoconjunctivitis sicca (KCS) has not been well established.
Aim: To verify the association between KCS and HTLV-1 proviral load.
Study design: 104 HTLV-1 infected patients (51 asymptomatic and 52 with HAM/TSP) from the HTLV reference center in Salvador, Brazil were followed from June 2008 to May 2010. Evaluation of tear secretion was performed by BUT (break-up time), Rose Bengal and Schirmer I tests. The diagnosis of KCS was based upon the presence of symptoms and when at least two of three tests were positive. HTLV-1 proviral load was determined using real-time PCR.
Results: The prevalence of KCS was 44.2%. KCS was more frequent among HAM/TSP patients ($p = 0.022$). Patients with KCS had higher proviral load (mean 134,672 ± 150,393 copies/10^6 PBMC) than patients without the disease (mean 66,880 ± 109,525 copies/10^6 PBMC) ($p = 0.001$). HTLV-1 proviral load > 100,000 copies/10^6 PBMC increased significantly the risk of developing KCS (OR = 4.05 and 95% CI = 1.40–11.76). After age > 45 years and HAM/TSP status were excluded *in stepway reward* analysis, the variables PVL > 100,000 (OR = 4.77 and 95% CI = 1.83–12.44) still remained statistically significant.
Conclusion: HTLV-1 proviral loads are higher in patients with KCS and may represent a relevant biological marker of disease.

1. Background

The human T-cell lymphotropic virus type 1 (HTLV-1) is etiologically linked with adult T cell leukemia (ATL),[1] tropical spastic paraparesis/HTLV-1-associated myelopathy (HAM/TSP),[2,3] infective dermatitis[4] and uveitis (HTLV-I-associated uveitis [HAU]).[5,6] Several diseases have been also associated with HTLV-1 infection, such as polymyositis, sinusitis, thyroiditis, bronchi alveolar pneumonia, Sjörgren syndrome indicating a multi-systemic involvement in this infection.[7,8]

In addition to HAU, keratoconjunctivitis sicca (KCS) has also been described in HTLV-1-infected individuals.[9–11] KCS causes an eye discomfort, a visual disturbance, and tear film instability with potential damage to the ocular surface.[12] The disease is more prevalent in women in their 50s.[13,14] In HTLV-1 infected individuals, the prevalence of KCS is higher in patients with HAM/TSP diagnosis, when compared to asymptomatic individuals.[15] A higher HTLV-1 proviral load (PVL) has been found in patients with HTLV-associated diseases, especially HAM/TSP,[16–19] infective dermatitis,[20] and uveitis[21] compared with proviral load from asymptomatic individuals. A recent study found that patients with KCS who were infected by HTLV-1 had a higher PVL than those without KCS. However, due to its small sample size, no statistically significant differences were observed in the study.[22]

2. Objective

This study aims to verify the association between KCS and HTLV-1 proviral load.

Abbreviations: ATL, adult T cell leukemia; BUT, break-up time; HAU, HTLV-I-associated uveitis; HTLV, human T-cell lymphotropic virus; KCS, keratoconjuntivitis sicca; TSP/HAM, tropical spastic paraparesis/HTLV-1-associated myelopathy; PBMC, peripheral blood mononuclear cells; PVL, proviral load.

* Corresponding author at: Centro Integrativo e Multidisciplinar de HTLV, Escola Bahiana de Medicina e Saúde Pública, Av. Dom João VI, nº 274, Brotas, Salvador, BA 40.290-000, Brazil. Tel.: +55 71 32768281; fax: +55 71 32768290.
E-mail address: bgalvao@bahiana.edu.br (C. Castro-Lima Vargens).

1386-6532/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jcv.2011.07.016

3. Study design

3.1. Population

An outpatient cross-sectional study was carried out between June 2008 and May 2010 at the Bahian School of Medicine and Public Health reference center for HTLV, Salvador, Bahia, Brazil, where an inter-disciplinary project including medical care, laboratory diagnosis, psychological assistance and physiotherapy is being carried out. Since 2002, a total of 1070 HTLV-1 infected patients were followed up in the center and 50% of them have been seen at least twice a year. The majority of patients of HTLV center was women, belonged to low social class and 30% had HAM/TSP.[23] Patients were sequentially invited at the moment of medical examination and were eligible if they had the following inclusion criteria: HTLV proviral load available and an ophthalmological exam, including complete tear film evaluation. All volunteers gave written informed consent before entering the research protocol.

3.2. Laboratory, clinical and ophthalmologic diagnosis

The HTLV-1 infection was assessed according to the algorithm recommended by the Brazilian Ministry of Health.[24] Plasma samples repeatedly positive in duplicate by ELISA (HTLV-1/HTLV-2 Ab-Capture ELISA Test System, Ortho. Clinical Diagnostic Inc. Raritan, New Jersey, USA) were confirmed and discriminated between HTLV-1 and HTLV-2 using Western Blot (HTLV Blot 2.4; Genelabs, Singapore). Polymerase chain reaction (PCR) analysis was performed in samples with undetermined results according to the technique described by Kashima.[25] The diagnosis of HAM/TSP was made according to the WHO guideline.[26] All patients had a full ophthalmologic examination in both eyes, including visual acuity measurement by Snellen table with optical correction, optical motility, applanation tonometry, biomicroscopy of the anterior and posterior chambers, binocular indirect ophthalmoscopy with or without depression and intraocular pressure. Evaluation of tear secretion was performed by BUT (break-up time), Rose Bengal and Schirmer I tests. Rose Bengal test was performed with 0.1% solution Rose Bengal staining and was considered abnormal when its total score was higher than three points (Van-Bijsterveld score).[27] Break-up time < 10 s and Schirmer I test < 5 mm were defined as abnormal. The diagnosis of KCS was based upon the presence of symptoms and when at least two of three tests were positive.[28,29]

3.3. HTLV-1 proviral load

HTLV-1 proviral load was quantified using a real-time TaqMan PCR method, as described previously.[30] SK110/SK111 primers were used to amplify a 186 pb fragment of the pol gene and dual TaqMan probe (5′FAM/5′VIC and 3′TAMRA) was located at 4829–4858 bp of the HTLV-1 reference sequence ($HTLV_{ATK}$). Albumin DNA was used as an endogenous reference. The value of HTLV-1 proviral load was reported as the [(HTLV-1 average copy number)/(albumin average copy number)] $\times 2 \times 10^6$ and expressed as the number of HTLV-1 copies per 10^6 cells in peripheral blood mononuclear cells (PBMCs.). PVL measurements for all patients were performed within a period of time ranging from 12 months prior to, or following, ophthalmologic examination.

3.4. Statistical analysis

Results were expressed as proportions for categorical variables and means ± standard deviation (SD) for continuous variables. The statistical tests used included Student's *t*-test for independent variables and chi-square test for comparison of sex and HAM/TSP between KCS and asymptomatic patients. Mann–Whitney test was used to evaluate PVL and this association with KCS. A logistic regression model was used to estimate the association between patients with KCS and variables such as age over 45 years, female sex, presence of HAM/TSP and CPV, measured by odds ratio (OR) and corresponding 95% confidence interval (CI). Differences of $p < 0.05$ were considered statistically significant. Data were stored and analyzed with SPSS 13.0 for Windows.

Table 1
Main ocular complaint and diagnosis of keratoconjunctivitis sicca.

Ocular complaint	*N*	%
Visual blurring	30	28.8
Pain/burning	29	27.9
Itching	15	14.4
Hyperemia	7	6.7
Foreign body sensation	5	4.8
Eyelid tumor	3	2.9
Tear flow	2	1.9
Flying flies	1	1.0
Dryness	1	1.0
No complaints	11	10.6
KCS diagnosis	46	44.2

KCS, keratoconjunctivitis sicca (tear secretion evaluated by break-up time, Rose Bengal and Schirmer I tests. The diagnosis of KCS was based upon the presence of symptoms and when at least two of three tests were positive).

Table 2
Association between KCS and age, sex, PVL and HAM/TSP diagnosis.

Variable	KCS		*p* value
	Present *N* = 46	Absent *N* = 58	
Age (years)	47.9 ± 11.7	45.2 ± 15.7	0.343*
Male *N* (%)	16 (55.2)	13 (44.8)	0.162**
PVL	134,672 ± 150,393	66,880 ± 109,525	0.001***
HAM/TSP diagnosis *n* (%)	29 (55.8%)	23 (44.2%)	0.022**

PVL, proviral load (copies/10^6 PBMC).
* Student's *t*-test, $p < 0.05$.
** Chi-square test, $p < 0.05$.
*** Mann–Whitney test, $p < 0.05$.

4. Results

There was a predominance of women (72.9%), patients' age ranged from 9 to 81 years (mean 46.4 ± 14.04). The main ocular complaints were described in Table 1. The prevalence of KCS was 44.2%. Fifty-two patients (50%) had HAM/TSP diagnosis and the presence of KCS was higher among TSP/HAM patients ($p = 0.022$) (Table 2). The frequency of KCS was higher in male patients, however this difference was not statistically significant ($p = 0.162$). The mean age of patients was not statistically significant ($p = 0.343$).

Table 3
Proviral load according to the presence or absence of KCS in HTLV-1-infected individuals.

PVL	KCS	
	Present *N* = 46	Absent *N* = 58
Min – Max	0.0 – 678,012	0.0 – 654,028
IQR	28,131–186,870	1,097–86,476
Median*	87,335	30,935
Mean ± SD	134,672 ± 150,393	66,880 ± 109,525
CV (%)	111.7	163.8

PVL, proviral load (copies/10^6 PBMC).
Min, minimum; max, maximum; IQR, interquartile range; SD, standard deviation; CV (coefficient of variation).
* $p = 0.003$ (median test).

Table 4
Multivariate analysis of patients with and without KCS.

Variable	Complete model		Final model[a]	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age < 45 years	1.0			
Age > 45 years	1.06 (0.46–2.47)	0.888		
Male	1.0		1.0	
Female	0.47 (0.18–1.23)	0.125	0.44 (0.17–1.13)	0.087
Asymptomatic	1.0			
TSP/HAM	1.36 (0.53–3.47)	0.519		
PVL 0–50,000	1.0		1.0	
PVL 50,000–100,000	2.26 (0.66–7.75)	0.191	2.58 (0.80–8.31)	0.111
PVL ≥100,000	4.05 (1.40–11.76)	0.010	4.77 (1.83–12.44)	0.001

[a] Age > 45 years and TSP/HAM status were excluded in *stepway reward*. PVL (HTLV-1 proviral load – copies/10^6 PBMC).

The mean ± SD of HTLV-1 proviral load in KCS patients was 134,672 ± 150,393 copies/10^6 PBMC) and was significantly higher than the mean (±SD) of proviral load of patients without KCS, 66,880 ± 109,525 copies/10^6 PBMC ($p = 0.001$) (Table 2). The coefficient of variations of PVL was 111.7% in patients with KCS and 163.8% in those without KCS. The measures of dispersion of PVL were described in Table 3.

The complete model for logistic regression analysis showed that patients with PVL > 100,000 copies/10^6 PBMC had a significantly increased risk of developing KCS (OR = 4.05 and 95% CI = 1.40–11.76). However, sex, HAM/TSP, status, age > 45 years and PVL between 50,000 and 100,000 did not positively correlate with the risk of developing KCS. The PVL > 100,000 copies/10^6 PBMC (OR = 4.77 and 95% CI = 1.83–12.44) variable remained statistically significant after exclusion of age > 45 years and HAM/TSP variables in the *stepway reward* analysis (Table 4).

5. Discussion

This study demonstrated that high HTLV-1 proviral load is associated with the presence of KCS in patients infected with HTLV-1. Moreover, KCS was observed in almost half of the infected individuals and was more frequent in HAM/TSP patients.

A broad spectrum of ophthalmic manifestations is described in HTLV-1-infected patients including HAU,[31] malignant infiltrates in patients with ATL,[32,33] interstitial keratitis,[34] Sjögren's syndrome with lacrimal involvement,[35] as well as an increased prevalence of KCS in patients with HAM/TSP.[15]

Several mechanisms have been proposed to explain the role of the virus for the development of these ophthalmological manifestations. The eye has a unique defense system to protect it from immunopathogenic mechanisms, including the blood ocular barrier that will stop activated T cells from entering the eye.[36–38]

The HTLV-1 proviral load is the amount of the DNA of virus (provirus) integrated into the host cell genome. Several evidence indicate that it may represent a biological marker of development of HTLV-1 associated diseases. There is strong evidence that high HTLV-1-proviral load is associated with HAM/TSP disease,[16–19] and also it is increased in patients with other HTLV-1-associated diseases, such as infective dermatitis,[20] ATL,[39] as well as patients with rheumatoid arthritis and other connective tissue diseases.[40] Recently, it has been suggested that a proviral load above 50,000 HTLV copies/10^6 PBMC is the best level of HTLV-1-proviral load to discriminate asymptomatic individuals from HAM/TSP patients.[19]

In the present study, only a proviral load above 100,000 copies/10^6 PBMC was associated with an increased risk of KCS, and there was an association between the presence of KCS and HAM/TSP disease corroborating previous data.[15] In KCS patients, Ferraz-Chaoui et al. demonstrated that the lesion of cornea was not due to the presence of autoantibodies elicited by the infection with HTLV-1. Moreover, the authors found a trend toward higher proviral load in HTLV-1 patients with KCS, compared with patients with preserved lacrimal function.[22] However, the mechanisms implicated in the development of KCS in these patients remain unclear. Interestingly, HAU was not diagnosed in this study, even in patients with high PVL, corroborating other studies performed in Brazil that found a lower prevalence of HAU, compared to Japanese studies.[10,11,41,42] It is possible that genetic background could play a role in the difference HAU prevalences.

We are aware that one of the limitations of the present study is its cross-sectional design. A single PVL measurement was taken no earlier than one year before, or after, the KCS diagnosis. In addition to an elevated PVL, it is also possible that the length of time in which a high load is sustained may play a role in the development of KCS. Although PVL varies widely among HTLV-1-infected individuals, there is evidence that it remains relatively constant within a given individual over a period of 24 months to 10.4 years.[43,44] An additional limitation of the present study is that the prevalence of KCS in the general population of Salvador is unknown.

In summary, the results presented herein suggest that HTLV-1 PVL may be a biological marker of KCS development. HTLV-1-infected patients with a high PVL should be encouraged to have regular ophthalmological examinations for KCS screening. This precaution can aid in the treatment of dry eye symptoms and avoid consequent corneal damage.

Funding

Support for this study was provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Coordenação Nacional de DST/aids-Ministério da Saúde.

Conflict of interest

The authors disclose any financial or other conflict of interest that might be construed to influence the contents of the manuscript, including the results or interpretation of publication.

Ethical approval

This study was approved by the Institutional Review Board of the Bahian School of Medicine and Public Health (Protocol Number 71/2006).

Acknowledgement

We would like to thank Dr. Raymond Césarie for providing HTLV/Albumina clones.

References

1. Yoshida M, Miyoshi I, Hinuma Y. Isolation and characterization of retrovirus from cells lines of human adult T-cell leukemia and its implications in the disease. *Proc Natl Acad Sci U S A* 1982;**79**:2031–5.
2. Gessain A, Barin E, Vernant JC, Gout O, Maurs L, Calender A. Antibodies to human T-lymphotropic virus type I in patients with tropical spastic paraparesis. *Lancet* 1985;**2**:407–10.
3. Osame M, Matsumoto M, Usuku K, Izumo S, Ijichi N, Amitani H, et al. HTLV-1 associated myelopathy, a new clinical entity. *Lancet* 1986;**1**:1031–2.
4. La Grenade L, Hanchard B, Fletcher V, Cranston B, Blattner W. Infective dermatitis of Jamaican children: a marker for HTLV-I infection. *Lancet* 1990;**336**:1345–7.
5. Nakao K, Ohba N, Matsumoto M. Noninfectious anterior uveitis in patients infected with human T lymphotropic virus type I. *Jpn J Ophthalmol* 1989;**33**:472–8.
6. Mochizuki M, Watanabe T, Yamaguchi K, Takatsuki K, Yoshimura K, Nakashima S, et al. Uveitis associated with human T-cell lymphotropic virus type I. *Am J Ophthalmol* 1992;**114**(2):123–9.

7. Eguchi K, Matsuoka N, Ida H, Nakashima M, Sakai M, Sakito S, et al. Primary Sjogren's syndrome with antibodies to HTLV-I: clinical and laboratory features. *Ann Rheum Dis* 1992;**51**:769–76.
8. Proietti FA, Carneiro-Proietti ACF, Catalan-Soares BC, Murphy EL. Global epidemiology of HTLV-1 infection and associated diseases. *Oncogene* 2005;**24**:6058–68.
9. Merle H, Smadja D, Le Hoang P, Bera O, Cabre P, Landau M, et al. Ocular manifestations in patients with HTLV-I associated infection: a clinical study of 93 cases. *Jpn J Ophthalmol* 1996;**40**:260–70.
10. Pinheiro SR, Martins-Filho OA, Ribas JG, Catalan-Soares BC, Proietti FA, Namen-Lopes S, et al. Immunologic markers, uveitis, and keratoconjuntivitis sicca associated with human T-cell lymphotropic virus type 1. *Am J Ophthalmol* 2006;**20**:1–5.
11. Rathsam-Pinheiro RH, Boa-Sorte N, Castro-Lima-Vargens C, Pinheiro CA, Castro-Lima H, Galvão-Castro B. Ocular lesions in HTLV-1infected patients from Salvador, State of Bahia: the city with the highest prevalence of this infection in Brazil. *Rev Soc Bras Med Trop* 2009;**42**(6):633–7.
12. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop. *Ocul Surf* 2007;**5**(2):75–92.
13. Schein OD, Munoz B, Bandeen-Roche K, West S. Prevalence of dry eye among the elderly. *Am J Ophthalmol* 1997;**124**(6):723–8.
14. Schaumberg DA, Sullivan DA, Buring JE, Dana R. Prevalence of dry eye syndrome among US women. *Am J Ophthalmol* 2003;**136**(2):318–26.
15. Merle H, Cabre P, Olindo S, Merle S, Smadja D. Ocular lesions in 200 patients infected by the T-cell lymphotropic virus type 1 in Martinique (French West Indies). *Am J Ophthalmol* 2002;**134**(2):190–5.
16. Nagai M, Usuku K, Matsumoto W, Kodama D, Takenouchi N, Moritoyo T, et al. Analysis of HTLV-I proviral load in 202 HAM/TSP patients and 243 asymptomatic HTLV-I carriers: high proviral load strongly predisposes to HAM/TSP. *J Neurovirol* 1998;**4**:586–93.
17. Olindo S, Lezin A, Cabre P, Merle H, Saint-Vil M, Edimonana Kaptue M, et al. HTLV-1 proviral load in peripheral blood mononuclear cells quantified in 100 TSP/HAM patients: a marker of disease progression. *J Neurol Sci* 2005;**237**:53–9.
18. Silva MT, Harab RC, Leite ACC, Schor D, Araujo A, Andrada-Serpa MJ. Human, T lymphotropic virus type 1 (HTLV-I) proviral load in asymptomatic carriers. HTLV-1-associated myelopathy/tropical spastic paraparesis, end other neurological abnormalities associated with HTLV-1 infection. *Clin Infect Dis* 2007;**44**:p.689–92.
19. Grassi MFR, Olavarria VN, Kruschewsky RA, Mascarenhas RE, Dourado I, Correia LCL, et al. Human, T cell lymphotropic virus type 1 (HTLV-1) proviral load of HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients according to new diagnostic criteria of HAM/TSP. *J Med Virol* 2011;**83**:1269–74.
20. Primo J, Siqueira I, Nascimento MC, Oliveira MF, Farre L, Carvalho EM, et al. High HTLV-1 proviral load, a marker for HTLV-1 associated myelopathy/tropical spastic paraparesis, is also detected in patients with infective dermatitis associated with HTLV-1. *Braz J Med Biol Res* 2009;**42**:761–4.
21. Ono A, Mochizuki M, Yamagushi K, Miyata N, Watanabe T. Increased number of circulating HTLV-1 infected cells in peripheral blood mononuclear cells of HTLV-1 uveitis patients: a quantitative polymerase chain reaction study. *Br J Ophthalmol* 1995;**79**(3):270–6 [Erratum in: Br J Ophthalmol 1995;69(6): 621].
22. Ferraz-Chaoui AK, Atta ML, Galvao-Castro B, Santiago MB. Study of autoantibodies patients with keratoconjunctivitis sicca infected by the human T cell lymphotropic virus type 1. *Rheumatol Int* 2010;**30**(6):775–8.
23. Nunes C, Mascarenhas-Batista AV, Maltês D, Brandão JCD, Ferreira TS, Seabra AML, et al. Características Clínicas–Epidemiológicas de 385 pacientes portadores de HTLV na Bahia. *Rev Soc Bras Med Trop* 2006:19–22.
24. Brasil, Ministério da Saúde. Secretaria de Vigilância em Saúde. Programa Nacional de DST e Aids. Guia do manejo clínico do HTLV/Ministério da Saúde, Secretaria de Vigilância em Saúde, Programa Nacional de DST e Aids. – Brasília: Ministério da Saúde; 2003. p. 52.
25. Kashima S, Alcantara LC, Takayanagui OM, Cunha MA, Castro BG, Pombo-de-Oliveira MS, et al. Distribution of human T cell lymphotropic virus type 1 (HTLV-1) subtypes in Brazil: genetic characterization of LTR and tax region. *AIDS Res Hum Retroviruses* 2006;**22**(10):953–9.
26. Osame M. Review of WHO Kagoshima meeting and diagnostic guidelines for HAM/TSP. In: Blattner W, editor. *Human retrovirology: HTLV*. New York: Raven Press; 1990. p. 91–197.
27. Van-Bijsterveld OP. Diagnostic tests in the Sicca syndrome. *Arch Ophthalmol* 1969;**82**:10–4.
28. Kanski JJ. *Clinical ophthalmology: a systematic approach*, vol. 5, fifth ed. Philadelphia: Butterworth Heinemann; 2003. p. 270–308.
29. Farris RL, Gilbard JP, Stuchell RN, Mandel ID. Diagnostic tests in keratoconjuntivitis sicca. *CLAO J* 1983;**9**:23–8.
30. Dehée A, Césaire R, Désiré N, Lézin A, Bourdonné O, Béra O, et al. Quantitation of HTLV-I proviral load by a TaqMan real-time PCR assay. *J Virol Methods* 2002;**102**:37–51.
31. Mochizuki M, Watanabe T, Yamaguchi K, Takatsuki K, Shirao M, Yoshimura K, et al. HTLV-1 uveitis: a distinct clinical entity caused by HTLV-1. *Jpn J Cancer Res* 1992;**83**:236–9.
32. Levy-Clarke G, Buggage R, Shen DF, Vaughn LO, Chan CC, Davis JL. Human T-cell lymphotropic virus type-I associated T-cell leukemia-lymphoma masquerading as necrotizing retinal vasculitis. *Ophthalmology* 2002;**109**: 1717–22.
33. Liu MM, Furusato E, Cao X, Shen D, Chan CC. Ocular manifestation and pathology of adult T-cell leukemia/lymphoma associated with human T-lymphotropic virus type 1. *Rare Tumors* 2010;**2**(63):179–82.
34. Merle H, Cabre P, Merle S, Gerard M, Smadja D. A description of human T-lymphotropic virus type I-related chronic interstitial keratitis in 20 patients. *Am J Ophthalmol* 2001;**131**:305–8.
35. Nakamura H, Eguchi K, Nakamura T, et al. High prevalence of Sjogren's syndrome in patients with HTLV-1 associated myelopathy. *Ann Rheum Dis* 1997;**56**:167–72.
36. Mochizuki M. Regional immunity of the eye. *Acta Ophthalmol* 2009;**88**(3): 292–9.
37. Ono A, Ikeda E, Mochisuki M. Provirus load in patients with human-T cell leukemia virus type I correlates with precedent Graves disease and disease activities. *Jpn J Cancer Res* 1998;**89**:608–14.
38. Sagawa K, Mochizuki M, Masuoka K, Katagiri K, Ktayama T, Maeda T, et al. Immunopathological mechanisms of human T cell lymphotropic virus type 1 (HTLV-I) uveitis. Detection of HTLV-I-infected T cells in the eye and their constitutive cytokine production. *J Clin Invest* 1995;**95**(2): 852–8.
39. Okayama A, Stuver S, Matsuoka M, Ishizaki J, Tanaka G, Kubuki Y, et al. Role of HTLV-1 proviral DNA load and clonality in the development of adult T-cell leukemia/lymphoma in asymptomatic carriers. *Int J Cancer* 2004;**110**: 621–5.
40. Yakova M, Lezin A, Dantin F, Lagathu G, Olindo S, Jean-Baptiste G, et al. Increased proviral load in HTLV-1-infected patients with rheumatoid arthritis or connective tissue disease. *Retrovirology* 2005;**2**:4.
41. Ikeda E, Ono A, Hikita N, Arima K, Mochizuki M, Yamaguchi K, et al. Estimated prevalence rate of HTLV-I uveitis in Chikugo. *Nippon Ganka Gakkai Zasshi* 1998;**102**:327–32.
42. Yamamoto JH, Segurado AA, Hirata CE, Sampaio MW, Souza EC, Nukui Y, et al. Human T-lymphotropic virus type 1 infection and ocular manifestations in São Paulo, Brazil. *Arch Ophthalmol* 1999;**117**(4):513–7.
43. Manns A, Miley WJ, Wilks RJ, Morgan OS, Hanchard B, Wharfe G, et al. Quantitative proviral DNA and antibody levels in the natural history of HTLV-I infection. *J Infect Dis* 1999;**180**(5):1487–93.
44. Taylor GP, Tosswill JH, Matutes E, Daenke S, Hall S, Bain BJ, et al. Prospective study of HTLV-I infection in an initially asymptomatic cohort. *J Acquir Immune Defic Syndr* 1999;**22**(1):92–100.