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ASSOCIATION OF CYTOKINES, NEUROLOGICAL DISABILITY, AND DISEASE DURATION IN HAM/TSP PATIENTS

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ABSTRACT - *Objective:* To identify clinical and immunological markers associated with HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP). *Method:* 237 HTLV-I infected individuals were clinically assessed. They were classified according to the Expanded Disability Status Scale (EDSS) and Osame's Motor Disability Score (OMDS). Cytokine levels were determined in HTLV-I seropositive individuals. *Results:* 37 patients had HAM/TSP. There was a correlation between the degrees of disability assessed by both scales. There was also a correlation between the duration of HAM/TSP and the severity of disability assessed by either EDSS or OMDS. Higher levels of IFN-γ were detected in unstimulated peripheral blood mononuclear cells (PBMC) from HAM/TSP patients as compared with HTLV-I carriers. *Conclusion:* This study shows the validity of the neurological scales to classify the degree of neurological disability in HTLV-I carriers and suggests a progressive behavior of HAM/TSP. This study also shows that IFN-γ in PBMC supernatants are markers of HAM/TSP.

KEY WORDS: human T-lymphotropic virus 1, nervous system diseases, cytokines, neurological disability.

Associação de citocinas, incapacidade neurológica e duração da doença em pacientes com mielopatia associada ao HTLV-I/paraparesia espástica tropical (MAH/PET)

RESUMO - *Objetivo*: Identificar marcadores clínicos e imunológicos associados com a mielopatia associada ao HTLV-I/paraparesia espástica tropical (MAH/PET). *Método:* 237 indivíduos infectados pelo HTLV-I foram clinicamente avaliados. Eles foram classificados de acordo com a escala expandida do estado de incapacidade de Kurtzke (EDSS) e escala de incapacidade motora de Osame (OMDS). Níveis de citocinas foram determinados nos indivíduos. *Resultados:* 37 pacientes tinham MAH/PET. Houve correlação entre os graus de incapacidade pelas escalas. Houve também correlação entre a duração da MAH/PET e o grau da incapacidade pelas escalas. Níveis elevados de IFN-γ foram detectados em células mononucleares de sangue periférico (CMSP) não estimuladas de pacientes com MAH/PET quando comparados com indivíduos HTLV-I positivos assintomáticos. *Conclusão:* Os dados demonstram a validade das escalas neurológicas para classificar o grau de incapacidade neurológica em portadores do HTLV-I e sugerem o comportamento progressivo da MAH/PET. Este estudo também demonstra que os níveis de IFN-γ em sobrenadante de CMSP são marcadores da MAH/PET.

PALAVRAS-CHAVE: vírus 1 linfotrópico T humano, doenças do sistema nervoso, citocinas, incapacidade neurológica.

Human T cell lymphotropic virus type I (HTLV-I) is an exogenous human retrovirus associated with adult T cell leukemia and a progressive neurological disease named HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP)[1-3]. About 20 million people are infected worldwide with endemic foci in Southern Japan, the Caribbean, Central and South America, Africa[4]. However, the vast majority of these individuals are clinically asymptomatic and less than 5% of them develop HAM/TSP[1]. The prevalence of HTLV-I infection is high in Brazil, particularly in the city of Salvador, where 1.35% of blood donors are infected[5].

How HTLV-I causes this disease is not completely

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understood, although virus-host immunological interactions have been suggested to play a role in the pathogenesis of the disorder[2]. The immunological response in HTLV-I infection is characterized by spontaneous T-cell proliferation with increasing secretion of interleukin (IL)-2 and expression of the IL-2 receptor[6]. Abnormalities in this response have been shown in patients with HAM/TSP compared to HTLV-I asymptomatic individuals, including elevated levels of cytokines such as tumor necrosis factor-α (TNF-α), IL-6, and IL-2 in sera and cerebrospinal fluid (CSF)[6-9].

The major aim of the current study is to evaluate the association among cytokines, neurological disability, and disease duration in HAM/TSP patients.

METHOD

Patients – Two hundred and thirty-seven patients referred from blood banks or from neurological clinics in Salvador, Brazil, were admitted to the HTLV-I Multidisciplinary Outpatient Clinic of Hospital Universitário Professor Edgard Santos, Federal University of Bahia, Brazil, from September 2000 to August 2001. In all cases the diagnosis was established by ELISA (Cambridge Biotech Corp., Worceste, MA, U.S.A) and confirmed by Western blot analysis, distinguishing HTLV-I from HTLV-II (HTLV blot 2.4, Genelab, Singapore). These subjects were assessed using a standardized questionnaire to determine the initial clinical manifestations of HTLV-I infection. These patients were then submitted to a complete clinical and neurological examination to evaluate if they had signs of HAM/TSP. Thirty-seven patients with HAM/TSP, according to consensus guidelines[10], were identified. Neurological disability was determined based upon Osame's Motor Disability Score (OMDS)[11] and Extended Disability Status Scale (EDSS)[12] by the assisting neurologist (André Muniz, MD). HTLV-I associated myelopathy was clinically defined as EDSS $\geq$3 and OMDS $\geq$1 for this study. Thirty-eight asymptomatic HTLV-I positive individuals served as the control group for the immunological studies. All asymptomatic subjects scored no points according to both scales.

All eligible subjects or minors' parents/guardians gave written informed consent to participate and the study was approved by the Hospital Universitário Professor Edgard Santos Ethical Committee.

Cytokine determination – The levels of interferon-γ, TNF-α, IL-5, and IL-10 were measured in culture supernatants of peripheral blood mononuclear cells (PBMC) from 21 patients with HAM/TSP and 38 HTLV-I asymptomatic carriers. Cytokine determination was performed in a seven-day period after neurological evaluation. Immunological studies were not performed in 16 patients with HAM/TSP because they were in use or had used immunosuppressors or immunomodulators in the previous 90 days of the enrollment visit. Briefly, PBMC, isolated from heparinized venous blood by lymphocyte isolation medium, were adjusted to $3x10^6$ cells per milliliter in RPMI 1640 plus 10% heat inactivated human serum AB Rh+. The cells were incubated at 37ºC in 5% CO_2 atmosphere for 72 hours without stimulus. Supernatants were collected and stored at –20ºC until use. Interferon-γ, TNF-α, IL-5, and IL-10 levels were measured by ELISA sandwich technique following instructions described by the manufactures (R & D system, Minneapolis, MN, USA). The results were expressed as picograms per milliliter based upon a standard curve generated using recombinant cytokines. To measure IFN-γ levels, the samples from HAM/TSP patients were diluted 5X because the levels were above the highest point of the standard curve.

Statistical analysis – Collected data were inserted in a data bank and analyzed with the help of a statistical package (SPSS version 9.0). The means and standard deviations (SD) of demographic data and cytokine levels were calculated for all the 21 patients with HAM/TSP selected for immunological evaluation, as well as for 38 HTLV-I asymptomatic carriers. Correlation among the cytokines levels, neurological scales, and time of disease evolution were made using the Spearman's correlation. Differences between the two groups were evaluated by a non-parametric Mann-Whitney *U* Test. A statistical significance was considered if $p<0.05$.

RESULTS

From the 237 HTLV-I positive individuals 51.1% were men. The overall mean age was 43±13 years. The 37 patients with HAM/TSP (15 men and 22 women) had 53±14 years mean age. The mean age of the 38 HTLV-I asymptomatic carriers (26 men and 12 women) was 42±11 years. The median EDSS of the 37 HAM/TSP patients was 4.0, ranging from 3.0 to 9.5. These patients also presented an OMDS ranging from 1 to 12 with a median of 4.0. The patients excluded from the immunological evaluation had a median EDSS of 4.0, ranging from 3.0 to 9.0, and a median OMDS of 5.0, ranging from 2 to 11.

All HAM/TSP patients presented pyramidal dysfunction as assessed by EDSS. Eighty-one percent had bladder dysfunction, 62% presented bowel impairment, 56% presented sensory alteration, and 51% complained of decreased libido or erectile dysfunction, or both. There was a high concordance between the neurological disability scores assessed by both scales (Fig 1). The patients in initial stages of neurological dysfunction with low grade of motor disability (OMDS=1) presented bladder compromise in 64%, bowel movement impairment in 33%, and sensory alterations in 11%.

There was also a correlation between the neurological disability evaluated by EDSS and duration of disease, as well as between the disability evaluated by OMDS and disease duration (Fig 2). Both groups of HAM/TSP patients and HTLV-I asymptomatic carriers had a high variability in IFN-γ levels (Fig 3).

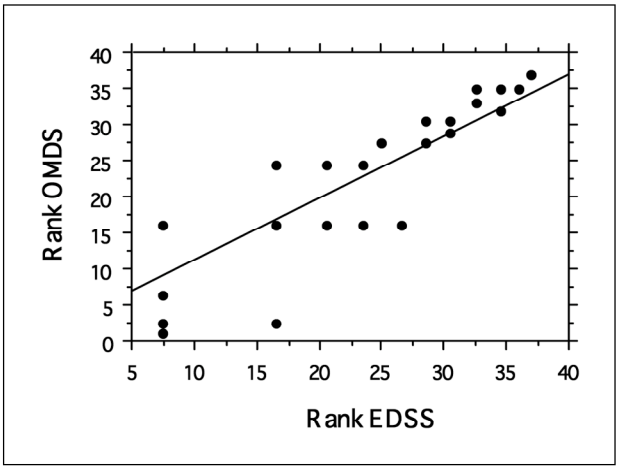

Fig 1. Correlation between the degree of neurological disabil - ity assessed by EDSS and OMDS of HAM/TSP patients (n=37); (r_s=0.857; p<0.001).

The mean (±SD) IFN-γ levels in myelopathy patients (4,456 ± 2,815 pg/ml, range: 375 to 10,750 pg/ml) was higher than IFN-γ levels observed in asymptomatic carriers (1,277±1,356 pg/ml, range: 0 to 6,995 pg/ml). To observe if these IFN-γ levels in PBMC did not reflect a transient situation, the levels were measured 2 months after the first evaluation in 05 HAM/TSP patients and in 05 asymptomatic HTLV-I carriers. No significant difference was observed between the mean (±SD) IFN-γ levels from the first to the second evaluation either in asymptomatic HTLV-I individuals (1,053±643 pg/ml and 1,403±896 pg/ml, respectively in the first and second evaluations; *p*=0.54) or in HAM/TSP patients (6,827±3,847 pg/ml and 6,846±1,374 pg/ml; *p*=0.69). No significant differences in other cytokine levels were observed between HAM/TSP patients and asymptomatic HTLV-I carriers: TNF-α levels (335±315 pg/ml versus 244±365 pg/ml; *p*=0.202), IL-5 levels (180±141 pg/ml versus 163±228

Table 1. Correlation between cytokines, evaluation scales, and duration of disease in HAM/TSP patients.

Cytokines	N° of cases	Spearman's *r*
IFN-γ / IL-5	21 / 15	0.560[a]
IFN-γ / IL-10	21 / 21	0.087
IFN-γ / TNF-α	21 / 17	0.304
TNF-α / IL-5	17 / 15	–0.043
TNF-α / IL-10	17 / 21	0.563[a]
IL-5 / IL-10	21 / 15	0.213

[a]p < 0.05.

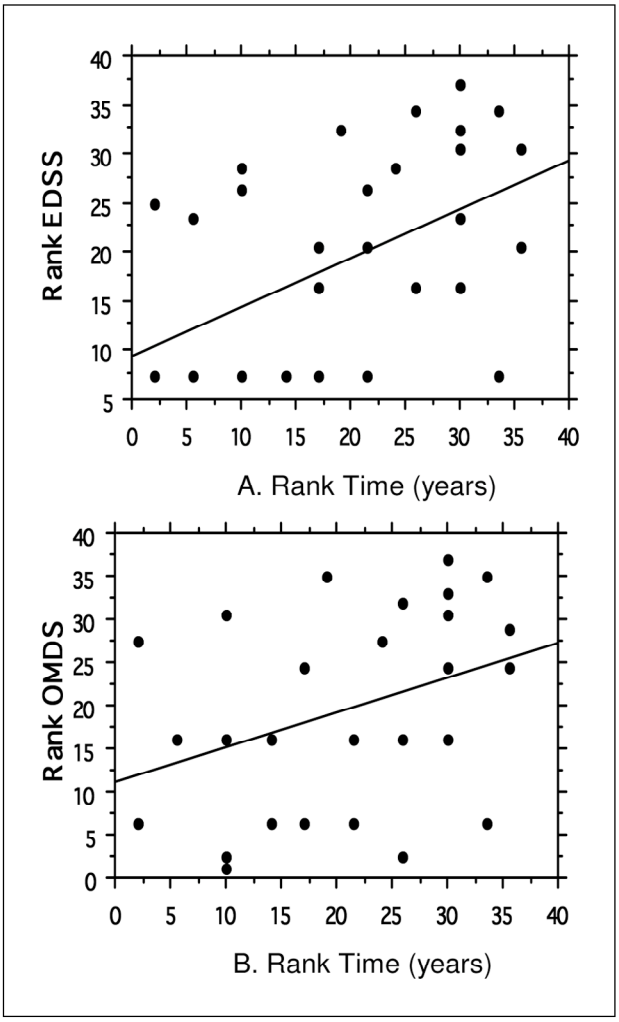

Fig 2. A) Correlation between the time of the initial signs of HAM/TSP and the degree of neurological disability assessed by EDSS from HAM/TSP patients (n=36); (r_s=0.511; p=0.001). B) Correlation between the time of the initial signs of HAM/TSP and the degree of neurological disability assessed by OMDS from HAM/TSP patients (n=36); (r_s=0.406; p=0.014).

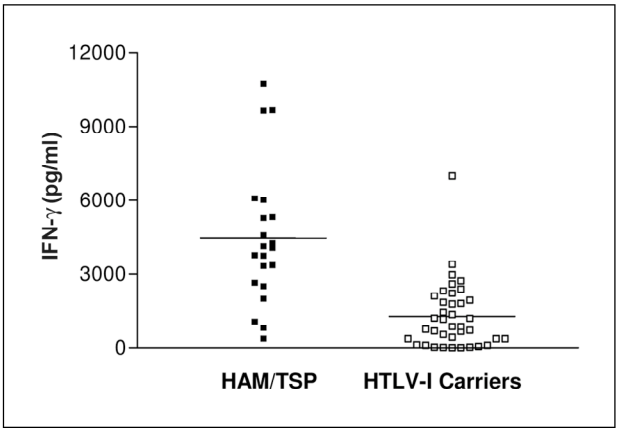

Fig 3. Interferon-γ levels in unstimulated peripheral blood mononuclear cells from HAM/TSP patients (n=21) as compared with asymptomatic HTLV-I positive individuals (n=38), p<0.0001; Mann Whitney U test.

pg/ml; *p*=0.113), and IL-10 levels (76±113 pg/ml versus 90±108 pg/ml; *p*=0.670).

We observed a correlation between IFN-γ and IL-5 levels and between TNF-α and IL-10 levels in HAM/TSP patients (Table 1). There was no significant correlation between IFN-γ, TNF-α, IL-5, IL-10 levels and the neurological disability or with the duration of illness in HAM/TSP patients.

DISCUSSION

The present study shows that although there is a similar proportion of males and females infected by HTLV-I, the frequency of females with HAM/TSP is higher than males (OR 3.178, 95% CI 1.23 – 8.20; p= 0.03). The HAM/TSP group present a high frequency of sensory alterations. The initial symptoms of HAM/TSP observed were bladder compromise, bowel movement impairment, and sensory alterations. Although these symptoms are unspecific, they should call the attention of clinicians to suspect and investigate HTLV-I infection and perform specialized neurological evaluation. The diagnosis might require a great degree of suspiciousness, especially in regions where HTLV-I infection is not endemic.

EDSS and OMDS are two scales broadly used presenting a good correlation with severity[10,13]. We extended this finding showing there is a positive correlation between the degrees of neurological disability assessed by the EDSS and OMDS. We also observed a positive correlation between each one of the functional incapacity scales and disease duration. Although this is a cross-sectional study, our data suggest a progressive worsening over time. However, it does not determine whether the disease progression was linear or fluctuating.

Despite many studies evaluating the HTLV-I immunological response in patients with HAM/TSP, few studies performed comparative analysis of the immunological response between HAM/TSP patients and asymptomatic HTLV-I carriers, as well as looked for the immune response in patients in different degrees of neurological disability and different periods of disease[14]. The large number of HTLV-I infected individuals followed at the HTLV-I Multidisciplinary Outpatient Clinic of the University Hospital in Salvador, has allowed, based upon neurological examination, the enrollment and evaluation of HTLV-I carriers and HAM/TSP patients.

This immunological evaluation shows that unstimulated PBMC from patients with HAM/TSP secrete higher amounts of cytokines such as IFN-γ and TNF-α as compared to HTLV-I asymptomatic carriers. Although IFN-γ synthesis was higher in patients with HAM/TSP, these responses were quite variable and in some asymptomatic carriers the IFN-γ production was similar to that found in patients with HAM/TSP, as also demonstrated in a recent publication[15].

There was no correlation between neurological disability and cytokine levels; on the other hand, there were elevated levels of helper T cell 1 (Th1) cytokines in PBMC with different degrees of neurological disability, suggesting a state of constant inflammatory activity (steady-state). However, there was a trend toward an inverse correlation of IFN-, TNF-, IL-10, and IL-5 levels in unstimulated PBMC with time of illness duration, showing a slight decrease of inflammatory response over time.

Among the evaluated cytokines, there was a trend of a positive correlation between IFN-γ and TNF-α, Th1 profile cytokines, as well as between IFN-γ and IL-5, and between TNF-α and IL-10. We have previously shown that a positive correlation between type 1 and type 2 cytokines may occur in HTLV-I carriers[6]. Herein we show this correlation also occurs in HAM/TSP patients. These correlations may reflect an attempt to modulate the proinflammatory response[16], once IL-10 is the main down regulatory cytokine of IFN-γ production[17]. Interleukin-10 has a suppressive effect over macrophages and T-cells cytokine production. It is also able to inhibit T-cells proliferation and to suppress macrophages activation mediated by IFN-γ[18], and suppresses TNF-α synthesis[19].

In combination, these results suggest a marked type 1 immune response with elevated IFN-γ as a marker of disease progression. However, in patients with already established neurological disease there is no association between severity of disease, cytokine levels, and illness duration. Since cellular activation and cytokine production are directly involved in the pathogenesis of HAM/TSP[8,20-23], prospective studies are necessary to trace the disease natural course. HTLV-I asymptomatic carriers with high production of IFN-γ should be conducted for a follow-up study to observe illness progression and assess early signs of neurological dysfunction as bladder compromise, bowel movement impairment, and sensory alterations.

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