
THROMBOSPONDIN-1 EXPRESSION IN HUMAN T-LYMPHOTROPIC VIRUS 1 ASYMPTOMATIC CARRIERS AND PATIENTS WITH HTLV-ASSOCIATED MYELOPATHY/TROPICAL SPASTIC PARAPARESIS

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ABSTRACT

Human T-lymphotropic Virus type 1 (HTLV-1) is associated with a myelopathy (named HTLV-associated myelopathy/Tropical Spastic Paraparesis–HAM/TSP). Thrombospondin-1 (TSP-1) is a matrix protein which interferes with cell adhesion, motility, and proliferation. The expression levels of the mRNA for this protein were evaluated in HTLV-1 infected individuals: 11 asymptomatic, 18 with myelopathy or oligosymptomatic, and 13 non-infected participants. RNA from peripheral blood mononuclear cells was submitted to TSP-1 RT-PCR analysis. The number of individuals expressing thrombospondin-1 was more frequent in the symptomatic group (14/18, $p=0.007$). In general, a tendency to higher values of the protein's mRNA was observed in the HTLV-1-infected group ($p=0.062$). The highest mRNA TSP-1 expression levels were detected at the beginning of the clinical symptoms of myelopathy. Additional studies with larger sample sizes are deemed to further elucidate the role of this matrix protein in the inflammatory network related to the HTLV-associated myelopathy/Tropical Spastic Paraparesis.

KEY WORDS: HAM/TSP. HTLV-1. Pathogenesis. Thrombospondin-1. Inflammation.

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RESUMO

Expressão de Trombospondina-1 em indivíduos infectados pelo HTLV-1 assintomáticos e pacientes portadores da mielopatia associada ao HTLV/Paraparesia Espástica Tropical.

O Vírus Linfotrópico de células T humanas tipo 1 (HTLV-1) está associado a uma mielopatia (chamada mielopatia associada ao HTLV - HAM/TSP). A trombospondina-1 (TSP-1) é uma proteína da matriz que interfere com a adesão, a motilidade, e a proliferação celular. Níveis de expressão de RNA mensageiro (mRNA) da trombospondina-1 foram avaliados em indivíduos infectados por HTLV-1: 11 pacientes assintomáticos, 18 com mielopatia ou oligossintomáticos, e 13 participantes não-infectados. O RNA de células mononucleares do sangue periférico foi submetido à análise de RT-PCR para trombospondina-1. O número de indivíduos que expressaram esta proteína foi maior no grupo com mielopatia/sintomas (14/18, $p = 0,007$). Em geral, a tendência para valores mais elevados de mRNA de trombospondina-1 foi observada no grupo de infectados pelo vírus ($p = 0,062$). Os níveis mais elevados de expressão do mRNA foram detectados no início dos sintomas clínicos da HAM/TSP. Estudos adicionais com maior número de amostras são necessários para elucidar melhor o papel desta proteína da matriz na rede inflamatória relacionada à HAM/TSP.

DESCRIPTORIOS: HAM/TSP. HTLV-1. Patogênese. Trombospondina-1. Inflamação.

INTRODUCTION AND OBJECTIVES

HTLV-1 is a retrovirus that is associated with two clinical entities, HTLV-1 Associated Myelopathy/Tropical Spastic Paraparesis - HAM/TSP and Adult T-cell Leukemia/Lymphoma – ATL (6). The majority of HTLV-1 infected individuals remain healthy carriers lifelong, suggesting that host and virus factors are involved in the outcome of HTLV-1 infection.

HAM/TSP is a chronic, neurodegenerative inflammatory disease more frequent in females (5). This demyelinating disease affects the spinal cord and white matter of the central nervous system (CNS). Symptoms include gait abnormalities, weakness and stiffness in the lower limbs, spasticity and; frequently, bladder and bowel dysfunction.

HAM/TSP pathogenesis involves host immune response and studies showed that HTLV-1-infected cells and Cytotoxic T Lymphocyte (CTL) could overcome the blood brain barrier and migrate from peripheral blood to CNS, inducing inflammatory response mediated by cytokines as IFN- γ and TNF- α , which causes damage to surrounding tissues (1). Immune cells migration to CNS depends on extracellular matrix (ECM) compounds. ECM functional molecules are a subset of components that appear only transiently in ECM during specific developmental or pathological events (3). Thrombospondin-1 (TSP-1) is a trimeric protein secreted by several cell types including endothelial and inflammatory cells. TSP-1 can be produced by and interact with T cells via $\alpha 4\beta 1$ integrins, CD47 and heparan sulfate proteoglycans (4). TSP-1 may interact with ECM and cell-surface receptors and promote or inhibit cell adhesion, motility, and proliferation. Considering the importance of peripheral immune cell migration events to CNS in the development of HAM/TSP and recent reports showing the involvement of TSP-1 in inflammatory

process, there could be an association between endogenous TSP-1 levels and HTLV-1 infection and progression to disease.

In order to evaluate TSP-1 as a marker of HTLV-1 infection progression, we have measured its messenger RNA (mRNA) in mononuclear cells from peripheral blood of HTLV-1 infected individuals presenting distinct clinical status, asymptomatic and with HAM/TSP, and compared the results with non-infected subjects.

MATERIALS AND METHODS

HTLV-1 infected individuals were classified in two groups: those with HAM/TSP or oligosymptomatic (HT), which means abnormal neurological exam for HTLV-1 without completing all the criteria for this myelopathy (n=18) and those who were asymptomatic carriers (AS, n=11). The criteria utilized for the neurological classification of the patients were those adopted by the ASIA scale for spine injury (7). The AS group represented HTLV-1 carriers with normal clinical and neurological examination. As controls, samples from a group of non-infected, seronegative individuals (NI, n=13) were tested. This control group was composed of healthy individuals, negative for all blood markers described below.

All subjects studied take part of the HTLV cohort study in Belo Horizonte, Minas Gerais, Brazil (Interdisciplinary HTLV Research Group - GIPH) and were negative to other blood-transmitted pathogens (HIV, hepatitis C and hepatitis B virus, syphilis and Chagas' disease).

Informed written consent was obtained from all subjects included in this study, which was approved by the institutional review boards of the participating institutions, Hemominas Foundation and Rede Sarah Hospital, Belo Horizonte, Minas Gerais, Brazil.

Blood samples (5 mL in ethylenediamine tetraacetic acid - EDTA) were collected and peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque gradient (Pharmacia Biotech Sweden). The accumulation level of TSP-1 transcripts was analyzed by RT-PCR. Total PBMC RNA was isolated using Trizol reagent (Invitrogen, USA), treated with DNase I (Invitrogen, USA). 10 mL of total RNA was reverse-transcribed (Reverse Transcription System - Promega, USA). Two ml of cDNA was used for PCR amplification, which was performed in 20 ml containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 3.5 mM MgCl₂, 200mM dNTP's, 0.5 U Taq DNA polymerase (Invitrogen, USA) and 2.5 pmol of primers TSP-F (5' -CACCAACAGCTCCACCAT-3') and TSP-R (5' -AGGTTGGCATCCTCGAT-3'). Forty cycles of 30s at 95°C, 45s at 50°C, and 1 min at 72°C were carried out in a thermal cycler (PTC-100, MJ Research, USA). b-actin was used as an internal control gene to normalize the expression of the target mRNA levels between different samples. PCR products were electrophoresed on 2% agarose gel and the image captured on UV light by ImageMaster[®] VDS

(Amersham). Analysis of densitometry was performed using TotalLab software version 2.00 (Amersham). The ratio of TSP-1 and b-actin densitometry in each sample represented the expression level of TSP-1 transcripts. Each PCR and electrophoresis procedure was repeated twice. ANOVA ONEWAY using PRISM[®] version 3.0 was used for statistical analysis. The means were compared using Mann Whitney test and a value of $p < 0.05$ was considered as of statistical significant difference.

RESULTS AND DISCUSSION

The gender and age distribution of the three groups studied are shown in Table 1.

Table 1. Gender and age distribution of the study participants according to the clinical status.

Groups	Gender distribution		Age range	Age, mean (SD)
	Female	Male		
Seronegative	4 (27%)	11 (73%)	20-56	34.27 (10.69)
Asymptomatic	4 (50%)	4 (50%)	19-56	39.63 (13.71)
HAM/TSP or other neurological symptoms	13 (61.9%)	8 (38.1%)	27-79	53.05 (12.22)

The level of TSP-1 expression in PBMC differed in the three groups evaluated as follows: 14/18 (77.7%) individuals of HT group, 6/11 (64.3%) of AS group, and 5/13 (38.5%) of NI group. The expression was more frequent in the HT group when compared to the seronegative group. No difference in the TSP-1 expression was found between HT and AS groups. When all HTLV-1-seropositive individuals were grouped, a difference in TSP-1 expression when compared to the seronegative group was observed ($p=0.01$). When the mean values of TSP-1 mRNA level were compared, similar levels were found in AS and HT groups. Comparisons of the TSP-1 levels means of the HTLV-1-seropositive individuals (0.23, SD=0.262) with the seronegative (0.04, SD=0.058) showed no statistical difference ($p=0.062$), although a statistical tendency could be considered (Figure 1). The highest mRNA TSP-1 expression levels were detected in three patients that were starting to have clinical symptoms of HAM/TSP (Figure 1, circled). In conclusion, TSP-1 mRNA level was shown to be higher in HAM/TSP patients.

Interestingly, the highest expression levels of TSP-1 mRNA, among all individuals tested, were observed in three individuals that presented clinical signals and symptoms indicative of incipient myelopathy, but not sufficient to be classified as HAM/TSP. This finding suggests that TSP-1 may play a role in the pathogenesis of HAM/TSP. This result is supported by previous reports of TSP-1 involvement in cell migratory potential and immunological cells activation in inflammatory

and degenerative disorders (4). Previous work by Cartier et al. (2004) showed that TSP-1 protein level was increased in cells from the cerebrospinal fluid in HAM/TSP patients.

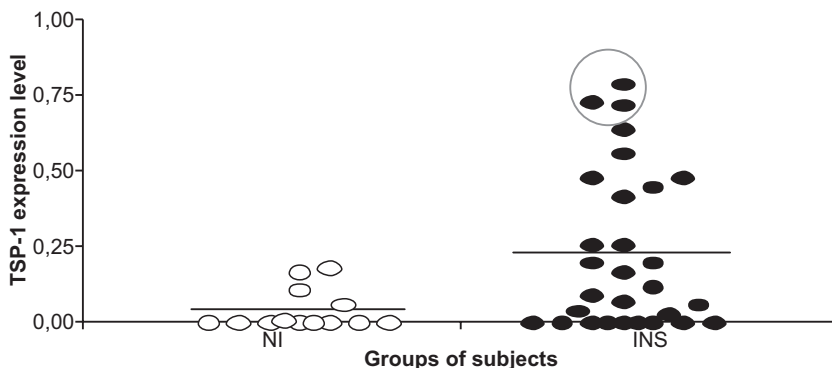


Figure 1. TSP-1 mRNA levels in PBMC from HTLV-1 infected (INF) and non-infected (NI) individuals. Horizontal lines show the mean values ($p < 0.05$). The median value was lower than the median (0 for NI and 0.12 for INF). Three infected oligosymptomatic individuals presented the highest expression levels (circle). GIPH Cohort, Brazil.

Although the time of diagnosis was known, the duration of the infection in these individuals could only be estimated from the possible route (s) of transmission detected during the interview (sexual, vertical, parenteral). Due to this uncertainty about the length of the presence of the virus in the organism, it was not possible to correlate the amount of time elapsed since infection with the results obtained.

The present work showed expression of TSP-1 mRNA in PBMCs from HTLV-1 seropositive individuals “ex-vivo”, without induction of cells in culture. More studies with larger samples of both infected and uninfected individuals are necessary to define TSP-1 role as a marker for clinical progression of HTLV-1 associated diseases, especially in patients in early stages of transition from acute to chronic disease.

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