Rev Soc Bras Med Trop 51(2):207-211, March-April, 2018
doi: 10.1590/0037-8682-0162-2017

Short Communication

The relationship between atopy and neurological manifestations in HTLV-1 infection

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Abstract

Introduction: Human T-cell lymphotropic virus type 1 (HTLV-1)induces exaggerated Th1 responses, whereas atopy is associated with exacerbated Th2 responses. **Methods:** Here, a cross-sectional study compared the prevalence of atopy in HTLV-1 carriers and HAM/TSP patients. It also compared the spontaneous cytokine production in HTLV-1-infected individuals. A retrospective cohort study evaluated the development of neurological manifestations in atopic and non-atopic carriers. **Results:** Atopic HAM/TSP patients with high IFN-γ production exhibited higher IL-5 levels than non-atopic patients. Allergic rhinitis accelerated the development of Babinski signals and overactive bladders. **Conclusions:** Abnormal Th1 and Th2 responses coexist in HTLV-1-infected individuals and allergic diseases may worsen the clinical course of HTLV-1 infections.

Keywords: HTLV-1. Atopy. Allergy. Immunology. Cytokines. HAM/TSP.

Human T- cell lymphotropic virus type 1 (HTLV-1) infection is characterized by T cell activation with T-helper 1 (Th1) cytokine overproduction[1]. It causes adult T-cell leukemia/lymphoma (ATLL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP)[2].The immune response is exacerbated in patients with HAM/TSP when compared to HTLV-1 asymptomatic carriers. They exhibit higher lymphocyte proliferation, overproduction of inflammatory cytokines, higher proviral loads, elevated levels of chemokines, and impaired modulation of the exaggerated immune response. HAM/TSP is characterized by lowerback pain, paresthesia in the inferior limbs, hyperreflexia, overactive bladder, Babinski signals and paraparesis[3-5].

Atopy is characterized by exacerbated production of T-helper 2 (Th2) cytokines [especially interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13)], B-lymphocyte proliferation, production of immunoglobulin E (IgE) and eosinophilia[6]. Immune responses are known to be modulated via the T cell activation routes, which consequently influence the clinical expression of chronic inflammatory diseases[6,7]. The frequency of skin reactivity to aeroallergens is lower in HTLV-1-infected individuals when compared to the uninfected individuals[8]. However, interferon gamma (IFN-γ) and IL-5 levels are observed to be higher in atopic HTLV-1 patient cultures stimulated with Derp-1 when compared to non-atopic patient cultures. In contrast, IL-10 levels are lower in the first group[8]. Thus, a decline in the production of IL-10, a cytokine that down regulates type1 and type 2 immune responses and prevents the development of chronic inflammatory diseases, may enhance type 1 and type 2 immune responses in atopic HTLV-1 patients. In such cases, HTLV-1-infected individuals, especially those with HAM/TSP, may present with atopic diseases.

It is unclear why most HTLV-1-infected individuals remain as carriers while others develop HAM/TSP. Patients with HAM/TSP are known to have higher proviral loads, T cell activation, and IFN-γ/IL-10 ratios than carriers[9]. Since a decrease in IL-10 is associated with atopy and HAM/TSP, we hypothesized that atopic HTLV-1 patients probably exhibited neurological manifestations more often. The present study aimed to evaluate whether atopy was associated with the development and severity of neurological manifestations associated with HTLV-1 and to compare the spontaneous production of cytokines in HTLV-1-infected individuals with and without atopy.

A cross-sectional study was conducted to compare the prevalence of atopy in HTLV-1 carriers and HAM/TSP patients and also to compare the spontaneously produced cytokine

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Received 21 April 2017
Accepted 17 November 2017

levels in HTLV-1- infected individuals. Participants in the study (enrolled from March 2011 to December 2012) included 46 HTLV-1 carriers and 45 patients with HAM/TSP. A retrospective cohort study was also conducted to compare the occurrence of neurological manifestations in 70 atopic and 79 non-atopic HTLV-1 carriers. For this study, we only included individuals that followed up for at least 3 years, since the admission at the Multidisciplinary Clinic. Babinski signals, overactive bladders, and HAM/TSP were considered to be neurological outcomes.

Participants were selected from the HTLV-1 Multidisciplinary Clinic at the *Complexo Hospitalar Universitário Professor Edgard Santos* in Salvador, Bahia, Brazil. HTLV-1 diagnosis was confirmed by detecting antibodies against viral antigens using enzyme-linked immunosorbent assay (ELISA) (CambridgeBi otechCorp.,Worcester,MA,USA) and Western Blotting (HTLV Blot 2.4; Genelab Diagnostics, Singapore). On admission and periodically, the laboratory analysis of patient samples included the determination of cytokines [interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), interleukin-5 (IL-5), and interleukin-10 (IL-10)] in the supernatants of unstimulated cell cultures. Every 6 months, these individuals were asked to answer a questionnaire and undergo physical and neurological exams in order to determine the presence of clinical manifestations associated with HTLV-1. All the data was entered into a database. Patients aged 18 years or older were included. We excluded patients with contraindications to prick-tests and other immune disorders. Patients with diabetes mellitus, neurological diseases, and neurological symptoms related to HTLV-1 at the beginning of the monitoring process in the cohort study were also excluded.

A questionnaire based on the International Study of Asthma and Allergies in Childhood (ISAAC) was provided to identify atopic related diseases[10]. Atopy was determined via a positive prick-test. The antigens in the prick-test panel included *Dermatophagoides pteronyssinus, Dermatophagoides farinae, Blomia tropicalis,* dog and cat epithelia*, Blattella germanica, Periplaneta americana*, and *Aspergillus* (ALK-Abello, Denmark).

The cytokine data was obtained by consulting the HTLV-1 Outpatient Clinic database. The cytokines levels considered for this study were the first determined in the database. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood samples via density gradient centrifugation using the Ficoll-Hypaque technique. A total of 3×10^6cells/mL were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) supplemented with 2mML-glutamine, 25mMHEPES, 10% heat-inactivated fetal bovine serum, and 0.05% gentamicin. Cells were incubated only with medium for 72 hours at 37°C in 5% CO_2. The IFN-γ, TNF-α, IL-5, and IL-10 levels in the supernatants of the unstimulated cultures were determined via ELISA using reagents purchased from BD Biosciences Pharmingen (SanJose, CA, USA)[11].

The Student's *t*-test was used to compare the age between the groups. The median cytokine levels in the unstimulated PBMC cultures, which did not follow a Gaussian distribution, were analyzed using the non-parametric Mann-Whitney *U* test. The association between the qualitative variables (gender, race, presence of atopy and atopic diseases, and neurological outcomes) was performed using the Chi-squared (χ^2) test. Disease-free survival until the development of neurological manifestations was estimated via the Kaplan-Meier method and presented as survival curves. The hypothesis test used to compare the survival distributions utilized the log-rank test. The Cox proportional hazards model was used to assess the association between atopy and the development of Babinski signals, overactive bladders, and HAM/TSP. Besides the main variable (presence of atopy), the covariates in the model included the presence of allergic rhinitis, asthma, and family history of atopy. The analysis was performed using the Statistical Package for the Social Sciences (SPSS) 17.0 software and the level of significance was set at 5%.

Ethical considerations

The study was approved by the Ethics Committee of the *Complexo Hospitalar Universitário Professor Edgard Santos, Universidade Federal da Bahia*. Written informed consent was also obtained from all the participants.

In the cross-sectional study, the demographic characteristics, prevalence of atopy, and cytokine levels in each group are shown in **Table1**. Patients with HAM/TSP demonstrated a 44.4% prevalence in atopy while HTLV-1 carriers demonstrated a 34.8% prevalence (p=0.346). The median cytokine levels in unstimulated PBMC cultures were compared between atopic and non-atopic individuals in both groups. In HTLV-1carriers, no difference was observed in IFN-γ (p=0.81), TNF-α (p=0.73), IL-10 (p=0.20), and IL-5 (p=0.56) concentrations between atopic and non-atopic individuals. Similarly, no difference was observed in IFN-γ(p=0.22), TNF-α(p=0.62), and IL-10 (p=0.95) concentrations between atopic and non-atopic individuals in patients with HAM/TSP. However, atopic patients with HAM/TSP exhibited higher levels of IL-5 (p=0.008, Mann-Whitney *U* test).

In the retrospective cohort, the demographic data, neurologic manifestation development, and mean of follow-up time in each group are shown in **Table 2**. During this study period, 10 individuals developed Babinski signals, 6 (8.6%) in the atopic HTLV-1 carrier group and 4 (5.1%) in the non-atopic HTLV-1 carrier group [hazard ratio (HR) =1.90; confidence interval 95% (95% CI)=0.55 to 7.13; p=0.29]. Forty-nine individuals developed overactive bladders, of which 24 (34%) were atopic and 25 (32%) were non-atopic (HR=1.15; 95%CI=0.65 to 2.02; p=0.62). The development of HAM/TSP occurred in 8 individuals, 5 (7.1%) in the atopic HTLV-1 carrier group and 3 (3.8%) in the non-atopic HTLV-1 carrier group (HR=2.24; 95% CI=0.53 to 9.45; p=0.27). No difference in the mean follow-up time between the 2 groups was observed (p=0.17). No difference was also observed between the time durations in developing Babinski signals (p=0.27), overactive bladders (p=0.61), and HAM/TSP (p=0.25).

Regarding the covariates included in the Cox regression, no association was found between asthma and family history

TABLE 1: Demographic features, prevalence of atopy and cytokine levels in HTLV-1 carriers and patients with HAM/TSP.

Features	HTLV-1carriers (n = 46)			HAM/TSP(n = 45)		
	n		%	n	%	p-value
Gender						
male	16		34.8	15	33.3	0.88*
female	30		65.2	30	66.7	
Age						
	52.4±12.46			54.7±14.13		0.41**
Race						
white	11		23.9	14	31.1	
mullatos	26		56.5	21	46.7	0.62*
black	9		19.6	10	22.2	
Prevalence of atopy	16		34.8	20	44.4	0.34*
Cytokine levels	Non-atopic	Atopic	p-value***	Non-atopic	Atopic	p-value***
IFN-γ	1,204	1,161		1,996	1,724	
	(98-1,759)	(433-1,548)	0.81	(853-3,021)	(810-2,151)	0.22
TNF-α	14	10		41	57	
	(0-98)	(0-308)	0.73	(0-273)	(7-118)	0.62
IL-5	2	10		11	54	0.008
	(0-26)	(0-107)	0.56	(0-52)	(29-234)	
IL-10	129	521		831	638	0.95
	(22-551)	(109-765)	0.20	(142-1,828)	(263-1,353)	

HTLV-1:human T-cell lymphotropic virus type 1; **HAM/TSP:** HTLV-1-associated myelopathy/tropical spastic paraparesis; **IFN-γ:** interferon gamma; **TNF-α:**tumor necrosis factor alpha**; IL-5:** interleukin-5**; IL-10:** interleukin-10**.** *Chi-squared test. **Student's *t*-test. ***Mann-Whitney *U* test. **Note:** Age (years) was presented as mean ± standard deviation and cytokine levels were expressed as pg/mL, median (interquartile range).

of atopy and the development of neurological manifestations (data not shown). While no association was observed between allergic rhinitis and the development of HAM/TSP (HR=2.66; 95%CI=0.51 to 13.79; p=0.24), an association was observed between the presence of allergic rhinitis and the development of Babinski signals (HR=9.09; 95%CI=1.11 to 74.07; p=0.03) and overactive bladders (HR=2.33; 95%CI=1.25to4.32; p=0.007) in HTLV-1carriers.The Kaplan-Meier method with the log-rank test for the development of overactive bladders and Babinski signals in HTLV-1 carriers with and without allergic rhinitis are shown in **Figure1A** and **Figure 1B**, respectively. We found that individuals with allergic rhinitis quickly developed Babinski signals (p=0.008) and overactive bladders (p=0.004).

HTLV-1 is characterized by an exaggerated type 1 immune response while atopy is mediated by an exacerbated production of Th2 cytokines. A previous study observed that the prevalence of atopy was lower among HTLV-1-infected individuals8; however, severe asthma was observed in patients with HTLV-1[12]. In this study, we found no difference in the prevalence of atopy among HTLV-1 carriers and patients with HAM/TSP, but found that allergic rhinitis in HTLV-1 carriers was associated with an early development of Babinski signals and overactive bladders.

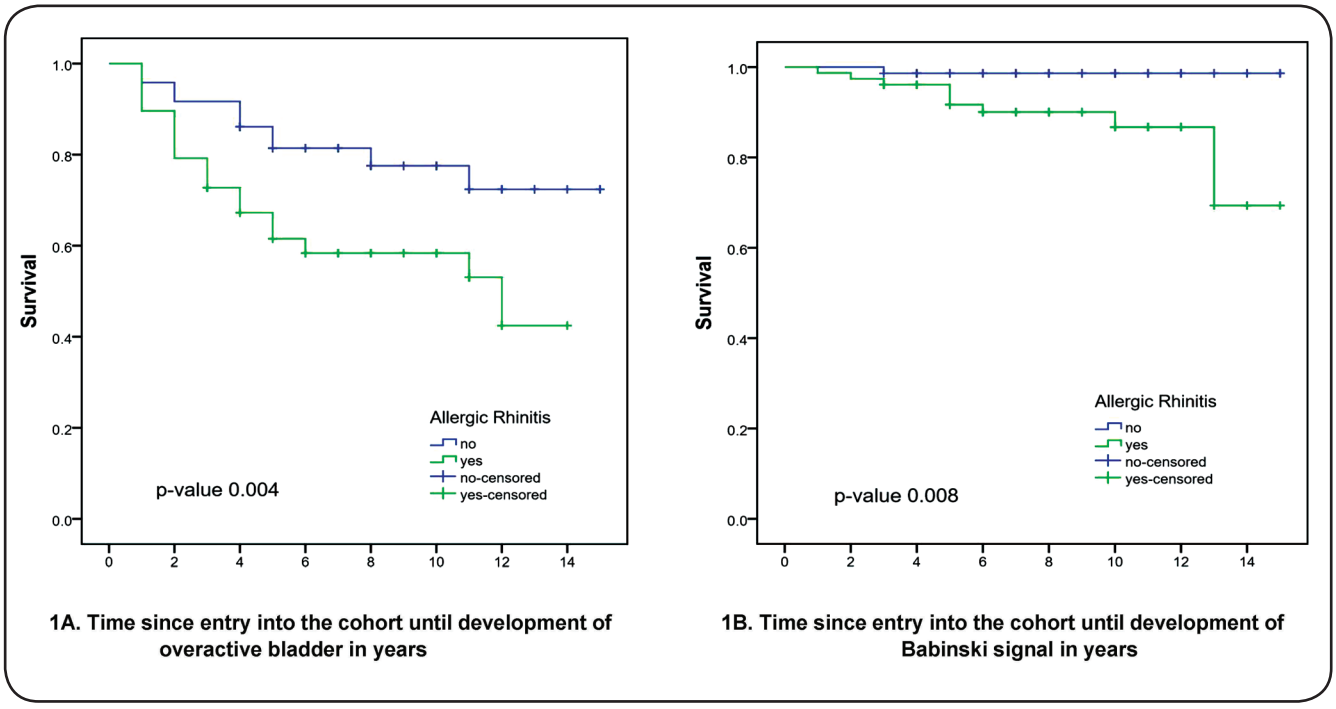
The spontaneous cytokine levels did not differ between atopic and non-atopic HTLV-1 carriers, and these results were similar to those previously documented in the literature[13]. There is no data in the literature that compares cytokine production in atopic and non-atopic HAM/TSP patients. Even though no difference in spontaneous IFN-γ, TNF-α, and IL-10 production between atopic and non-atopic HAM/TSP patients was observed in this study, increased spontaneous IL-5 production was observed in atopic HAM/TSP patients when compared to non-atopic HAM/TSP patients, both exhibiting high spontaneous production of IFN-γ. This shows that exaggerated Th1 response does not always have the ability to negatively modulate Th2 response and that high IFN-γ and IL-5 production can occur in patients with atopy and HAM/TSP.

Although the immune response modulation was impaired in atopic HTLV-1 carriers as these patients had lower IL-10 concentrations in cultures stimulated by Derp-1[13], we hypothesized that the frequency at which neurological manifestations developed would be higher in atopic HTLV-1 carriers than in non-atopic HTLV-1 carriers. Our data showed no statistical difference in the incidence of Babinski signals, overactive bladders, or HAM/TSP among the

TABLE 2: Demographic features, mean follow-up time, neurologic manifestation development, and time to outcome development in atopic and non-atopic HTLV-1carriers.

	Non-atopic(n = 79)		Atopic(n = 70)		
Features	**n**	**%**	**n**	**%**	**p-value**
Gender					
male	33	41.8	20	28.6	0.09**
female	46	58.2	50	71.4	
Age*					
	52.8±12.88		51±11.52		0.37***
Race					
white	21	27.3	16	23.9	
mullatos	32	41.6	27	40.3	0.81**
black	24	31.2	24	35.8	
Outcomes					
Babinski signal	4	5.1	6	8.6	0.29**
overactive bladder	25	32.0	24	34.0	0.62**
HAM/TSP	3	3.8	5	7.1	0.27**
Mean follow-up time(years)	8.95		8.27		0.17***
Time to outcome development(years)					
Babinski signal	14.4		13.6		0.27****
Overactive bladder	11.0		9.4		0.61****
HAM/TSP	14.5		14.1		0.25****

HTLV-1: human T-cell lymphotropic virus type 1; **HAM/TSP:** HTLV-1-associated myelopathy/tropical spastic paraparesis. *Mean and standard deviation.**Cox proportional hazard models. ***Student's *t*-test. ****Kaplan-Meier method.



1A. Time since entry into the cohort until development of overactive bladder in years

1B. Time since entry into the cohort until development of Babinski signal in years

FIGURE 1-1A. Overactive bladder and **1B.** Babinski signal (1B) development in HTLV-1 carriers with and without allergic rhinitis. **HTLV-1:** human T-cell lymphotropic virus type 1.

groups. Nevertheless, we observed that HTLV-1 carriers with allergic rhinitis developed early neurologic manifestations of myelopathy as Babinski signals and overactive bladders. Skin reactivity to histamine and aeroallergens was also observed to decline in HTLV-1 infected individuals[8]. This may explain why rhinitis, and not atopy, was associated with neurological manifestations as observed in this study.

One limitation of this study was the sample size, which may have influenced the statistical significance of the study. Atopy prevalence is possibly underestimated as HTLV-1 reduces positive histamine outcomes. There are a limited number of cohort studies that determine the incidence of neurologic manifestations in HTLV-1-infected individuals[14,15]. We observed that the development of HAM/TSP, overactive bladders, and Babinski signals occurred in 5.4%, 6.7%, and 33% of the HTLV-1-infected individuals (data not shown). We also found that the atopic disease possibly modified the clinical course of the HTLV-1 infections. Our data showed that not only did exacerbated Th1 and Th2 responses likely coexist in HTLV-1-infected individuals, but also that allergic diseases like rhinitis probably accelerated the development of neurologic manifestations in HTLV-1 carriers.

In conclusion, although there was no statistically significant association between atopy and the development of neurological manifestations in HTLV-1-infected individuals, we observed that allergic rhinitis accelerated the development of neurological manifestations as Babinski signals and overactive bladders in HTLV-1 carriers. We also observed that not only did exacerbated Th1 and Th2 responses coexist in HTLV-1-infected individuals, but also that allergic diseases probably interfered with the clinical course of HTLV-1 infection.

Conflict of interest

The authors declare that there is no conflicts of interest.

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