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Brain volume measurements in patients with human T-cell lymphotropic virus-1–associated tropical spastic paraparesis

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Human T-cell lymphotropic virus (HTLV)-1 is associated with a chronic progressive neurologic disease known as HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP) that affects 0.2% to 3% of HTLV-1–infected people. The authors aimed at exploring, *in vivo*, whether brain volume reduction occurs in patients with HAM/TSP through the use of magnetic resonance imaging (MRI). T1 pre/postcontrast spin echo–weighted images (WIs) and T2WIs of the brain were obtained in 19 HAM/TSP patients and 14 age- and sex-matched healthy volunteers. Both patients and healthy individuals were imaged at a 1.5-Tesla magnet by employing a conventional head coil. Focal T1 and T2 abnormalities were calculated and two measurements of brain parenchyma fraction (BPF) were obtained by using SIENAx (Structural Image Evaluation,using Normalisation, of Atrophy; University of Oxford, Oxford, UK) and MIPAV (Medical Image Processing, Analysis, and Visualization; National Institutes of Health, Bethesda, USA) from T1WIs. No significant differences in BPF were found between patients and healthy subjects when using either SIENAx or MIPAV. Analysis of individual patients detected that BPF was lower by 1 standard deviation (SD) relative to patients' average BPF in one patient. The authors conclude that reductions in BPF do not occur frequently in patients with HAM/TSP. However, the authors believe that one individual case of significant brain atrophy raises the question as to whether atrophy selectively targets the spinal cord of HAM/TSP patients or may involve the brain as well. A larger patient population analyzing regional brain volume changes could be helpful in determining whether brain atrophy is a marker of disease in patients with HAM/TSP. *Journal of NeuroVirology* (2006) **12,** 349–355.

Keywords: atrophy; brain; magnetic resonance imaging; spinal cord; tropical spastic paraparesis

Introduction

Human T-cell lymphotropic virus-1 (HTLV-1) infects approximately 10 to 20 million individuals worldwide (Jacobson, 2002). HTLV-1 is particularly prominent in Southern Japan, equatorial regions of Africa, Central and South America, and the Caribbean (Jacobson, 2002). HTLV-1 transmission follows three potential routes: (i) transmission from infected mothers to newborns via breast milk, (ii) sexual intercourse, (iii) and blood transfusion (Kannagi *et al*, 2004). Of those infected people, approximately 0.2%

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to 3% develop an inflammatory disease of the central nervous system (CNS) known as HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP), whereas the majority remain healthy asymptomatic carriers of HTLV-1 (Jacobson, 2002). Patients with HAM/TSP generally exhibit a greater frequency of HTLV-1–infected T cells in their peripheral blood mononuclear cells (PBMCs) and a higher immune response against HTLV-1 antigens compared to asymptomatic carriers (Yamano *et al*, 2002). Postmortem studies have shown inflammation and degeneration at the level of the spinal cord as the most relevant pathological features of HAM/TSP. However, focal pathology in the brain of HAM/TSP patients mirroring abnormal spinal cord alterations has been demonstrated in some postmortem studies (Aye *et al*, 2000).

Magnetic resonance imaging (MRI) is a highly sensitive tool for investigating and monitoring diseases of the CNS *in vivo*. MRI studies have indicated that HAM/TSP may affect patients at the level of the spinal cord and brain. The latter is in accordance with clinical findings suggestive of disability of either spinal cord structures or brain areas (Cruickshank *et al*, 1989; Fukushima *et al*, 1994; Osame, 1990). The incidence of spinal cord and brain focal lesions on MR images has been reported to be highly variable among the different populations examined (Howard *et al*, 2003; Godoy *et al*, 1995; Fukushima *et al*, 1994; Cruickshank *et al*, 1989; Kasahata *et al*, 2003; Melo *et al*, 1993; Bagnat *et al*, 2005). As a result, it is unclear to what extent focal abnormalities in either the spinal cord or brain can be considered as markers of disease duration and severity in patients with HAM/TSP. This finding could also suggest that focal lesions, though easily disclosed by brain and spinal cord MRI, may not embody the prominent pathological processes.

On the contrary, diffused reduction of CNS structures occurs frequently in patients with HAM/TSP as a result of chronic degenerative changes in white matter (WM) and gray matter (GM) tissues over time.

In vivo spinal cord volume measurements are still challenging due to the small diameter of the cord and the consequent poor resolution of spinal cord images. Measurements of brain volume changes (i.e., brain atrophy) have been applied extensively in patients with other CNS diseases. Brain atrophy computation is a sensitive and reproducible metric for monitoring tissue loss during the natural history and treatment phases of several diseases such as multiple sclerosis (MS) (Miller *et al*, 2002) and Alzheimer disease (Rusinek *et al*, 2004). Fuelled by previous hystopathological evidence showing that spinal cord disease mirrors brain pathology in HAM/TSP patients and by the absence of studies exploring whether a diffuse reduction in brain volume is present in patients with HAM/TSP, we aimed at investigating whether reduction in brain volume occurs in HAM/TSP patients. T1-weighted brain images (WIs) of 19 patients were acquired and brain volumes were computed and compared with a group of healthy volunteers matched for age and sex.

Two different segmentation techniques obtained from two independent laboratories were employed in order to strengthen the validity of our results and minimize possible bias given by the methodology used for measuring brain volumes, as suggested by an expert panel (Miller *et al*, 2002).

Results

Of the patient cohort, nine (47.4%) had abnormalities on the T2-WIs, and 10 (52.6%) patients had normal brain MRIs. Three (15.8%) patients exhibited T2 lesions in the spinal cord. No abnormalities were found on the images of healthy subjects. Details on findings from conventional brain and spinal cord MRIs of these patients have been previously reported (Bagnato *et al*, 2005).

Brain atrophy measurements

Results on normal brain volume (NBV) and brain parenchyma fraction (BPF) obtained through SIENAx (Structural Image Evaluation, using normalisation, of Atrophy) and MIPAV (Medical Image Processing, Analysis, Visualization) are summarized in Table 1. When using SIENAx, no differences ($t\,(31) = 1.150$, $P = .26$) were found between patients and healthy controls in NBV. There were also no differences ($t\,(31) = 1.229$, $P = .23$) in BPF between patients and healthy persons. Similar results were obtained when comparing BPF of patients and healthy subjects that were obtained using MIPAV ($t\ (31) = -0.447$, $P = .64$). The two software programs had a coefficient of variation of $\leq 0.04\%$ regarding intra patient and intravolunteer comparisons. Given these findings, subsequent statistical analyses were performed using brain volume outputs obtained from the SIENAx software exclusively.

MRI and clinical correlates of brain atrophy measurements

Correlations between age and NBV ($r = -.28$, $P = .25$) or BPF ($r = -.29$, $P = .23$) measures within patients did not reach statistical significance. A weak, but not significant, correlation was seen among healthy

Table 1 NBV and BPF in patients and HV

	Patients	*Healthy volunteers*
NBV (cc^3)	1507.8 ± 291.3	1,374.3 ± 375.9
BPF Sienax	0.86 ± 0.04	0.88 ± 0.02
BPF MIPAV	0.86 ± 0.02	0.85 ± 0.02
	Patients without T2 lesions	*Patients with T2 lesions*
NBV (cc^3)	1263.5 ± 398.9	1555.6 ± 124.9
BPF Sienax	0.86 ± 0.03	0.87 ± 0.03

volunteers for NBV ($r = -.46$, $P = .09$) but not BPF ($r = .09$, $P = .77$).

A slight trend (t (17) = 2.051, $P = .06$) indicating decreased NBV in patients without T2 lesions was observed when compared to patients with T2 abnormalities. No differences (t (17) = 0.803, $P = .43$) were found in terms of BPF between patients with and those without T2 lesions.

Analyses of an individual case

Although there was no significant change in BPF and NBV between patients and healthy controls, individual case analyses were performed. The latter revealed that one patient appeared to have significantly lower BPF, which could not be explained by the relatively young age of the patient. This patient was found to have a BPF of 0.79 (Figure 1). Such a measurement corresponded to the lowest value in the distribution of BPF measures in the entire cohort of patients and was lower than the average value of the patient cohort by 1 SD.

At 39 years of age, this Caribbean female was enrolled with an Expanded Disability Status Scale (EDSS) of 6.5 and disease duration of 2 years. The neurological impairments in this patient consist of severe paraparesis associated with impairment of the third cranial nerve and sensory loss. The proviral load at the time of the study was 10.3 copies of HTLV-1 per 100 cells. The brain MRI shows the presence of diffuse and confluent WM lesions along the course of the pyramidal tract and atrophy of the frontal and temporal lobes (see Figure 1). An age- and sex-matched healthy volunteer is shown in Figure 2 for comparison. The spinal cord MRI, performed at the time of the brain MRI, showed the presence of enhancements in many segments of the spinal cord. Those enhancements were no longer present on the spinal MRI performed 4 months later, although edema did not appear to be completely resolved by the second MRI.

Discussion

The purpose of the present study was to determine if diffused brain volume changes are present in patients with HAM/TSP compared to healthy volunteers as well as to assess whether the reduction in brain volume is an additional surrogate marker of HTLV-1–associated neurological disease duration

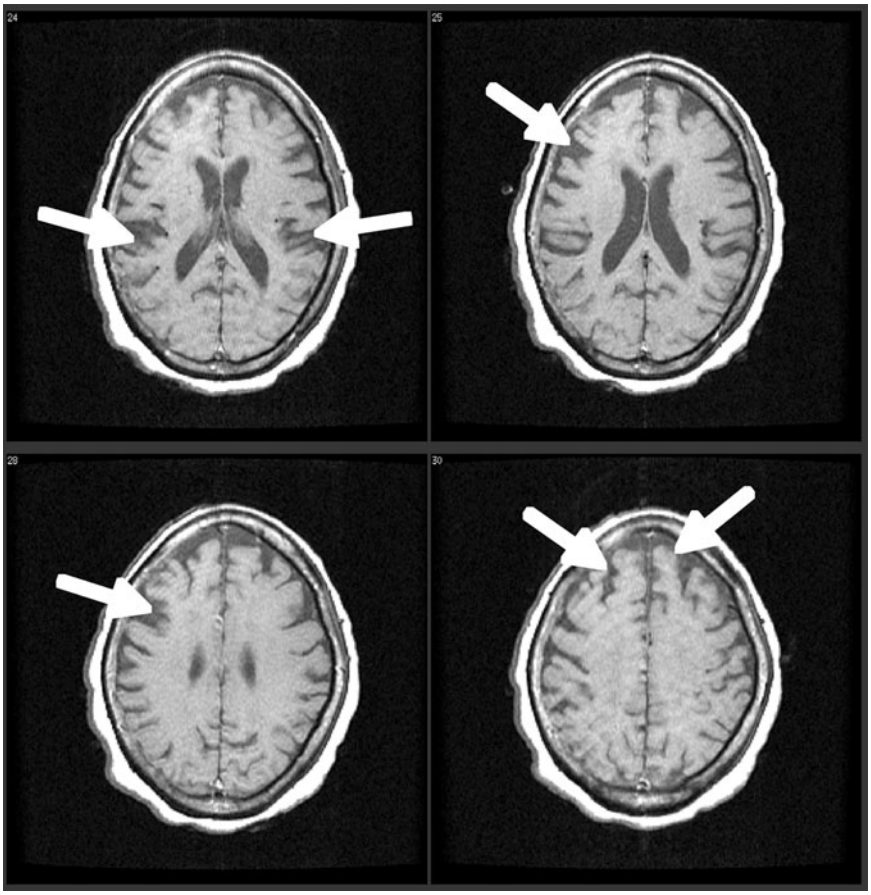

Figure 1 FLAIR images of patient with HAM/TSP depicting diffuse and confluent lesions around ventricles and along the pyramidal tract. Arrows highlight areas of decrease in brain parenchyma.

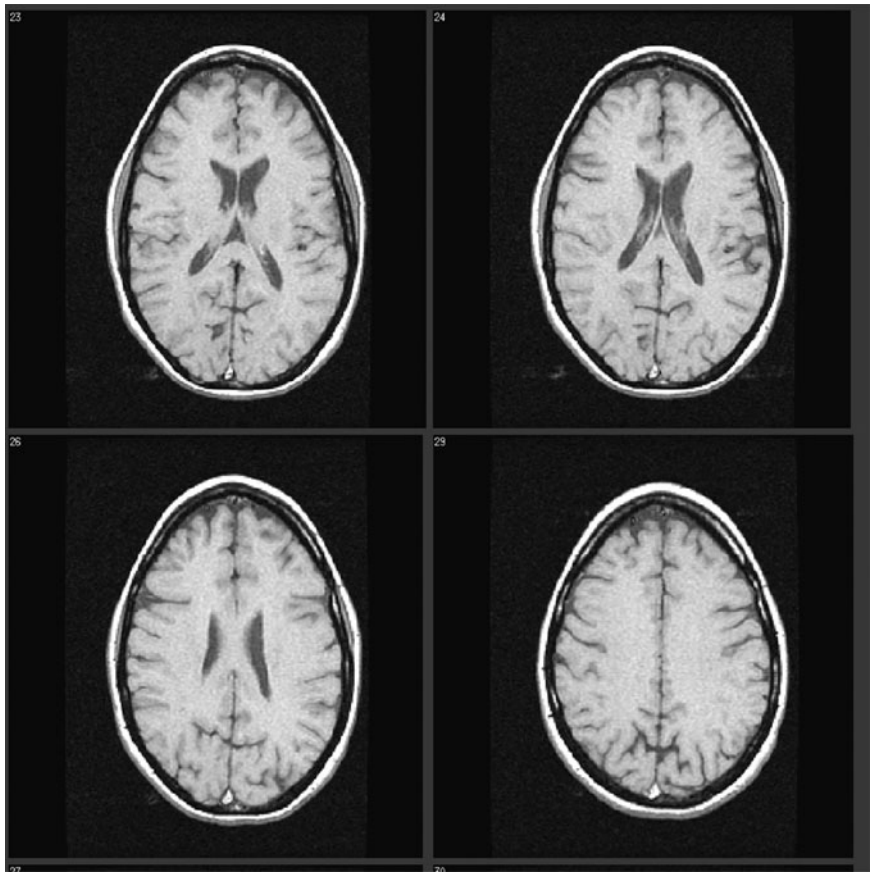

Figure 2 Brain atrophy appears to be absent in the FLAIR images of an age- and sex-matched healthy volunteer who corresponds with the patient in Figure 1.

and severity. We were unable to detect any differences in both NBV and BPF between our cohort of HAM/TSP patients and healthy persons. Additionally, a nearly significant trend indicated decreased NBV measures in patients without T2 abnormalities compared to those with T2 lesions, but no differences were found in BPF between the two groups of patients. Although such a trend is an intriguing observation, suggesting that focal pathology (as seen by lesions) and diffused disease (as measured by decrease in brain volume) do not parallel one another in HAM/TSP individuals, no definitive conclusions can be drawn given the small number of patients included.

The fact that brain volume reduction is absent or does not frequently occur in patients with HAM/TSP, unlike in patients with other CNS inflammatory-degenerative diseases, such as MS (Miller *et al*, 2002), is an important clinical observation. It is well known that MS may present clinical patterns similar to HAM/TSP, especially in its primary progressive form. Thus, the absence of BPF reduction in patients with HAM/TSP could provide an important tool in differentiating MS and HAM/TSP at the time of diagnosis. An additional distinguishing marker is that only one patient who showed a decrease in BPF did not exhibit any enlargement of the ventricles (as more frequently seen in MS), but rather volume reduction of the cerebral lobes and expansion of peripheral intracranial spaces. The cross-sectional nature of the study does not allow the authors to report any detailed information regarding the progression of brain tissue loss in such a patient and we believe that the latter is worthy of further investigations.

From one side, the observations of this study could be explained by assuming that HAM/TSP is a degenerative process selectively targeting the spinal cord and sparing the brain, even in those patients with focal T2 abnormalities. However, one can still postulate that regional brain volume changes not detectable by measuring global BPF still occurs in patients with HAM/TSP. Clinical observations suggest that HAM/TSP selectively affects the motor areas of the spinal cord, leading to a lower limb paraparesis in the majority of cases (Takenouchi *et al*, 2004). Similar selective regional processes may occur at the level of the brain. If this is true, studies computing the measurement of cortical thickness or volumes of specific brain regions rather than global measurement of the

brain size may be more sensitive in disclosing disease pathology and are certainly warranted. We believe that the latter would provide relevant insights in explaining the clinical disability of HAM/TSP patients, with particular regard to the occurrence of cognitive impairment affecting a small proportion of HAM/TSP patients (Osame, 1990).

Alternatively, however, one could still believe that decreases in BPF occur in HAM/TSP patients and that our study may have failed to depict this phenomenon. Interestingly, a significant reduction in brain volume was reported in one of our youngest patients. Both focal (i.e., T2 and T1 lesions) and diffuse CNS damages were demonstrated in this patient. Precisely, confluent WM lesions around ventricles and along the course of the pyramidal tracts were observed. In addition, transient edema and swelling were observed in the spinal cord. The BFP value of this patient was lower than the average value of the patient cohort by 1 SD. Such a reduction could not be explained by the young age of the patient. This isolated case raises the question as to whether global degeneration of the brain might occur in a subpopulation of patients with HAM/TSP. Although confirming that more than one pathogenetic mechanism may be associated with damage formation in the spinal cord and brain of patients with HAM/TSP, such an observation calls for further investigations in larger study cohorts.

To this regard, possible limitations that may confound results in this study need to be considered before drawing any definitive conclusion. Those include (i) small sample size, (ii) cross-sectional design of the study, and (iii) time at which patients were imaged. It is possible that significant differences in BPF between patients and healthy volunteers may have been found if a larger number of subjects were included in the study. We also believe that if small changes in brain volume occur in HAM/TSP patients, it may be difficult to detect through a single MRI. Yearly longitudinal follow-up of disease progression could provide better insight as to whether BPF decreases over time in HAM/TSP patients. Finally, possible technical limitations of the study might have also influenced the outcome of our results. In the future, three-dimensional (3D) volume anatomical rather than two-dimensional (2D) spin echo images may improve the validity of the present observations.

In conclusion, our preliminary findings suggest that global brain atrophy does not appear to be a common marker of disease in patients with HAM/TSP. Nevertheless, the assumption that brain atrophy does not occur in any patient with HAM/TSP cannot be unambiguously ruled out by our findings. Longitudinal studies in larger patient cohorts and analysis of regional areas of the brain may clarify the role of volume loss as a marker of disease and cause of disability for patients with HAM/TSP.

Materials and methods

Patients

The present study was conducted at the National Institutes of Health (NIH), Bethesda, Maryland. The National Institute of Neurological Disorders and Stroke Institutional review board approved the study and each patient and healthy volunteer signed an informed written consent. Patients with other concomitant systemic or CNS disease known to cause changes at the level of the brain volume were excluded from the present analysis. Administration of immunomodulatory or immunosuppressive drugs within 1 month prior to enrollment was an exclusionary criterion.

Nineteen patients with HAM/TSP according to World Health Organization criteria (Osame, 1990) and no other concomitant disease that might affect the brain and spinal cord MRI (Bagnato *et al*, 2005) were consecutively enrolled. Each patient underwent (i) a clinical examination by rating disability through the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) score, and (ii) HTLV-I proviral DNA load detection in the peripheral blood mononuclear cells (PBMCs) as measured by an ABI PRISM 7700 Sequence Detector (Applied Biosystems, Foster City, CA) (Yamano *et al*, 2002). Clinical and demographic characteristics of patients are summarized in Table 2. Fourteen age and gender matched healthy individuals were enrolled. Each healthy control underwent a clinical examination for ascertaining his/her status as normal. Brain MRI was performed for obtaining brain volume measurement and comparing it with patients.

MRI acquisition

All patients and healthy individuals underwent brain MRIs at the time of the clinical and biological evaluations. Concomitant spinal cord MRI was obtained in patients. A second brain or spinal cord MRI was obtained 4 to 6 months later in patients if contrast-enhancing lesions or edema were seen on the first examination. MRI examinations were preformed on a 1.5 Signa Unit (General Electric, Milwaukee, WI) with

Table 2 Demographic and clinical features of the patients' cohort

Gender	12 females/7 males
Age*	49.3 ± 10.9 years
Ethnicity	Caribbean (7), African-American (7), Caucasian (3), Latin (2)
Disease duration*	8.2 ± 5.5 years
PBL viral load*	25.3 ± 21.3 copy number of HTLV-1 per 100 cells
EDSS*	5.6 ± 1.9
Paraparesis/Spasticity	16 patients
Cranial nerves impairment	2 patients
Cerebellar impairment	13 patients
Sensory impairment	16 patients

*Mean ± standard deviation.

a standard quadrature head coil. Brain MRI sequences included (i) fast spin echo (FSE) (proton density/ T2-weighted images [T2WIs]) with a reception time (TR) of 2000 to 4000 ms and echo time (TE) of 20 to100 ms; (ii) fluid attenuated inversion recovery (FLAIR) with a TR of 10,000 ms, TE of 160 ms, and inversion time of 2000 to 2200 ms; and (iii) T1 SE WIs with TR of 400 to 600 ms and TE of 8 to16 ms, which was obtained before and within 15 min after intravenous (IV) administration of gadopentate dimeglumine (Magnevist, Berlex Labs, Cedar Knolls, NJ) at 0.1 mmol/kg. Axial contiguous slices of 3-mm thickness with 22 to 24 cm of field view were obtained for all the brain studies described above. Spinal cord MRIs included (i) routine sagital sequences of T1 and T2 pre- and postcontrast WIs on the entire spinal cord, and (ii) axial sequences as needed in case of an identified lesion along the spinal cord.

Image analysis

Each MRI was analyzed by one neurologist and one neuroradiologist who reached a final agreement in the detection of T2/T1 abnormalities on brain and spinal cord MRIs of patients and healthy individuals, as previously described (Bagnato *et al,* 2005). Images were then stored in dicom format, transformed into analyze format, and processed using a Linux computer. Brain volumes were computed from T1WIs obtained before contrast injection, and by using two different brain extraction and segmentation tools. We first used SIENAx (Structural Image Evaluation using Normalization of Atrophy), an automated procedure (Smith *et al,* 2001, 2002). SIENAx is incorporated in the FMRIB Software Library, Oxford University, United Kingdom (http://www.fmrib.ox.ac.uk/fsl). Its methodology has been previously described (Smith *et al,* 2001, 2002). All the segmented images were reviewed using MEDx 3.42 visualization and analysis software (Medical Numerics, Sterling, VA; www.medicalnumerics.com), and extracranial regions were manually deleted if still included in the mask. Normalized brain volume (NBV) and brain parenchyma fraction (BPF) were obtained from the mask of each individual.

A second calculation of BPF was performed on the same images using the MIPAV (Medical Image Processing, Analysis, and Visualization) platform developed by the Biomedical Imaging Research Services Section at the NIH (http://mipav.cit.nih.gov/). The brain surface extraction tool (BSE) in MIPAV, modeled after the original BSE (Shattuck *et al,* 2001), was used to extract the brain from nonbrain tissue on the T1WIs. Manual editing was required to delete regions of nonbrain tissue that the software was unable to identify. A fuzzy-connectedness segmentation was then used to segment GM, WM, cerebrospinal fluid (CSF), and background (Pham *et al*, 2000). BPF was calculated using the formula: BPF = (WM + GM)/(WM + GM + CSF) (Rudick *et al,* 1999; Pelletier *et al*, 2004).

Statistical analyses

Differences between patients and healthy individuals were analyzed using Fischer's Exact Test for gender and a two-sample independent *t* test for age. Differences in NBV and BPF between patients and healthy subjects as well as between patients with and without T2 abnormalities were analyzed using a two-sample independent *t* test. A coefficient of variance was obtained to measure intersoftware (SIENAx and MIPAV) variability in the computation of BPF, and was calculated as the standard deviation (SD) divided by the mean of the two measurements.

Correlations between NBV or BPF and age within patients' or the control group were performed using a Pearson's correlation test.

All reported *P* values were based on two-tailed statistical tests, with a significance level of .05. The statistical analyses were performed using SPSS version 12.0.

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