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ORIGINAL ARTICLE

Brain Atrophy in Clinically Isolated Syndrome

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KEYWORDS
Clinically isolated syndrome;
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Abstract
Introduction: Previous reports have shown that brain atrophy appears early in the course of multiple sclerosis (MS). The aim of the present study was to evaluate whether brain atrophy already exists in clinically isolated syndrome (CIS) by comparing with a control sample.
Methods: Patients with CIS were included prospectively from June 2008 to June 2009. A control group of healthy persons, matched by age and gender with CIS, was also included during the same period of time. An automated analysis tool, SIENAX, was used to obtain total brain volume (TBV), gray matter volume (GMV) and white matter volume (WMV). Mann-Whitney U test was used to analyze the data.
Results: Twenty CIS patients and 30 healthy controls were included (8 vs. 17 females, p=0.11). Mean age for CIS was 35±6 years vs. 34.4±5 in controls (p=0.61). Mean EDSS in CIS was 1.1±0.5. Eighteen patients with CIS (90%) had abnormal baseline MRI. The TBV in CIS was 1.6μl±0.22μl$\times 10^6$ vs. 1.65±0.15$\times 10^6$ in controls (p=0.005), the GMV in CIS was 0.58±0.05$\times 10^6$ vs. 0.67±0.03$\times 10^6$ in controls (p = 0.001) and the WMV in CIS was 1±0.1$\times 10^6$ vs. 1.12±0.02$\times 10^6$ in controls (p=0.03).
Conclusions: This is the first study dealing with brain atrophy in a CIS sample from Latin America in which brain atrophy, mainly grey matter atrophy, was shown in early stages of the disease compared with healthy individuals.

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PALABRAS CLAVE
Síndromes desmielinizantes aislados;
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SIENAX;
Esclerosis múltiple

Atrofia cerebral en pacientes con síndrome desmielinizante aislado

Resumen
Introducción: Estudios previos demostraron que la atrofia cerebral (AC) aparece precozmente en esclerosis múltiple. Es nuestro objetivo evaluar la AC en pacientes con síndrome desmielinizante aislado (SDA) respecto a un grupo control en una población argentina.
Métodos: Se incluyó prospectivamente a pacientes con SDA durante el período junio de 2008 a junio de 2009. El grupo control estaba formado por sujetos sanos apareados por edad y sexo. Se utilizó el programa SIENAX para medir el volumen cerebral total (VCT), el volumen de sustancia gris (VSG) y de sustancia blanca (VSB) en cada grupo. Los datos se compararon con la prueba de Mann-Whitney. Se consideró significativo $p < 0,05$.
Resultados: Se incluyó a 20 pacientes con SDA y 30 controles sanos (8 frente a 17 mujeres; $p = 0,11$). La media de edad en SDA fue 35 ± 6 frente a $34,4 \pm 5$ años en controles ($p = 0.61$). El EDSS de los pacientes con SDA fue $1,1 \pm 0,5$; 18 pacientes (90%) con SDA tenían lesiones en la resonancia magnética cerebral. El VCT en SDA fue $1,6 \pm 0,22$ µl $\times 10^6$ frente a $1,65 \pm 0,15 \times 10^6$ en controles ($p = 0,005$); el VSG en SDA fue $0,58 \pm 0,05 \times 10^6$ frente a $0,67 \pm 0,03 \times 10^6$ en controles ($p = 0,001$), y el VSB en SDA fue $1 \pm 0,1 \times 10^6$ frente a $1,12 \pm 0,02 \times 10^6$ en controles ($p = 0,03$).
Conclusiones: Éste es el primer estudio en una población latinoamericana con SDA que demostró atrofia cerebral con predomino en la sustancia gris, respecto a un grupo control. Esta herramienta es coste-efectiva para la medición de la AC, aspecto poco estudiado en nuestro medio.

Introduction

Multiple sclerosis (MS) is characterised by recurrent episodes of neurological dysfunction in 80%of patients. Most patients initially present a clinically isolated syndrome (CIS), characterised by a well-defined, neurological event lasting more than 24 hours and affecting the cerebral white matter, brainstem, spinal cord, cerebellum and optic nerves, among others, after ruling out other diseases.[1]

Traditionally, MS has been described as an exclusive disease of the white matter.[1] However, recent research has shown that along with the myelin damage, there is also significant axonal damage in the cerebral grey matter.[2,3] This has been demonstrated using advanced brain imaging techniques (magnetic resonance imaging [MRI]) and confirmed by histopathological studies. Consequently, these studies have become the pillars of the neurodegenerative hypothesis as a determinant factor of disease progression and disability in affected patients.[4-8]

While in MS there is clear evidence of early axonal loss and subsequent atrophy, this has not been sufficiently evaluated in patients after CIS.[9] Taking this into account, the objective of this study was to evaluate the volume of cerebral atrophy (CA) in patients with CIS in comparison with a control group of healthy individuals.

Methods

Cases

We prospectively included CIS patients evaluated at the *Sección de Enfermedades Desmielinizantes* (Department of Demyelinating Diseases) of the *Hospital Italiano* in Buenos Aires from June 2008 to June 2009. Inclusion criteria were: presenting a clinical syndrome indicative of central nervous system (CNS) demyelination affecting the optic nerves, brainstem, spinal cord or other regions, not attributable to other diseases. Patients who upon examination reported a history of focal neurological disorder that had lasted more than 24h were excluded. The initial clinical evaluation was performed in all cases by a neurologist with extensive experience in the management of demyelinating diseases, considering clinical, demographic and complementary studies. All patients underwent a cerebral and cervical spine MRI at 1.5-3 months of the event. This was performed using a Siemens 1.5 Tesla resonator with standard image acquisition techniques for patients with demyelinating diseases (proton density, conventional T2, FLAIR, T1 with and without intravenous contrast). The MRI scan was evaluated by neuroradiologists blinded to the study objective. We excluded patients who had received glucocorticoids in the month prior to the completion of the MRI.

Controls

The control group consisted of healthy subjects matched with CIS cases by age and gender, with no history of organic and/or functional neurological disorder. At least one control was identified for each patient. When controls were identified, they were invited to participate; upon acceptance and after obtaining their written informed consent, they were included in the study. The MRI protocol for controls was the same as for cases. Cases were paired with controls by frequency, selecting the controls who had equal age and gender as the selected cases.

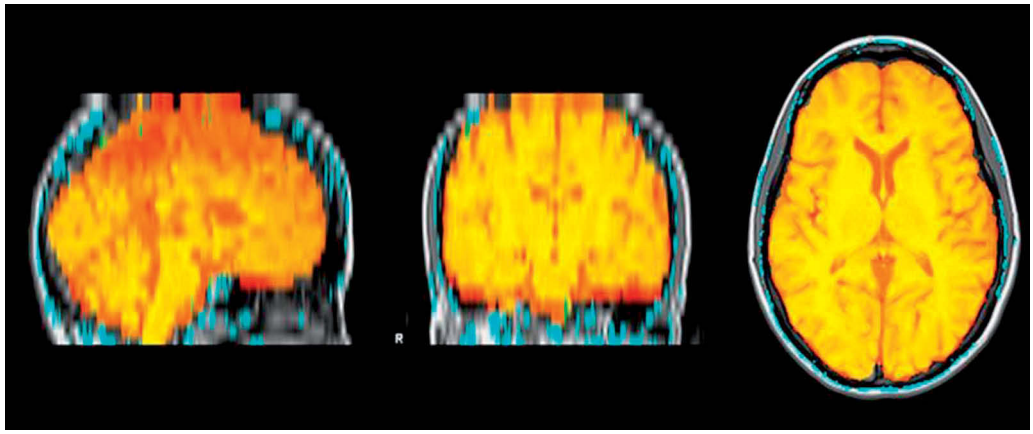

Figure 1 Brain extraction tool (BET), used to extract the brain and skull from magnetic resonance images.

Measurement of Cerebral Volume

Measurements of brain volumes (total brain volume [TBV], neocortical grey matter volume [GMV] and white matter volume [WMV]) were obtained using MRI T1 sequences, applying the automated method and the measurement software for cerebral atrophy, SIENAX.[10] To carry out the measurement, SIENAX used the brain extraction tool (BET), as part of the FSL-FMRIB software library, to extract the brain and skull content from the MRI images (fig. 1). Once extracted, a tissue segmentation program (FAST, another part of the FSL library software)[11] segmented the image obtained into neocortical grey matter, white matter and CSF, thereby estimating the segmented TBV, GMV and WMV.[16] SIENAX was thus able to obtain accurate, automated brain volume readings (fig. 2). The brain volume obtained was then multiplied by a pre-established normalisation factor incorporated into the software, which determined the final, normalised brain volumes for the patient (fig. 3).

The study was approved by the Ethics Committee for Research Protocols of the *Hospital Italiano* in Buenos Aires. All cases and controls gave their informed consent before entering the study.

Statistical Analysis

The baseline characteristics of the population studied were reported in percentages for categorical data, and in mean and standard deviation (SD) for dispersion measurement of continuous data. The data were compared using Fisher's exact test and the Mann-Whitney U test for categorical and continuous data, respectively. Values of $P<.05$ were considered statistically significant. To calculate the sample size, the formula for continuous data calculation was used, considering the estimate of the difference between two means, using the mean TBV difference of patients versus controls for that purpose. From previous data in patients with relapsing-remitting MS (RRMS) in early stages,[12] a p-value type I error of 0.05 and type II error of 0.8 were accepted. Considering the above, the sample size was estimated as at least 20 patients, with a minimum of 20 controls. Data collection and analysis were performed using the Stata 9.1 program.

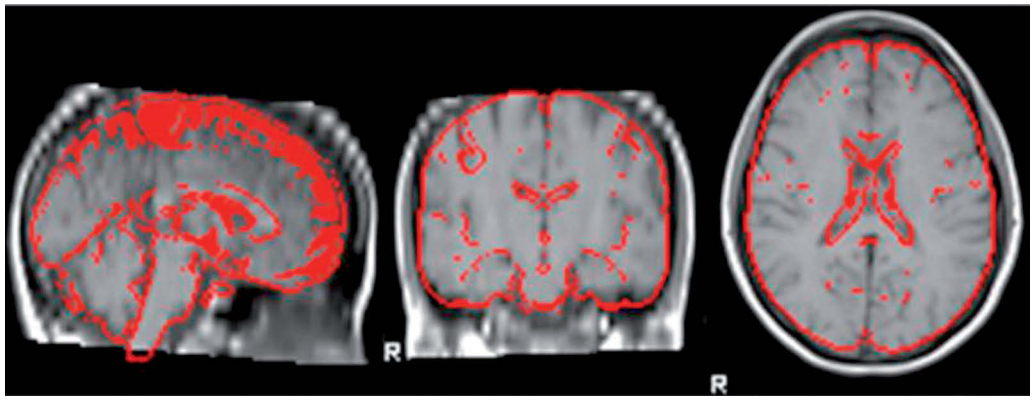

Figure 2 FAST tool, used to segment the image obtained by the brain extraction tool (BET) into neocortical grey matter, white matter and cerebrospinal fluid.

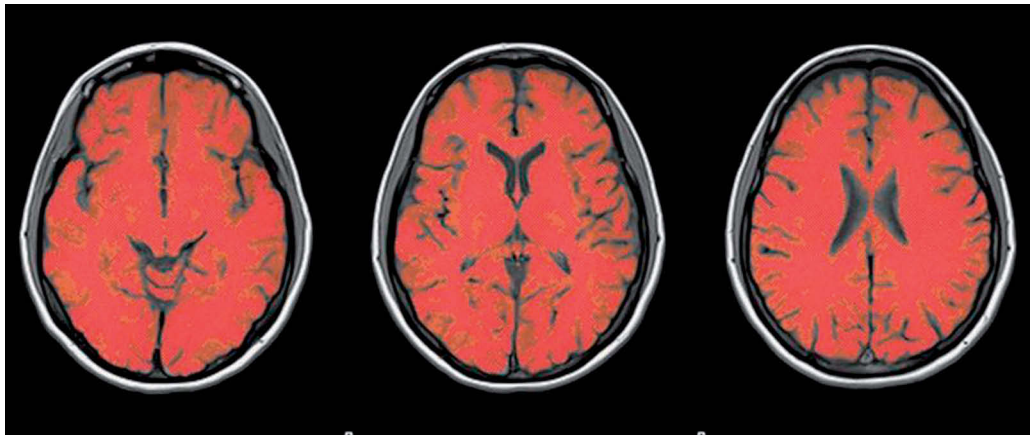

Figure 3 Final segmentation and measurement of final volumes.

Table 1 Brain volumes in patients and controls

	Cases (n=20)	Controls (n=30)	*P*
TBV ($\times 10^6 \mu l$)	1.6±0.22	1.65±0.15	0.005
GMV ($\times 10^6 \mu l$)	0.58±0.05	0.67±0.03	<0.001
WMV ($\times 10^6 \mu l$)	1±0.08	1.12±0.02	0.03

GMV: grey matter volume; TBV: total brain volume; WMV: white matter volume.
Data expressed as mean±standard deviation.

Results

The study included 20 patients with CIS and 30 healthy controls (ratio, 1:1.5). The mean age of CIS cases was 35±6 years; in controls, it was 34.4±5 years (*P*=.11). A total of 41%(8) of the CIS cases were female, compared to 58%(17) in the control group (*P*=.61). The clinical presentation of CIS was as follows: 8 (40%) with optic neuritis; 6 (30%) with cerebral parenchyma involvement; 4 (20%), brainstem; and 2 (10%) cervical spine. The extended disability status score (EDSS) of patients with CIS at the time of MRI was 1.1±0.5. On MRI, 90%(18) of patients with CIS presented demyelinating lesions in the brain and/or cervical spine. Of the 18 patients with MRI and lesions, 11 (61%) presented 2 Barkhof criteria (BC),[2] 6 (33%) presented 3 BC and only 1 (6%) patient presented 4 BC. With respect to the volumetric data, the TBV in patients with CIS was 1.6±0.22µl?10^6 versus 1.65±0.15?10^6 in controls (*P*=.005), the GMV was 0.58±0.05x10^6 in CIS patients versus 0.67±0.03?10^6 in controls (*P*=.001) and the WMV in CIS patients was 1±0.1?10^6 versus 1.12±0.02?10^6 in controls (*P*=.03) (table 1).

Discussion

The measurement of cerebral atrophy is a fundamental process that provides knowledge about damage to the cerebral parenchyma.[8,13] The present work shows the presence of CA, predominantly in the grey matter, in a population of patients with CIS compared to healthy controls.

While there are various reports in the literature describing the phenomenon of early CA in patients with MS,[3,12,14,15] there have been few studies to date that assess the phenomenon of atrophy at the time of CIS.

The exact mechanism and cause of this general and mainly neocortical atrophy are unknown. It is known that white matter inflammation by axonal transection leads to neuronal loss and consequent cortical atrophy.[3] However, it is difficult to argue that this mechanism causes the atrophy in the presence of scarce or possibly no inflammation and/or underlying white matter demyelination.[12]

Based on previous studies and our observation, it would appear that there are two mechanisms that cause the atrophy; the one described previously (secondary to demyelinating damage and axonal transection)[3] and another process (independent from the observable changes in the white matter and demyelination), which would explain why atrophy is scarce or nonexistent in the presence of inflammation.[12,15,16] The matter of elucidating the molecular mechanisms of this neurodegenerative process would still remain, but an apoptotic process induced via inflammatory mechanism could have a role in it.

Although atrophy was thought to begin with the clinical onset of the disease, this study and others have shown that CA may start to take place long before the indicative clinical event. Filippi et al[17] have used spectroscopy studies to demonstrate the presence of widespread, irreversible axonal damage independent of inflammatory lesions in the cerebral white matter at very early stages of the disease, long before the clinical onset. The consequent early brain atrophy measured by quantitative techniques, such as that found in the present report, could therefore be expected.

It is important to highlight the possible role that certain disease-associated factors that could adversely affect CA and generate an even greater rate of progression would have in each patient. Among these, it has been postulated that some genetic factors would lead to a higher rate of atrophy. Some studies have pointed to the relationship between APOE-Â4 and a higher rate of CA; however, the

association found in the various studies analysed was weak and occasional.[7,18,19] Another factor associated with a higher rate of CA has been the high number of brain lesions identified in the white matter from the beginning of the disease; however, this association has not been completely demonstrated yet.[20]

In conclusion, this is the first study in a Latin American population to explore atrophy in patients with CIS. The early atrophy identified in this population compared to the control group takes place mainly in the grey matter, with little inflammatory white matter demyelination. This suggests that an independent neurodegeneration mechanism other than axonal transection would be acting from the initial moments of the disease (even much earlier than the clinical onset) and progressively damaging the cerebral parenchyma. Future research will help to clarify the molecular mechanisms that intervene in this process and the prognostic value of the finding.

Congress

It should be noted that this work has been submitted at only the Argentine Congress of Neurology in Mar del Plata 2009, not at the SEN, and has not received any funding.

Conflict of interests

The authors declare no conflict of interests.

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