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Structural sex differences at disease onset in multiple sclerosis patients

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Abstract

Background: Male sex is associated with worsening disability and a more rapid progression of multiple sclerosis (MS). This study analysed structural sex differences in magnetic resonance images of the brain, comparing women whose disease started before and after the menopause with a control group of men.

Methods: This was a case control study in which female patients whose MS started before (Group 1) and after (Group 2) the menopause were included. The control group was matched by age, disease duration, Expanded Disability Status Scale and disease-modifying treatment. Patients were analysed according to demographic and clinical variables, as well as in terms of radiological measurements at disease onset and during the first 12 months of follow-up. These measurements included normalised total brain volume (NTBV), normalised cortical volume (NCV), normalised white matter volume, left and right hippocampus, the thalamus, brain stem volume, lesion load and percentage brain volume change. A linear regression model was used to analyse the data.

Results: A total of 97 patients were included: 53 in Group 1 (27 females) and 44 in Group 2 (22 females). In Group 1, we observed a reduction in brain volume in males compared with females at disease onset in NTBV ($p = 0.01$), NCV ($p = 0.001$) and brain stem volume ($p = 0.01$). We did not observe differences in Group 2 at disease onset in the brain volumes analysed.

Conclusion: We observed structural sex differences in brain volume at disease onset in the pre-menopausal group. However, no structural differences were observed at disease onset between the sexes after the menopause had started.

Keywords

Multiple sclerosis, sex differences, structural

Introduction

Multiple sclerosis (MS) is a complex demyelinating disease of the central nervous system in which several environmental factors act together in a genetically susceptible individual to cause disease.[1] Over the past years, several studies on MS epidemiology have reported differences in the frequency and the clinical course of the disease between men and women.[2–4] These studies have shown a higher incidence of the disease in women and a more aggressive course (reaching the milestones of disability measured by the Expanded Disability Status Scale (EDSS) at an early stage) in men.[5] The observations have led to a consideration of the male sex as a marker of worse prognosis of the disease.[5] Despite these observations, recent studies have shown that the differential in the clinical course in women compared with men occurs if the disease begins before menopause as a possible consequence of hormonal regulation, but that if the disease starts after menopause, the course is similar in both sexes.[4] These studies have focused on the evaluation of clinical sex differences, but there is scant information regarding differences in the structural magnetic resonance images (MRI) between the sexes.

Consequently, we designed the present case control study to analyse structural differences in brain MRI at disease onset, comparing women who started their disease before and after the menopause with a control group of men.

Methods

Setting

The study was conducted at the MS Center of the Italian Hospital of Buenos Aires, Argentina, between June 2010

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and June 2015.[6] The study was approved by the local ethics committee (internal number 1492) and written informed consent was obtained from all patients and subjects before they were included in the study.

Patients with a MS diagnosis according to validated criteria were included in the study.[7–10] Female patients were split into two groups: those whose MS disease started before (Group 1) and after (Group 2) the menopause was defined. Menopause was defined as the permanent cessation of the menstrual cycle in women for at least 12 months, and the condition was defined by a trained physician in the field. The onset of MS was defined as the appearance of the first signs/symptoms suggestive of central nervous system demyelination involving the optic nerve, brainstem, cerebellum or spinal cord not attributable to other diseases. The initial clinical evaluation and follow-up was performed in all cases by a neurologist with expertise in the management of demyelinating diseases. Female patients were excluded if they were undertaking hormone replacement therapy, and if they had prior exposure to mitoxantrone or cyclophosphamide in order to minimise potential confounding effects of iatrogenic menopause. We included a male control group for female patients who had not started the menopause as well as for the group of female patients who had started the menopause. Each control group was matched by age, disease duration, EDSS and disease-modifying treatment. Finally, to be included, all patients must have had at least one brain MRI within the first 60 days of the first demyelinating event and after 30 days of steroids (if used) and a second MRI after 12 ± 3 months of the first MRI in order to perform longitudinal brain volume changes.

MRI assessment

A brain MRI was performed on a 1.5 Tesla machine with a standard head coil (Siemens Avanto) within two months of the first demyelinating event. The MRI study included images obtained in the following sequences: T1-weighted conventional spin-echo; T2-weighted fast spin-echo; FLAIR spin-echo; T1-weighted conventional spin-echo after a single dose of gadolinium (0.1 mg/kg); and 3D-T1 image sequence (MPRAGE). All images had a section thickness of 3 mm, an intersection gap of 0.3 mm with $256 \times 256 \times 170$ acquisition matrix and a voxel size of $1.25\,\text{mm} \times 1.25\,\text{mm} \times 1.2\,\text{mm}$. 3D T1-weighted image sequence parameters were: 176 partitions; flip angle 15°, 1.2 mm slices, matrix size 256×256, voxel size $1\,\text{mm} \times 1\,\text{mm} \times 1\,\text{mm}$ after interpolation, repetition time (TR) 1900 ms; echo time (TE) 4.0 ms; inversion time (TI) 300 ms, FOV $192\,\text{mm} \times 192\,\text{mm}$. The MRI software and hardware did not change during the study period.

MRI post-processing

Lesion volume (LV) analyses were assessed by an experienced neuroradiologist unaware of each subject's condition and were subsequently reviewed by one MS specialist who was blinded to the formal reading. Labelling of T2-W and T1-W LV was then performed by employing a semi-automated segmentation technique based on user-supervised local thresholding (SepINRIA software).[11] The value of both T2-W and T1-W total brain LV was calculated by multiplying the lesion area by the slice thickness.

Brain volumes were measured on 3D-T1 image sequence (MPRAGE) by using the cross-sectional version of the Structural Image Evaluation using Normalization of Atrophy (SIENA) software, SIENAX, which forms a part of FSL.[12] SIENAX allows global measurements of normalised total brain volume (NTBV) as well as selective measurements of normalised cortical volume (NCV) and normalised white matter volume (NWMV).[13] Normalisation was done for skull size. To avoid misclassification due to WM lesions, this was masked out and refilled with intensities matching the surrounding normal-appearing WM (NAWM) before each tissue-class segmentation analysis.[14,15]

The volume of the hippocampus (HV), brainstem (BV) and thalamus (TV) was estimated using FIRST software (FMRIB's Integrated Registration and Segmentation Tool) incorporated into the FSL library (www.fmrib.oxlibrary.ac.uk).[12] The longitudinal percentage of brain volume change (%BVC) between the first MRI and the second MRI performed 12 ± 3 months later was done by using SIENA.

Statistical analysis

The population was separated into those female patients whose disease began before the menopause with their corresponding male control group (Group 1) and those female patients whose disease began after the menopause with their corresponding male control group (Group 2). Baseline characteristics of the sample evaluated were reported as percentages for categorical data and means with SDs for continuous data. Data were compared between groups (females and males) using Fisher's exact test and Mann–Whitney's *U*-test for categorical and continuous data, respectively. A linear regression model was used to analyse the entire data. *p*-Values of < 0.05 were considered significant. The collection and data analyses were performed using Stata v10.1.

Results

A total of 97 patients were included, of whom 48 (49.4%) were women. When patients were separated into the two groups, MS had started in 26 females before the menopause, and it has started after the menopause in 22 women (Groups 1 and 2, respectively). A total of 27 and 22 males were included in Groups 1 and 2, respectively, in order to provide sex comparisons. The average time between the onset of disease

and performing the baseline MRI was 45 ± 12 days (range 25–58 days). No significant differences in demographic and clinical variables were observed between the groups and sexes (Table 1). Regarding the structural analysis of MRI at disease onset, differences between men and women in Group 1 in the NTBV ($p = 0.01$), NCBV ($p = 0.001$) and BT ($p = 0.01$) were observed without other differences. A trend without significant differences was observed regarding %BVC in Group 1 between males and females ($p = 0.09$). No significant differences were observed between men and women in Group 2 (Table 2).

Discussion

Our study showed structural differences at disease onset between women whose disease started before menopause and the control group of men, while no differences were observed between the sexes in patients whose disease began after the menopause.

The results of this study are in line with recent cross-volumetric findings in patients with MS. Antulov et al.[16] showed a pronounced commitment of NTBV and NCBV in men compared with women matched by age and disease onset. Another recent study that evaluated clinical aspects between men and women before and after the menopause found no differences in disability as measured by the EDSS, relapse rate and the rate of atrophy measured longitudinally. However, that study did not assess more sensitive quantitative aspects of neurodegeneration.[17] Klistorner et al. also showed sex-specific differences in the progressive change of lesional diffusivity, mainly an increase in parallel and perpendicular diffusivity during the 12-month follow-up in males compared with female RRMS patients.[18]

A possible explanation of our finding is the possible role that sex hormones may have on immune response.[16,17] Oestrogens may mediate neuro-inflammatory signals and protect against neurodegeneration. In other inflammatory diseases such as systemic lupus erythematous and rheumatoid arthritis, the onset of the menopause has been associated with a modification of the disease course, presumably by

Table 1. Clinical characteristics of included patients at study entry.

	Before menopause started (Group 1)			After menopause started (Group 2)		
Variable	Males	Females	*p*	Males	Females	*p*
n	27	26		22	22	
Age at onset, years ± SD	34 ± 2.9	35.4 ± 3.5	0.27	52.1 ± 3.1	53.4 ± 1.1	0.17
RRMS, *n* (%)	27 (100)	26 (100)	1	12 (54.5)	11 (50)	0.82
PPMS, *n* (%)	0	0	–	10 (45.5)	11 (50)	0.75
EDSS	2 ± 0.5	1.7 ± 1	0.23	4 ± 1	3.5 ± 1.1	0.32
Positive oligoclonal bands in CSF, *n* (%)	26 (96)	24 (92)	0.68	22 (100)	21 (95)	0.64
Disease-modifying treatment, *n* (%)	27 (100)	26 (100)	1	14 (63.6)	13 (59)	0.19

RRMS: relapsing remitting MS; PPMS: primary progressive MS; EDSS: Expanded Disability Status Scale.

Table 2. Comparison of magnetic resonance imaging (MRI) variables among groups. Statistically significant values are shown in bold.

	Before menopause started (Group 1)			After menopause started (Group 2)		
Variable	Males	Females	*p*	Males	Females	*p*
n	27	26		22	22	
NTVB $\times 10^6$ mm^3, mean ± SD	1.56 ± 0.22	1.66 ± 0.2	**0.01**	1.47 ± 0.11	1.48 ± 0.8	0.31
NCV $\times 10^6$ mm^3, mean ± SD	0.60 ± 0.09	0.66 ± 0.05	**0.001**	0.52 ± 0.03	0.51 ± 0.09	0.21
NWMV $\times 10^6$ mm^3, mean ± SD	1 ± 0.12	1.1 ± 0.09	0.1	0.89 ± 0.02	0.92 ± 0.05	0.1
HV left (cm^3), mean ± SD	3.9 ± 0.1	4.1 ± 0.06	0.12	3.4 ± 0.01	3.6 ± 0.03	0.04
HV right (cm^3), mean ± SD	4 ± 0.09	4.1 ± 0.08	0.13	3.5 ± 0.08	3.7 ± 0.05	0.06
BV (cm^3), mean ± SD	17.6 ± 0.2	18 ± 0.5	**0.01**	15.6 ± 0.4	16 ± 0.49	0.11
TV right (cm^3), mean ± SD	7.9 ± 0.07	8 ± 0.2	0.09	7.4 ± 0.04	7.6 ± 0.23	0.2
TV left (cm^3), mean ± SD	8 ± 0.1	8.1 ± 0.2	0.13	7.4 ± 0.1	7.5 ± 0.3	0.86
Lesion load (mm^3), mean ± SD	1252 ± 452	1324 ± 331	0.22	1548 ± 526	1489 ± 478	0.54
%BVC	−0.46 ± 0.05	−0.40 ± 0.03	0.09	−0.62 ± 0.08	−0.60 ± 0.05	0.10

NTVB: normalized total brain volume; NCV: normalized cortical volume; NWMV: normalized white matter volume; HV: hippocampus volume; BV: brainstem volume; TV: thalamus volume; %BVC: percentage of brain volume change.

modulating immunological activity.[3,17,19] Hormone replacement therapy has been associated with modulating effects on some neurological and inflammatory conditions, but this has not been evaluated in depth in MS.[20] The protective effect against neurodegeneration by oestrogen has been demonstrated in animal models, but the exact mechanism that could lead to this effect has not been defined. The role of testosterone should also be mentioned. Physiological levels of testosterone have been shown to be protective in EAE, since the castration of young male mice leads to the disease worsening,[21,22] while supplemental testosterone treatment has shown to be protective in gonadally intact male mice.[21] These results suggest that a mechanism of disease protection conferred by endogenous testosterone exist. However, the exact role in humans is not completely understood.

Important limitations of our study are the observational design and the low number of patients included. However, on balance, the strict inclusion criteria and the automated evaluation of the study variables reduce the possibility of bias and confounding factors.

In summary, our study shows the presence of structural sex differences when MS occurs before the menopause, while no differences were observed between the sexes when MS began after the menopause. The differences may be mediated by hormonal factors and could explain the difference between men and women in terms of clinical course and prognosis. Future studies will help to confirm our initial observations and establish the possible role that sex hormones could have on the evolution of the disease.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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References

1. Marrie RA. Environmental risk factors in multiple sclerosis aetiology. *Lancet Neurol* 2004; 3: 709–718.
2. Confavreux C, Hutchinson M, Hours MM, et al. Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in Multiple Sclerosis Group. *N Engl J Med* 1998; 339: 285–291.
3. Voskuhl RR and Gold SM. Sex-related factors in multiple sclerosis susceptibility and progression. *Nat Rev Neurol* 2012; 8: 255–263.
4. Bove R, Musallam A, Healy BC, et al. No sex-specific difference in disease trajectory in multiple sclerosis patients before and after age 50. *BMC Neurol* 2013; 13: 73.
5. Confavreux C and Vukusic S. Age at disability milestones in multiple sclerosis. *Brain* 2006; 129: 595–605.
6. Cristiano E, Patrucco L, Giunta D, et al. Incidence of multiple sclerosis in Buenos Aires: a 16-year health maintenance organization-based study. *Eur J Neurol* 2010; 17: 479–482.
7. McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001; 50: 121–127.
8. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the 'McDonald Criteria'. *Ann Neurol* 2005; 58: 840–846.
9. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011; 69: 292–302.
10. Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol* 1983; 13: 227–231.
11. INRIA. SepINRIA software, http://www-sop.inria.fr/asclepios/software/SepINRIA/ (2011, accessed 15 January 2016).
12. FSL. FMRIB software library, www.fmrib.ox.ac.uk/fsl/ (2015, accessed 15 January 2016).
13. Smith SM, Zhang Y, Jenkinson M, et al. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. *Neuroimage* 2002; 17: 479–489.
14. De Stefano N, Stromillo ML, Rossi F, et al. Improving the characterization of radiologically isolated syndrome suggestive of multiple sclerosis. *PLoS One* 2011; 6: e19452.
15. Vrenken H, Jenkinson M, Horsfield MA, et al. Recommendations to improve imaging and analysis of brain lesion load and atrophy in longitudinal studies of multiple sclerosis. *J Neurol* 2013; 260: 2458–2471.
16. Antulov R, Weinstock-Guttman B, Cox JL, et al. Gender-related differences in MS: a study of conventional and nonconventional MRI measures. *Mult Scler* 2009; 15: 345–354.
17. Spence RD, Hamby ME, Umeda E, et al. Neuroprotection mediated through estrogen receptor-alpha in astrocytes. *Proc Natl Acad Sci USA* 2011; 108: 8867–8872.
18. Klistorner A, Wang C, Yiannikas C, et al. Progressive injury in chronic multiple sclerosis lesions is gender-specific: a DTI study. *PLOS ONE* 2016; 11: e0149245.
19. Gold SM and Voskuhl RR. Estrogen and testosterone therapies in multiple sclerosis. *Prog Brain Res* 2009; 175: 239–251.
20. Tomassini V and Pozzilli C. Sex hormones: a role in the control of multiple sclerosis? *Expert Opin Pharmacother* 2006; 7: 857–868.
21. Palaszynski KM, Loo KK, Ashouri JF, et al. Androgens are protective in experimental autoimmune encephalomyelitis: implications for multiple sclerosis. *J Neuroimmunol* 2004; 146: 144–152.
22. Bebo BF Jr, Zelinka-Vincent E, Adamus G, et al. Gonadal hormones influence the immune response to PLP 139-151 and the clinical course of relapsing experimental autoimmune encephalomyelitis. *J Neuroimmunol* 1998; 84: 122–130.