T2 Hypointensity in the Deep Gray Matter of Patients With Multiple Sclerosis

A Quantitative Magnetic Resonance Imaging Study

Rohit Bakshi, MD; Ralph H. B. Benedict, PhD; Robert A. Bermel, BA; Shelton D. Caruthers, PhD; Srinivas R. Puli, MD; Christopher W. Tjoa; Andrew J. Fabiano, BS; Lawrence Jacobs, MD

Context: While gray matter T2 hypointensity in multiple sclerosis (MS) has been associated with physical disability and clinical course, previous studies have relied on visual magnetic resonance imaging (MRI) assessments.

Objective: To quantitatively determine if T2 hypointensity is associated with conventional MRI and clinical findings in MS.

Design: Case-control study.

Setting: University-affiliated community-based hospital.

Subjects: Sixty patients with MS and 50 controls.

Main Outcome Measures: T2 intensities of the substantia nigra, red nucleus, thalamus, putamen, globus pallidus, and caudate; third ventricular width; total brain T1 (hypointense) and T2 (hyperintense) lesion volumes; Expanded Disability Status Scale (physical disability) score; and disease course.

Results: Deep gray matter T2 hypointensity was present in patients with MS in all structures (P<.005) except for the substantia nigra. T2 hypointensity was associated with third ventricle enlargement and higher T2 but not T1 plaque load. The regression model predicting third ventricle width included caudate T2 hypointensity (P = .06). The model predicting T2 lesion load included globus pallidus T2 hypointensity (P = .01). Caudate T2 hypointensity was the only variable associated with disability score in regression modeling (P = .03). All T2 hypointensities differentiated the secondary progressive from the relapsing-remitting clinical courses. The final model (P<.001) predicting clinical course retained T2 hypointensity of the thalamus, caudate, and putamen but not MRI plaques or atrophy.

Conclusions: Gray matter T2 hypointensity in MS is associated with brain atrophy and is a stronger predictor of disability and clinical course than are conventional MRI findings. While longitudinal studies are warranted, these results suggest that pathologic iron deposition is a surrogate marker of the destructive disease process.

Arch Neurol. 2002;59:62-68

Multiple sclerosis is increasingly thought of as a globally destructive disease process. Pathologic and positron emission tomography imaging studies indicate that cortical and subcortical gray matter involvement is common in MS. Recently we showed that hypointensity on T2WI (purported iron deposition) occurred commonly in the subcortical gray matter of patients with MS and was associated with physical disability, disease duration, disease course, brain MRI lesion load, and brain atrophy. These and other studies of T2 hypointensity in MS used qualitative (visual) rating systems that limited the findings. In the present study, we performed a computer-assisted quantitative MRI study of T2 hypointensity in patients with MS and controls. We compared the degree of T2 hypointensity with
SUBJECTS AND METHODS

SUBJECTS

Sixty patients clinically confirmed to have MS and 30 controls were scanned with the same MRI unit at a tertiary care facility. None of the patients with MS had other major medical illnesses, were younger than 20 years or older than 60 years, used corticosteroids within 4 weeks, or had a history of substance abuse. Forty-two patients had the relapsing-remitting and 18 had the secondary progressive MS clinical disease course. Physical disability was assessed by the Expanded Disability Status Scale (EDSS) within 1 week of the MRI by a single experienced neurologist blind to the MRI findings. Scores ranged from 0 to 8.0 (mean ± SD, 3.7 ± 1.9). The duration of MS ranged from 0.5 to 38 years (mean ± SD, 10.6 ± 9.4 years). The average number of lifetime courses of high-dose intravenous methylprednisolone taken by all 60 patients with MS was 1.8. Four (7%) were receiving bi-monthly intravenous methylprednisolone for disease progression. Controls included normal volunteers recruited from hospital staff and consecutive patients referred to the MRI center for dizziness, headaches, and seizure disorder, who had normal neurologic and MRI findings. An experienced observer reviewed the scans of controls to ensure normal findings and discarded 3 controls due to the presence of bright lesions on T2-weighted images (53 control scans screened, 50 retained). Visual determination of gray matter T2 intensity was not used to exclude control scans. Patients with MS and controls were sex-matched (68% women and 70% women, respectively) and age-matched (mean ± SD age, 42 ± 9 years and 42 ± 10 years, respectively) (P > .05).

MAGNETIC RESONANCE IMAGING

Fast spin-echo T2WI (repetition time [TR]/echo time [TE]/number of signal averages [NSA]=2300/120/2; 6-mm slice thickness; 0.6 mm slice gap; echo train length 18), fast spin-echo fluid-attenuated inversion-recovery (FLAIR) images (TR/TE/NSA/inversion time = 8000/120/2/2200; 5-mm slice thickness; interleaved; echo train length 20), and T1-weighted images (TR/TE/NSA = 585/20/1, 5-mm slice thickness, interleaved) were obtained in the axial plane on an ACS-NT MRI scanner (Philips Medical Systems, Best, the Netherlands). The in-plane spatial resolution was approximately 1 × 1 mm. The FLAIR protocol was detailed previously. Images were transferred to Sun workstations (Sun Microsystems, Mountain view, Calif) on which images were analyzed quantitatively at the Buffalo Neuroimaging Analysis Center (Buffalo, NY). A trained observer who was blind to clinical information performed the quantitative MRI analysis. Based on a localization technique, standardized circular regions-of-interest (ROIs) were placed on T2WI in the substantia nigra pars compacta, substantia nigra pars reticulata, red nucleus, anterior thalamus, posterior thalamus, head of the caudate nucleus, and in the cerebrospinal fluid (CSF) of the right lateral ventricular body. To sample the structure while minimizing partial volume effects, the ROIs were 2-mm in diameter for the substantia nigra pars reticulata, pars compacta, and red nucleus and were 5 mm for the other structures. Since the lateral ventricular size varied among subjects, the largest circular ROI (not exceeding 5 mm) was placed in the ventricle without including the adjacent parenchyma or choroid plexus. Freehand ROIs were also manually traced for the putamen and globus pallidus. One axial slice was used for each measurement (the slice showing the largest part of the structure). Care was taken to avoid placing small hyperintensities (lesions or perivascular spaces) into ROIs. To correct for potential interscan variations in system scaling and gain, mean signal intensity in each ROI was divided by the mean signal intensity of lateral ventricular CSF as adapted from Pujol et al.21 Because T2 intensity was expressed as a ratio to the right lateral ventricular CSF, it is important to note that absolute right vs left CSF intensities did not differ in patients (P = .44) or controls (P = .26), and neither right (P = .44) nor left (P = .27) absolute CSF intensities differed between the groups. The data from the anterior and posterior thalami ROIs were averaged to produce a value for the thalamus. The data from the substantia nigra pars reticulata and pars compacta ROIs were averaged to produce a value for the substantia nigra. Separate measurements were taken from each hemisphere, which were then collapsed across both hemispheres by calculating the mean in each ROI (to reduce the number of statistical variables).

We assessed the validity of right/left collapsing of the variables and assessed the symmetry of the gray matter signal changes. The Pearson correlation r values between right and left T2 intensities were 0.92 for the caudate, 0.88 for the thalamus and putamen, 0.90 for the red nucleus, 0.87 for the substantia nigra, and 0.92 for the globus pallidus.

RESULTS

Univariate comparisons indicated that T2 hypointensity was widespread throughout the deep gray matter in patients with MS before and after adjusting for age, affecting the caudate, putamen, globus pallidus, thalamus, and red nucleus (P < .005) (Table). The magnitude of hypointensity was largest in the globus pallidus, caudate, and putamen (6% to 7% lower than controls, P < .001) (Table). Representative MRIs of T2 hypointensity in patients with MS vs controls are shown in Figure 2. The substantia nigra was the only structure that did not show abnormal T2 hypointensity in patients with MS compared with controls. Third ventricular width was larger in patients with MS (mean ± SD, 4.0 ± 2.4) than in controls (2.2 ± 1.0) (P < .05), indicating central atrophy in patients with MS.

Within the MS group, T2 hypointensities in deep gray matter structures were associated with higher third ventricle width and higher T2 lesion volume. The regression model predicting third ventricle width included T2 hypointensity of the caudate (partial r with age = −0.36; P = .006; R2 = 0.23). The model predicting total T2 lesion load included T2 hypointensity of the globus pallidus (partial r with age = −0.41; P = .001; R2 = 0.18). In
Paired sample t tests showed no significant right vs left difference for the caudate, thalamus, and red nucleus. While the putamen, substantia nigra, and globus pallidus showed significant right vs left differences in T2 intensities, the effect sizes were quite small: 0.3 or less (effect sizes = difference between means divided by pooled SDs). Thus, in general, the T2 intensity in the gray matter ROIs showed symmetry in the MS group.

After collapsing the ROIs, a total of 6 ROIs were used for further analysis (substantia nigra, red nucleus, thalamus, caudate, putamen, globus pallidus). The typical ROI placement in a patient with MS is shown in Figure 1. The last spin-echo T2 sequence was used in the analysis of T2 hypointensity. Previous studies analyzing iron deposition have used conventional spin-echo to detect T2 shortening. In this study, we used fast spin-echo T2 because of the faster scanning time and increased clinical utility in the evaluation of patients with MS (see “Comment” section).

The total T2 hyperintense parenchymal plaque lesion load was determined by manual tracing of lesions on FLAIR images and was the sum of the volume of each lesion seen on each FLAIR axial slice (area multiplied by slice thickness, non-gapped images); artifacts and other normal hyperintensities seen in the normal population on FLAIR images were avoided. To assess central atrophy, third ventricular width was measured from FLAIR images using our previously established method. The analysis of gray matter and CSF T2 intensity, T2 hyperintense lesion volume, and third ventricular width was performed using EasyVision software (Release 2.1.2; Philips Medical Systems, Best, the Netherlands). Since hypointense T1 lesions are difficult to delineate manually, the analysis of T1 lesion volume was performed using a semiautomated edge finding and local thresholding technique (Java Image, Version 1.0; Xinapse Systems, Leicester, England [http://www.xinapse.com]). The operator clicks on the edge of hypointense area and the program examines a region 5 pixels around the mouse click and computes the maximum intensity gradient within the region. The pixel with the highest intensity gradient is then used as the starting point for contour following, thus outlining the region where the intensity is locally lower than at the starting pixel.

RELIABILITY

The same individual reanalyzed the MRI scans of 10 randomly chosen patients with MS at least 2 weeks after the initial analysis. Intraobserver coefficients of variation for the T2 intensity measurements ranged from 1.0% to 2.7% as follows: caudate, 1.2% (right)/1.6% (left); anterior thalamus, 2.1%/1.2%; posterior thalamus, 1.5%/2.2%; red nucleus, 1.4%/1.8%; substantia nigra-pars reticulata, 2.4%/2.7%; pars compacta, 1.3%/1.8%; putamen, 1.3%/1.1%; globus pallidus, 1.0%/1.0%; ventricular CSF, 1.2%. The interobserver coefficients of variation were 1.2% for total T2 hyperintense parenchymal lesion volume, 1.7% for total T1 hypointense parenchymal lesion volume, and 5.7% for third ventricular width. A second trained observer analyzed the same 10 patients for gray matter and CSF T2 intensity; the interobserver coefficients of variation ranged from 0.6% to 2.9%. To test the stability of the T2 intensity measurement technique, 2 healthy volunteers, aged 26 (man) and 31 (woman) years, each underwent the MRI protocol twice (1 week apart). The scan/rescan intrasubject coefficients of variation for the various gray matter intensities ranged from 1.2% to 2.9%.

GROUP DIFFERENCES

Group differences were assessed by independent sample t tests. The Pearson r statistic was used for correlations between continuous variables and the Spearman rank correlation test was used to compare continuous data with ordinal ratings. We used a conservative threshold for statistical significance (P < .01) in all univariate comparisons and controlled for multiple correlations via regression models. Within the MS group, linear and logistic regression models were used to predict conventional MRI findings (total hyperintense parenchymal lesion volume, third ventricular width) and clinical parameters (EDSS, disease duration, relapsing-remitting [secondary progressive] clinical course) using only T2 intensity measures as predictors that differed significantly between patients with MS and controls (ie, abnormal T2 hypointensity). All models controlled for age by entering age in block 1 and holding age in the final model. Otherwise, each model used a forward stepwise selection procedure, with P to enter .05 and P to exit .10. Two types of analyses were performed. First, regression models with individual gray matter ROI T2 intensities were fitted to detect significant predictors of total T1 or T2 parenchymal lesion volume, third ventricular width, or clinical parameters while controlling only for age. Second, total T1 and T2 parenchymal lesion volume and third ventricular width were added to the clinical parameter models.

contrast, T2 hypointensities were not significantly associated with T1 lesion load.

The regression model predicting disease duration retained only T1 lesion load (partial r with age = .32; P = .01; R² = .43). When third ventricular width, T1, and T2 lesion load were removed from the analysis, there were no significant T2 hypointensity predictors retained in the model.

Within the MS group, Spearman rank correlations between EDSS score and MRI variables were as follows: caudate, r = −.26; thalamus, r = −.01; red nucleus, r = −.03; putamen, r = −.05; globus pallidus, r = −.10; substantia nigra, r = 0.23; whole-brain hyperintense T2 lesion volume, r = 0.23; whole-brain hypointense T1 lesion volume, r = 0.20; third ventricular width, r = 0.30. Caudate T2 hypointensity was retained in the model predicting EDSS (partial r with age = −0.27, P = .03, R² = 0.47). Putamen T2 hypointensity was also retained in step 3 of the analysis but the unique variance contributed by this predictor was provided by a positive correlation and the partial r with age only was not significant. The model predicting EDSS included no general MRI measures, indicating that caudate T2 hypointensity was a stronger predictor of EDSS than were third ventricular width, T2 lesion volume, and T1 lesion volume.

All T2 hypointensities were significantly associated with the secondary progressive course after age adjustment. T2 hypointensity in the thalamus (P = .02) was
the strongest predictor of secondary progressive (vs relapsing-remitting) disease course among the T2 hypointensity ROIs and general MRI measures. In the final model ($R^2 = 0.46; P < .001$), T2 intensity of the caudate and putamen were also retained but there were no general measures included. Thus, gray matter T2 hypointensity was a stronger predictor of secondary progressive vs relapsing-remitting disease course than were third ventricular width, T2 lesion load, and T1 lesion load.

**COMMENT**

This quantitative study shows that abnormal hypointensity on T2-weighted images (BT2 ["black T2"]) is present in MS, occurs throughout the deep gray matter, and is associated with clinical and MRI markers of disease severity. BT2 is associated with brain atrophy and is a stronger predictor of disability and clinical course than are conventional MRI findings. Our findings extend previous studies that were based on visual (qualitative) assessments of BT2. A previous study of 47 patients with MS, using an ordinal rating scale to measure BT2, found that 23 patients with MS had abnormal hypointensity in the thalamus and putamen. The degree of hypointensity was correlated with the degree of T2 white matter plaques (also rated visually). Another group used visual rating of BT2 and reported mild hypointensity in the thalamus (not in the putamen or brainstem). They used the cortical gray matter as a visual standard of normal T2 intensity but the cortical gray matter may also develop BT2 in patients with MS, so it is not a reliable standard of reference. Previous studies may have been limited by sample size, lack of a quantitative approach, or both. In our recent study of BT2 in 114 patients with MS, we used a visual rating and found that BT2 was commonly detected in the basal ganglia and thalamus and was
related to disease duration, physical disability, clinical course, MRI lesions, and atrophy. The present study confirmed that BT2 occurs in patients with MS in previously recognized areas (ie, the basal ganglia, thalamus) and also in the brainstem (red nucleus). The degree of BT2 showed a stronger relationship to physical disability and clinical course than did T1 plaque load, T2 plaque load, or central atrophy. This suggests that BT2 reflects important disease effects relating to brain function. These same gray matter structures showed hypointensity on positron emission tomography scans of patients with MS.

BT2 in MS has not yet been correlated with pathologic findings, but it is probably due to pathologic iron deposition. It is not known whether pathologic iron accumulation is a secondary process (related to neurodegeneration), a primary process contributing to injury, or both. Previous studies have implicated disturbed iron homoeostasis in MS. Iron accumulates in reactive microglia, microglia, and macrophages in the brains of patients with MS and ferritin levels are elevated in the CSF of patients with progressive disease. The normal pattern of transferrin and ferritin binding was impaired and he-mosiderin and ferritin deposits were identified in the brains of patients with MS. Increased chelatable iron can cause neurotoxicity by transferring electrons to molecular oxygen to produce free radicals. Iron deposition has been described in a host of neurodegenerative diseases and aging, in which BT2 is also observed by MRI, suggesting a common theme underlying a variety of neurologic conditions. Blood-brain barrier dysfunction (increased delivery), disrupted clearance of iron byproducts, or dysregulation of brain iron transport proteins may play a role in iron deposition, potentially offering new therapeutic opportunities in MS.

While BT2 is most likely due to iron deposition, other possibilities include magnetization transfer effects, diffusional changes, and tissue oxygenation differences. If cellular structure is degraded and the free diffusion constant rises, fast spin-echo T2WI could theoretically show reduced signal. However, fast spin-echo is relatively insensitive to diffusion effects compared with echoplanar techniques. Using the moderate echo train length in the present study, diffusion effects would likely be minimized (if detectable). Deoxyhemoglobin causes T2 shortening while oxyhemoglobin causes T2 prolongation on heavily weighted T2WI. Thus, if patients with MS have higher ratios of deoxyhemoglobin to oxyhemoglobin in gray matter than controls, this might lead to relative T2 hypointensity. A pathologic correlation of the T2 hypointensity on MRI is warranted to confirm that iron deposition is the cause.

BT2 was related to third ventricular width, a marker of central brain atrophy that was previously shown to increase in patients with MS during a 2-year period and to predict physical disability. In a recent study, we showed that BT2 was related to third ventricular enlargement and cortical atrophy but the data were obtained visually. In the present study, our quantitative approach confirms that BT2 is associated with third ventricular width, suggesting a relationship between iron deposition and atrophy in MS. This might relate to neuronal loss and abnormal iron accumulation caused by tissue destruction or iron-mediated neurotoxicity. The presence of BT2 in the deep gray matter and its relationship to brain atrophy in the brains of patients with MS supports a degenerative disease process.

BT2 was related to the severity of total T2 plaque volume. In a previous study we showed that visually rated BT2 was related to T2 lesion load and showed a less robust relationship to T1 lesion load. The present study confirms that BT2 is related to total brain T2 lesion load but not T1 lesion load. Hypointense T1 lesions in MS represent areas of severe irreversible tissue loss in most instances, and less commonly, transient changes. Hyperintense T2 lesions are much more nonspecific and may include a wide range of pathologic changes, such as Wallerian degeneration. One possible explanation for the correlation of BT2 with bright T2 lesions but not with dark T1 lesions is the contribution of tract degeneration, which will prolong T2 but not T1 relaxation time. Consistent with this hypothesis, BT2 showed a close association with brain atrophy in a previous study. However, BT2 was not related to gadolinium enhancement. These data suggest that iron deposition is a marker of the global disease process. However future studies should determine if longitudinal changes in BT2 occur to a degree that could serve as a sensitive surrogate disease marker.

The reason for the general symmetry of BT2 in MS is not entirely clear. Multiple sclerosis is increasingly recognized as a global (whole-brain) disease process that ex-

| Gray Matter T2 Hypointensity in Patients With Multiple Sclerosis (MS) vs Controls |
|-----------------------------------------------|-----------------|-----------------|-----------------|
| Patients With MS, Mean (SD)* (n = 60) | Controls, Mean (SD)* (n = 50) | Difference, Patients With MS vs Controls, %† | P Value ‡ |
| Head of caudate | 0.45 (0.04) | 0.48 (0.04) | −6.2 | <.001 |
| Putamen | 0.38 (0.03) | 0.41 (0.04) | −5.2 | .002 |
| Globus pallidus | 0.30 (0.03) | 0.32 (0.03) | −6.2 | <.001 |
| Thalamus | 0.37 (0.03) | 0.39 (0.03) | −4.6 | .001 |
| Red nucleus | 0.30 (0.03) | 0.31 (0.03) | −5.4 | .003 |
| Substantia nigra | 0.32 (0.03) | 0.33 (0.03) | −3.4 | .08 |

*Gray matter T2 hypointensity values are normalized (ratio to the signal intensity in the ventricular cerebrospinal fluid).
†Means in MS group – control group/control group = 100%.
‡T Test.
tends beyond focal white matter plaques to include pathologic changes in normal-appearing white matter,\textsuperscript{5,8,9} tract degeneration,\textsuperscript{33} diffuse brain atrophy,\textsuperscript{25,34} and widespread hypometabolism.\textsuperscript{11,12} Thus, conventional MRI plaques (which may appear asymmetric) are probably only the "tip of the iceberg" in appreciating disease effects. Focal T1 hypointensities were not associated with BT2 in the current study. Other important disease processes that are only weakly associated with foci of demyelination may be present in the brain, such as axonal injury, atrophy, and pathologic iron deposition. Thus, global and focal disease effects may be related but different.

We used fast spin-echo T2 since this is more practical to implement than conventional spin-echo and has increased sensitivity for MS plaque detection.\textsuperscript{22} However, conventional spin-echo is more sensitive to the susceptibility effects of iron.\textsuperscript{36} Future studies should compare the 2 spin-echo methods and gradient echo methods in detecting BT2 in MS.\textsuperscript{37} We used a method of estimating T2 relaxation time by calculating intensity as a ratio to the intensity of CSF, a method shown to accurately reflect T2 relaxation times in iron-containing gray matter structures.\textsuperscript{22} It could be argued that CSF changes related to MS could invalidate the use of CSF T2 intensity as a standard of reference for gray matter T2 intensity. However, the typical protein elevations in the CSF of patients with MS do not rise to a threshold that affects T2 relaxation time,\textsuperscript{38} and there was no difference in the absolute intensity of CSF between MS and controls in our study. Direct measurement of T2 relaxation time is con-

\begin{figure}
\centering
\includegraphics[width=\textwidth]{image}
\caption{Four representative subjects illustrating the deep gray matter T2 hypointensity in multiple sclerosis (MS). A-F, 3 patients with MS in A 55-year-old healthy female control in G-L. A and B, A 43-year-old man with secondary progressive MS and moderate physical disability (Expanded Disability Status Scale [EDSS] score 3.5). Note the hypointensity of the red nuclei (arrow) in comparison with the normal control scan (G). In figure B, note the hypointensity of the thalami and lenticular nuclei and enlargement of the third ventricle (central atrophy) compared with normal control scan (H). C and D, A 33-year-old woman with relapsing-remitting MS and an EDSS score of 3.5. Note the hypointensity of the thalami, putamen, and globus pallidus compared with normal control scans (I, J). E and F, A 28-year-old man with secondary progressive MS and moderate to severe physical disability (EDSS score 6.5). Note the hypointensity of the caudate nuclei (arrows) compared with the normal control scans (K, L).}
\end{figure}
sidered the gold standard for quantification of iron concentration in gray matter and should be used in future studies to extend the present findings.21,22,23,38

Accepted for publication August 30, 2001.

Author Contributions: Study concept and design (Drs Bakshi, Benedict, Caruthers, Jacobs); data interpretation (Drs Bakshi, Benedict, and Caruthers); drafting of the manuscript (Dr Bakshi); data analysis and editing of the manuscript (Dr Benedict, and Caruthers); acquisition of data and technical support (Messrs Bermel, Tjoa, and Fabiano); critical revision (Drs Benedict, Caruthers, Jacobs); supervision (Drs Benedict, Caruthers, Jacobs); and obtaining funding (Drs Bakshi, Jacobs).

This research was supported by grant NIH-NINDS 1 K23 NS42379-01 from the National Institutes of Health, Bethesda, Md (Dr Bakshi), University at Buffalo State University of New York Pilot Project Program Grant (Dr Bakshi), American Academy of Neurology Student Interest in Neurology Summer Scholarship, Minneapolis, Minn (Mr Bermel), Alpha Omega Alpha Medical Student Research Fellowship, Menlo Park, Calif (Mr Bermel) and University at Buffalo State University of New York Medical Student Research Fellowship (Mr Bermel).

This work was presented in part at the annual meeting of the Americas Committee for Treatment and Research in Multiple Sclerosis, Boston, Mass, October 15, 2000; the annual meeting of the American Academy of Neurology, Philadelphia, Pa, May 9, 2001; and the annual meeting of the American Neurological Association, Chicago, Ill, October 1, 2001. We thank Mark Horsfield, PhD, for developing and installing the neuroimaging analysis software. We are grateful to Jack Simon, MD, PhD, for critically reviewing the manuscript.

Corresponding author and reprints: Rohit Bakshi, MD, Buffalo Neuroimaging Analysis Center, 100 High St, Suite E-2, Buffalo, NY 14203 (e-mail: rbakshi@buffalo.edu).

REFERENCES