Assessing Abnormal Iron Content in the Deep Gray Matter of Patients with Multiple Sclerosis versus Healthy Controls

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BACKGROUND AND PURPOSE: It is well known that patients with MS tend to have abnormal iron deposition in and around the MS plaques, in the basal ganglia and the THA. In this study, we used SWI to quantify iron content in patients with MS and healthy volunteers.

MATERIALS AND METHODS: Fifty-two patients with MS were recruited to assess abnormal iron content in their basal ganglia and THA structures. One hundred twenty-two healthy subjects were recruited to establish a baseline of normal iron content in deep GM structures. Each structure was separated into 2 regions: a low-iron-content region and a high-iron-content region. The average phase, the percentage area, and the total phase of the high-iron-content region were evaluated. A weighting was also assigned to each subject depending on the level of iron content and its deviation from the normal range.

RESULTS: A clear separation between iron content in healthy subjects versus patients with MS was seen. For healthy subjects 13% and for patients with MS 65% showed an iron-weighting factor >3 SDs from the normal mean (P < .05). The results for those patients younger than 40 years are even more impressive. In these cases, only 1% of healthy subjects and 67% of patients with RRMS showed abnormally high iron content.

CONCLUSIONS: Iron-weighting factors in the basal ganglia, THA, and the midbrain appeared to be abnormal in roughly two-thirds of patients with MS as measured by SWI.

ABBREVIATIONS: CN = caudate nucleus; EDSS = Expanded Disability Status Scale; GM = gray matter; GP = globus pallidus; ICC = intraclass correlation coefficient; PT = pulvinar thalamus; PUT = putamen; RI = normal iron-content region; RII = high iron-content region; RN = red nucleus; RR = relapsing-remitting; SN = substantia nigra; SP = secondary progressive; SPIN = signal processing in nuclear magnetic resonance; THA = thalamus

MS has been considered as both an autoimmune inflammatory demyelinating disease and a disease in which venous involvement is recognized as a possible biomarker or represent some specific damage to the tissue. Recently, the inter-relationship between venous abnormalities, obstructed flow, and a possible role for iron in tissue damage has been considered.

To a large degree but not exclusively, the imaging pathogenic landmarks of MS have been well documented mostly in WM. To a lesser degree, investigators have noticed abnormalities in cortical regions as well, specifically near the GM/WM boundary and in GM as well. However, whether the starting point is in the GM or WM is still unclear. Currently, there is an increased interest in studying how GM is abnormal in roughly two-thirds of patients with MS as measured by SWI.

Brain iron accumulation in neurodegenerative diseases, including MS, is not new and has been shown historically in the past. In MS, its source is likely due to myelin or oligodendrocyte debris, concentrated iron in the macrophages, or as a product of local microhemorrhages following venule wall damage. As the wall breaks down, free iron may escape outside the vessel. This process has typically been seen in the basal ganglia, neurons, oligodendrocytes, macrophages, and microglia. Generally, free iron is known to lead to the formation of highly reactive hydroxyl radicals that can trigger cell membrane dysfunction and chronic microglial activation. Thus, iron from any of the above-mentioned sources could lead to inflammation and a further buildup of iron, causing the system to be self-sustainable. When iron is present, the result is a hypointense signal intensity on T2- or T2* -weighted images and a change in the phase for SWI, which makes it possible to quantify iron changes in vivo. Different results have been reported in studying iron involvement in MS. The variations seen in these results have been related to many factors, including the type of MS studied, the sample size recruited, and the methodologies used to assess iron deposition. A number of studies have now shown that there are increases in iron in the basal ganglia and the THA. In a recent study, high iron was found in the PT, the THA, and GP, and the authors considered the iron measured by SWI to be a strong indicator of disability progression, lesion volume accumulation, and atrophy.

Another study by Burgetova et al by using T2 relaxometry...
showed that iron does increase in the basal ganglia and the
THA of patients with MS, but it showed an inverse correlation
with lesion load. Zhang et al23 also reported a correlation be-
tween T2 hypointensities (representing iron deposition) and
the patients’ disabilities in the RRMS group they studied. Cec-
carelli et al22 investigated deep GM T2 hypointensity in pa-
tients with clinically isolated syndrome MS patients and
showed that iron-related changes and neurodegeneration can
both occur in the early stages of MS. Recent work investigated
the potential role that extravasated iron might play by study-
ing CSF ferritin levels, which are considered an indirect mea-
sure of iron in the brain. They found no difference between
the MS population and the control group. Another study24
noted that iron deficiency was reported in the MS group stud-
ied, and iron supplementation was prescribed to these pa-
tients. Evidently, this treatment resulted in a partial recovery
of their symptoms. Thus, the role of iron in MS and its corre-
lation with the clinical outcomes is still unclear, and new
approaches to investigate brain iron in patients with MS are
still needed. In this multidisciplinary work, we study a group
of 52 patients with MS by using SWI, which is a powerful MR
imaging methodology known to be sensitive to iron. We also
present a new weighting scheme to evaluate iron abnormali-
ties and better differentiate between what is considered nor-
mal and abnormal iron deposition.

Materials and Methods

Data Acquisition

Fifty-two patients with clinically definite MS were imaged (mean age, 43 ± 11.73 years; range, 17–66 years) under 4 separate internal review
board approved protocols. One hundred twenty-two healthy subjects
(mean age, 44 ± 13.49 years; range, 20–69 years) were included in this
study to establish a normal range of iron deposition in the struc-
tures of interest. The patient population studied covered 2 types
of MS including patients with RRMS (n = 31) and SPMS (n = 21)
(RRMS mean EDSS score, 2.19; EDSS range, 1–5.5; mean disease
duration, 8 years; SPMS mean EDSS score, 5.92; EDSS range, 3–7.5;
mean disease duration, 19 years). All patients and controls consented
to be subjects in this study. A velocity-compensated 3D gradient-echo
sequence was used to generate SWI images.

Site 1. Twelve patients with MS were recruited at the Detroit Med-
cal Center, Detroit, Michigan, for this study. SWI data were acquired
on a 1.5T Sonata scanner (Siemens Healthcare, Erlangen, Germany)
equipped with an 8-channel head coil. Imaging parameters for SWI
were the following: TR = 57 ms, TE = 40 ms, flip angle = 20°, band-
width = 80 Hz/pixel, FOV = 256 × 192 mm², matrix size = 512 ×
448, with a resolution of 0.5 × 0.5 × 2 mm³.

Site 2. Thirty-one patients with MS and 18 healthy subjects were
scanned on a 3T Signa Excite HD 12.0 Twin Speed 8-channel scanner
(GE Healthcare, Milwaukee, Wisconsin) at the University of Buffalo,
Buffalo, New York. A multichannel head and neck coil was used to
acquire the SWI data. The imaging parameters were the following:
TR = 40 ms, TE = 22 ms, flip angle = 12°, and FOV = 256 × 192
mm² (512 × 256 matrix with phase FOV = 0.75) and image resolu-
tion of 0.5 × 1 × 2 mm³.

Site 3. Nine patients with MS were scanned by using a 3T TIM
Trio system (Siemens Healthcare) at Jena University Hospital, Jena,
Germany. A 12-channel receive head-matrix coil was used to acquire
the SWI data. The imaging parameters were the following: TR = 29
ms, TE = 20 ms, flip angle = 15°, bandwidth = 120 Hz/pixel, FOV =
256 × 192 mm², matrix 512 × 256 with FOV phase = 0.75 corre-
sponding to an image resolution of 0.5 × 1 × 2 mm³, integrated par-
allel acquisition techniques = 2 (24 reference lines), 128 partitions.

Site 4. The SWI data of another 104 healthy subjects (previously
studied25) were added to the 18 healthy subjects studied at site 2 to
create a baseline for iron content in the deep GM nuclei of healthy
subjects as a function of age.

The ability to use and compare data from different sites, acquired
at different field strengths, lies behind the concept that the phase
values will remain constant if the product of field strength to the TE is
kept constant and is otherwise independent of field strength or system
manufacturer.26 Thus, no matter what field strength is used, evaluat-
ing iron content by using phase images should report consistent re-
sults. To experimentally test this concept, we recruited 36 healthy
controls from site 1, 2, and 3 who were scanned by using the param-
eters specific to each site, and we analyzed their data. The results were
compared with those of 104 healthy subjects from site 4 and indeed
showed that these measured values (from sites 1, 2, and 3) lay within
the normal range deduced from site 4 data.

Data Analysis

A 64 × 64 (or equivalent) low-spatial-frequency kernel matrix was
used to complex-divide the original k-space data to create an effective
high-pass-filtered phase image.26 The resulting SWI filtered-phase
images were used as a means to quantify iron content. Seven deep GM
structures were studied for iron content (methodology described in
previous studies,18,19,25) including the following: the GP, the head of
the CN, PUT, THA, SN, RN, and PT.

Our in-house software SPIN (MRI Institute for Biomedical Re-
search, Detroit, Michigan) was used for the data analysis. Each struc-
ture was separated into 2 regions of interest: RI and RII. Because our
interest was to quantify high iron content, our main focus was on RII
and the total region (RI + RII). To achieve this quantification, we
used the same process described in more detail in Haacke et al.25 To
separate these 2 regions automatically, we based thresholds on the
data from recent articles.7,20 For the structures that were not in-
cluded in these articles (PT and THA), appropriate thresholds were
calculated by analyzing 20 healthy subjects ranging from 20 to 39
years of age. Otherwise, the thresholds were set by using the mean
value measured minus 2 times the SD across an elderly population, to
be on the conservative side as outlined in Haacke et al28 and across the
above-mentioned 20 healthy subjects. The boundary of each structure
was drawn manually by 3 well-trained graduate students, and the
high-iron-content region was found automatically by using threshold
values. The ICC reliability was evaluated by comparing the results of 5
different subjects from 3 different observers by using the Statistical
Package for Social Sciences (SPSS, Chicago, Illinois) software.

All 3 observers evaluated the average iron content of same struc-
ture in RII and the normalized area, and the total iron in the PUT. The
ICC values for these measures were 0.71, 0.93, and 0.81, respectively.
SPIN can output the statistical measures of the total structure and the
high-iron-content region for later analysis. We evaluated different
measures23,25: 1) the fraction of the structure that had high iron con-
tent (percentage area), 2) the average putative iron per voxel in RII, and
3) the total putative iron content in the high-iron-content region.
The main criterion to differentiate normal from abnormal is that the
measured iron content should lie outside the 95th prediction interval
lines of the healthy population. Moreover, iron deposition measures
were weighted according to their spread around the mean values. This
abnormality weighting, \( AW(m) \), was achieved by subtracting the measured mean value (MV) from the estimated values of normal iron deposition of age-matched controls, \( MV_n \) (calculated from the linear regression), then subtracting \( m \) times the estimated SD (SD\(_n\)) (\( m = 2 \) while considering a 95% confidence interval) and dividing the results by SD\(_n\).

\[
AW(m) = \frac{(MV - MV_n) - mSD_n}{SD_n}
\]

In this article, the results will be shown for both \( m = 2 \) and \( m = 3 \).

**Results**

The main purpose of this study was to quantify iron content in the basal ganglia and THA of patients with MS by using phase information acquired from SWI data and to create a new weighting scheme that allows us to better differentiate normal and abnormal iron deposition in patients with MS compared with control subjects. Iron overload was well visualized in patients with MS in a number of regions related to the basal ganglia and THA. Figure 1 displays the iron content visualized in an age-matched control (age, 39 years) and a patient with MS (age, 40 years) in the basal ganglia and midbrain by using SWI. Note that both have iron content in the RN and SN; however, iron overload was only seen in the patient with MS. High iron deposition can be seen in the CN, GP, PUT, and PT of the patient with MS. In the healthy subject, iron deposition in these brain structures is much less and is evenly distributed.

Abnormal iron changes were seen on visual examination in Fig 1, with the quantified results appearing in Fig 2. Generally, the degree of abnormal iron accumulation varied depending on whether one measures total iron in RII, average iron in RII, or the normalized percentage area of RII. In Fig 2, we display these 3 measurements for the CN and PT as an example to show the abnormal iron content of patients with MS relative to healthy subjects.

Figure 3 displays the individual weighting results calculated by using the equation for the PT and RN. A clear separation between healthy subjects and patients with MS was visualized with patients having iron content higher than 3 times the SD of healthy subjects. Figure 4 displays the subtotal weighting of the 4 structures for each measured parameter, including average phase, normalized area, and total phase. The last graph, Fig 4D, shows the total weighting of all the parameters of the CN, PT, RN, and SN combined. The figure also demonstrates that there were no healthy controls younger than roughly 40 years of age with abnormal iron content, as reflected by a weight factor of >1.0.

The percentages of individuals with abnormal iron content in both control subjects and patients with MS are shown in Tables 1–3. Note that in these tables, the evaluations and calculations were done for both hemispheres, thereby resulting in a total that is 2 times the number of subjects. In Tables 1 and 2, individual weights (1 parameter for 1 structure), subtotal weights (1 parameter for all the structures), and the total weights (all parameters for all the structures) are displayed for \( m = 2 \) and \( m = 3 \) (>2 and 3 times the SD, respectively). When we compared healthy subjects with patients with MS (\( P < .05 \)), the total weights showed that 76% of all patients with MS had abnormally high iron content (higher than 2 SDs) in at least 1 of the structures, whereas only 27% of controls had abnormally high iron content. Similar results were seen for subtotal and individual weights (except in the PUT). Results for subjects with iron deposition higher than 3 SDs still show high values for patients with MS.

In Table 3, we display the total and subtotal weights calculated from the CN, PT, RN, and SN iron content that again lie above 2 and 3 times higher than the SD (\( m = 2 \) and 3 in the equation). Patients with MS were divided into RR and SP and into 2 age ranges (equal or younger than 40 years and older than 40 years). We found that 27% of the healthy population, 74% of patients with RRMS, and 79% of those with SPMS had iron content above 2 SDs from the mean, while 13% of control subjects had iron content higher than 3 SDs compared with 67% of patients with SPMS and 65% of those with RRMS. The younger RRMS population tended to have a higher percentage of abnormal iron deposition compared with healthy controls for \( m = 2 \) and \( m = 3 \) except for the subtotal weight–average phase.
Fig 3. Plots showing individual weighting of different parameters (average phase: upper row; normalized area: middle row; and the total phase: lower row) of the PT (A–C) and the RN (D–F). The asterisks represent the patients with RRMS, the triangles represent those with SPMS, and the diamonds represent the healthy subjects. Patients with MS and healthy subjects with weighting higher than 1 are shown in the plots (these results correspond to m = 3; for quantitative evaluation, please refer to Tables 1 and 2).

Fig 2. Average phase, normalized area, and total phase of RII in the CN and PT. Small dots represent healthy subjects. The solid line is the regression line, and the outer dashed lines represent the 95% prediction interval of the regression. Hollow squares and triangles represent patients with RRMS, and the solid squares and triangles represent patients with SPMS. The squares and triangles represent the left and right hemispheres respectively. Many of the patients with MS have brain iron content beyond the 95% prediction intervals.
Discussion

The interest in and association of MS with veins and iron deposition are not new. We know that the basal ganglia from the dentate nucleus, the midbrain, and up to the thalamostriate system are all drained by the medial venous drainage system out into the straight sinus. It is just these regions that have increased iron content as seen with SWI and with conventional MR imaging. The recent work of Haacke et al suggests that the iron increase seen in MR imaging occurs at the confluence of the small draining veins out of the structures of interest such as the PUT, GP, and CN. Given the previous evidence that MS is a perivenular disease and that iron builds up in the venule wall, it may be that these increases in iron represent venous endothelial damage. However, it is still unknown whether iron deposition is a cause or a consequence of the inflammatory demyelinating aspect in MS pathology. On the other hand, the work by Neema et al showed the presence of deep GM T2 hypointensities and suggested that excessive iron deposition is associated with the progression of disease.

Table 1: Percentage of healthy and patient subjects with iron deposition higher than 2 SDs (P < .05) from the mean based on individual structures

| Weightsa | RII-Average Phase | RII-Normalized Area | RII-Total Phase |
|----------|-------------------|---------------------|----------------|
| N  | P  | N  | P  | N  | P  | N  | P  |
| Subtotal | 12.3 | 62.5 | 16.4 | 63.5 | 16.4 | 51.9 |
| Individual | | | | | | |
| CN | 3.7 | 18.3 | 4.5 | 20.2 | 5.3 | 12.5 |
| PT | 2.0 | 24.0 | 4.5 | 27.9 | 4.1 | 23.1 |
| RN | 4.5 | 33.7 | 6.6 | 34.6 | 6.1 | 30.8 |
| SN | 3.7 | 16.3 | 4.1 | 31.7 | 2.5 | 22.1 |
| GP | 4.5 | 7.7 | 3.3 | 6.7 | 4.5 | 5.8 |
| THA | 2.9 | 16.3 | 3.3 | 3.8 | 2.0 | 3.8 |
| PUT | 5.3 | 3.8 | 4.1 | 2.9 | 5.3 | 1.0 |

Note:—N indicates healthy subjects; P, patients.

*Total weight for healthy subjects was 27.01, and for patients, 75.81.

Table 2: Percentage of healthy and patient subjects with iron deposition higher than 3 SDs (P < .05) from the mean based on individual structures

| Weightsa | RII-Average Phase | RII-Normalized Area | RII-Total Phase |
|----------|-------------------|---------------------|----------------|
| N  | P  | N  | P  | N  | P  |
| Subtotal | 5.7 | 43.3 | 6.1 | 52.9 | 5.7 | 41.3 |
| Individual | | | | | | |
| CN | 1.2 | 14.4 | 2.0 | 12.5 | 0.4 | 8.7 |
| PT | 1.2 | 13.5 | 1.2 | 18.3 | 0.8 | 15.4 |
| RN | 0.8 | 21.2 | 0.8 | 30.8 | 2.9 | 27.9 |
| SN | 2.0 | 10.6 | 2.0 | 21.2 | 2.5 | 10.6 |
| GP | 2.0 | 4.8 | 0.8 | 1.0 | 2.0 | 1.0 |
| THA | 0.8 | 6.7 | 0.0 | 1.0 | 0.8 | 0.0 |
| PUT | 2.0 | 0.0 | 3.3 | 1.0 | 2.5 | 1.0 |

Note:—N indicates healthy subjects; P, patients.

*Total weight for healthy subjects was 12.7, and for patients, 65.4.

MR phase information has become more frequently used to evaluate iron content as a function of age in the human brain. In previous articles, we introduced the concept of a high-iron content region and normal-iron-content region. This made it possible to study not only smaller increases in iron content more confidently but also to study both the average iron content per pixel and the normalized area of increased iron content with age, which showed different and interesting results as mentioned earlier. This approach revealed subtle changes in iron content that cannot be seen with the single-region approach because even large increases in iron in a small area would be washed out when looking at iron over the whole region. The variability shown in our results demonstrates that evaluating abnormal iron content is not best achieved by using total iron content measurements. Rather, more information can be obtained by evaluating the area of the high-iron-content region (RII) as well as its average iron content per pixel. These 2 measures appear to present better quantitative indicators of iron content abnormality. In this study, the CN, PT, RN, and SN are shown to be more susceptible to abnormal iron deposition than the other structures studied (with RN being the most susceptible [30%] followed by the PT [27.9%], see Tables 1 and 2). However, no
significant correlation of iron content with EDSS was found with any of the measures we obtained, regardless of the structure studied. This high iron content seen in the basal ganglia, the THA, and the midbrain structures may be consistent with the hypothesis of venous hypertension.4

Conclusions
Abnormal iron content by using the total iron-weighting scheme described herein was clearly identified in patients with RRMS, especially those younger than 40 years of age, with almost no abnormal iron seen in the healthy population. Evidently, iron in the basal ganglia, CN, PT, and the midbrain (RN and SN) may be a biomarker for MS. Further work in this direction would be to compare iron content with the severity of venous disease in chronic cerebrospinal venous insufficiency.4,33

Disclosures: James Garbern—Speaker Bureau Genzyme, Lundbeck; Details: I am on the speakers’ bureau for neurologic conditions other than MS (lysosomal storage disease-Genzyme; Huntington disease-Lundbeck); Consultant: EMD-Serono, Biogen, Details: I have done consulting for both companies regarding pharmaceuticals for treatment of MS. Other Financial Relationships: PMD Foundation, European Leukodystrophy Association; Details: I have research grants from both of these nonprofit agencies to support my work on Pelizaeus-Merzbacher disease. Robert Zivadinov—Speakers' bureau for neurologic conditions other than MS (lysosomal storage disease-Speaker Bureau

Table 3: Percentage of healthy and patient subjects with iron deposition higher than 2 and 3 SDs from the mean based on subtotal and total weights

| Weights          | 2 SDs          | 3 SDs          |
|------------------|----------------|----------------|
|                  | RR MS/TMS     | RR TMS         | SP MS/TMS     | SP TMS         | Healthy MS/TMS | Healthy TMS |
| Subtotal-normalized area |               |                |               |                |               |               |
| 20–40            | 23/36 (64%)    | 6/8 (75%)      | 15/112 (13%)  | 24/36 (67%)    | 4/8 (50%)      | 1/122 (1%)   |
| 41–70            | 25/66 (74%)    | 33/42 (79%)    | 51/132 (38%)  | 16/26 (62%)    | 24/34 (71%)    | 30/122 (23%) |
| Subtotal-average phase |               |                |               |                |               |               |
| 20–40            | 39/62 (63%)    | 27/42 (64%)    | 40/244 (16%)  | 31/62 (50%)    | 24/42 (57%)    | 15/244 (6%)  |
| 41–70            | 22/56 (50%)    | 25/42 (64%)    | 31/122 (26%)  | 12/26 (46%)    | 20/42 (59%)    | 15/122 (11%) |

Note:—TMS indicates total number of patients with MS.

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