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Non-Motor Symptom Burdens Are Not Associated with Iron Accumulation in Early Parkinson's Disease: a Quantitative Susceptibility Mapping Study

Chaewon Shin,[1] **Seon Lee**,[2] **Jee-Young Lee**,[3] **Jung Hyo Rhim**,[4] **and Sun-Won Park**[4,5]

[1]Department of Neurology, Kyung Hee University Medical Center, Seoul, Korea
[2]Department of Mechanical and Aerospace Engineering, Seoul National University, Seoul, Korea
[3]Department of Neurology, Seoul National University-Seoul Metropolitan Government Boramae Medical Center, Seoul, Korea
[4]Department of Radiology, Seoul National University-Seoul Metropolitan Government Boramae Medical Center, Seoul, Korea
[5]Department of Radiology, Seoul National University College of Medicine, Seoul, Korea

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Address for Correspondence:
Sun-Won Park, MD, PhD
Department of Radiology, Seoul National University-Seoul Metropolitan Government Boramae Medical Center, 20 Boramae-ro 5-gil, Dongjak-gu, Seoul 07061, Korea.
E-mail: swpark8802@gmail.com

ORCID iDs
Chaewon Shin
https://orcid.org/0000-0002-4157-492X
Seon Lee
https://orcid.org/0000-0002-7451-1706
Jee-Young Lee
https://orcid.org/0000-0002-9120-2075
Jung Hyo Rhim
https://orcid.org/0000-0001-5822-9770
Sun-Won Park
https://orcid.org/0000-0002-5063-2685

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ABSTRACT

Background: Quantitative susceptibility mapping (QSM) has been used to measure iron accumulation in the deep nuclei of patients with Parkinson's disease (PD). This study examined the relationship between non-motor symptoms (NMSs) and iron accumulation in the deep nuclei of patients with PD.
Methods: The QSM data were acquired from 3-Tesla magnetic resonance imaging (MRI) in 29 patients with early PD and 19 normal controls. The Korean version of the NMS scale (K-NMSS) was used for evaluation of NMSs in patients. The patients were divided into high NMS and low NMS groups. The region-of-interest analyses were performed in the following deep nuclei: red nucleus, substantia nigra pars compacta, substantia nigra pars reticulata, dentate nucleus, globus pallidus, putamen, and head of the caudate nucleus.
Results: Thirteen patients had high NMS scores (total K-NMSS score, mean = 32.1), and 16 had low NMS scores (10.6). The QSM values in the deep were not different among the patients with high NMS scores, low NMS scores, and controls. The QSM values were not correlated linearly with K-NMSS total score after adjusting the age at acquisition of brain MRI.
Conclusion: The study demonstrated that the NMS burdens are not associated with iron accumulation in the deep nuclei of patients with PD. These results suggest that future neuroimaging studies on the pathology of NMSs in PD should use more specific and detailed clinical tools and recruit PD patients with severe NMSs.

Keywords: Parkinson Disease; Iron; Basal Ganglia; Magnetic Resonance Imaging

INTRODUCTION

Iron accumulation in the brain increases with age in the substantia nigra (SN), putamen (PU), globus pallidus (GP), caudate nucleus (CN), and cortex.[1] The accumulation is greater in patients with Parkinson's disease (PD) who exhibit widespread neurodegeneration in the basal ganglia (BG). Neuropathological studies have revealed that iron concentrations are

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increased in the SN pars compacta (SNc) and reticulata (SNr) in patients with PD.[2,3] Although the mechanisms underlying the increased accumulation of iron in patients with PD are unclear, researchers have used various methods to measure the in vivo levels of iron accumulation.[1]

Iron deposits in the deep nuclei of the brain influence regional magnetic susceptibility, which can be detected with brain magnetic resonance imaging (MRI).[4] Previous studies using brain MRI in patients with PD have measured the transverse relaxation rates (R_2 and R_2^*) and revealed that R_2^* was increased in the SNc and related to motor severity.[5,6] Longitudinal studies have suggested that R_2^* values increase with PD progression.[7-9] However, R_2^* is limited because it cannot exclude the magnetic susceptibility of the surrounding tissue. To solve this problem, a new method, quantitative susceptibility mapping (QSM), was developed.

Studies that have used QSM to measure iron accumulation in patients with PD have found that magnetic susceptibility was increased in the SNc compared with that in healthy controls,[10-16] and these increases were also found in patients in the early stage of PD.[11,13,16] Several studies have also found increased susceptibility in the GP, red nucleus (RN), and thalamus of patients with PD.[10,11,16] As expected, QSM measures are more precise than R_2^*.[12-14] The QSM values in the SNc of patients with PD increase with disease duration[12,16] and correlate with disease severity.[10,12,16] In addition, QSM values are increased in the dentate nucleus (DN) in patients with the tremor dominant subtype of PD.[17] A recent study reported that the Movement Disorder Society-sponsored revision of the Unified PD Rating Scale (MDS-UPDRS) Part I (non-motor experiences in daily life) scores positively correlated with magnetic susceptibility in the GP, CN, SN, and PU.[10]

Interest is increasing in the non-motor symptoms (NMSs) of patients with PD.[18,19] Some NMSs precede the motor symptoms and would be used for diagnosis of early or prodromal stage of PD. Furthermore, several early NMSs are related to the prognosis of the patients with PD.[20] However, because few studies have evaluated the relationships of the in vivo pathology and NMSs, the current understanding of the pathophysiology of NMSs depends on indirect anatomic correlations from post-mortem pathology studies.[21] Based on neuroanatomic knowledge, NMSs originate from various brain lesions beyond the motor circuit in the BG.[21] Hyposmia is related to the anterior olfactory nucleus; depression and sleep disorders are related to the pontine nuclei such as the locus ceruleus, raphe, lateral tegmental nuclei; executive, emotional, and behavior problems are related to the nucleus basalis Meynert and amygdala; dementia and psychosis are related to the neocortex. According to the current pathologic staging system for PD, the more extensive pathologic changes exist, the severer pathology can be found.[22] Therefore, patients with more NMSs probably have more extensive and severe pathology in the brain.

In this study, we assumed that wider iron accumulation, which reflects pathologic changes, would be observed in the brain of patients with more NMSs. To prove this hypothesis, we examined the relationship between NMSs and iron accumulation in the deep nuclei, which was measured with QSM, in patients with PD.

METHODS

Patients and clinical assessments

Participants eligible for this study were patients who were diagnosed with PD and who underwent brain MRI within 5 years of the onset of motor symptoms at the Seoul National University-Seoul Metropolitan Government (SNU-SMG) Boramae Medical Center. The

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diagnosis of PD was confirmed by movement disorder specialists using the UK brain bank clinical diagnosis criteria.[23] Patients with other neurodegenerative diseases or secondary brain lesions were excluded. Control candidates were selected from the brain MRI database of the SNU-SMG Boramae Medical Center. Individuals with a history of neurological diseases or abnormalities on brain MRI were excluded from the control group.

The clinical data were acquired with retrospective reviews of medical records. The demographic data included sex, age at acquisition of brain MRI, and age at the onset of motor symptoms. Disease duration was defined as the time from the age at the onset of motor symptoms to the age at acquisition of brain MRI. The severity of PD was evaluated with the Hoehn and Yahr (HY) stage, and MDS-UPDRS part III scores[24] that were evaluated within 1 month of the acquisition of brain MRI. The levodopa-equivalent daily dose (LEDD) was calculated by reviewing the medication history of each patient.[25] The NMS burden of the patients were evaluated using the Korean version of the NMS scale (K-NMSS).[26] The patients were further divided into a high NMS group and low NMS group according to the cut-off value of 20 in K-NMSS. This value was adopted from the comprehensive grading system of NMS severity in PD,[27] in which the value discriminates mild vs. more severe NMS burden in patients with PD.

MRI protocol and data reconstruction

All patients were scanned with 3-Tesla brain MRI (Achieva 3.0T; Koninklijke Philips N.V., Amsterdam, the Netherlands) using an 8-channel head coil. All scans were in the axial plane. A three-dimensional single gradient-echo sequence was acquired; the parameters were the following: echo time = 25.7–28.5 ms; repetition time = 18.2–20.0 ms; field of view = 200 × 200 mm^2; matrix = 256 × 256; slice thickness = 2 mm, no gap; and flip angle = 10°. The voxel size was same (0.390625, 0.390625, 2) for all subjects. The entire slices of 3D scan data were reconstructed in QSM images using a susceptibility tensor imaging software suite (version 2.2, Updated on Jan. 8, 2014; Brain Imaging & Analysis Center, Durham, NC, USA).[4,28] All other parameters which are required for the calculation of susceptibility were the same; Tol_LSQR was 0.01, D2_thres was 0.1, and Max_iter was 100.

Region-of-interest analysis

The region-of-interest (ROIs) were manually segmented on the reconstructed QSM images by one investigator using ITK-SNAP (www.itksnap.org).[29] No normalization on the brain size was done. The targeted deep nuclei were the following: SNc, SNr, RN, GP, DN, PU, and head of the CN (HCN, **Fig 1**). The ROIs were drawn on the slices that covered the SNc, SNr, RN, and DN. We selected the 2–5 most representative slices of the GP, HCN, and PU to minimize the influence of the surrounding structures due to unclear margins. The QSM values of deep nuclei of the patients and controls were calculated separately by averaging the values of selected ROIs.

Statistical analyses

All statistical analyses were performed with SPSS (version 21.0; IBM Corporation, Armonk, NY, USA). Non-parametric tests (Fisher's exact and Kruskal-Wallis tests) were used to compare the demographics of the patients with high and low NMS scores and controls. Mann-Whitney U tests were used to compare the clinical data between the patients with high and low NMS scores. The QSM susceptibility value in each ipsilateral and contralateral deep nucleus to the dominant side of motor symptoms of the patients was compared with mean QSM value of left and right deep nuclei of controls with Kruskal-Wallis tests. Bonferroni correction was used for multiple comparison (P value threshold = 0.025). The Pearson's

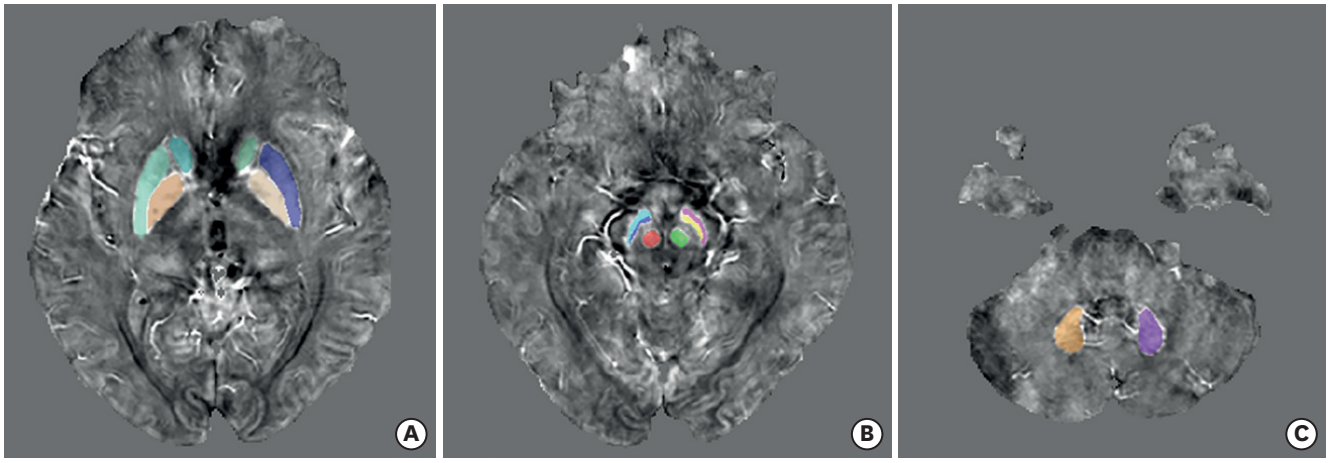

Fig. 1. Representative image of quantitative susceptibility mapping (QSM) with color overlay of the deep nuclei in the region-of-interest segmentation in a 64-year-old female patient with Parkinson's disease. (**A**) Axial section at the level of the basal ganglia showing the head of the caudate, putamen, and globus pallidus. (**B**) Axial section at the level of the midbrain showing the substantia nigra pars compacta, substantia nigra pars reticulata, and red nucleus. (**C**) Axial section at the level of the cerebellum showing the dentate nucleus.

partial correlation analyses adjusting the age at acquisition of brain MRI were performed to evaluate the linear correlations between the clinical characteristics and QSM data in the entire patient group. Two-tailed *P* values less than 0.05 were considered statistically significant.

Ethics statement

This study protocol was approved by the Institutional Review Board of the Seoul National University-Seoul Metropolitan Government Boramae Medical Center (No. 26-2016-107). A waiver of informed consent met the requirements and was granted.

RESULTS

Clinical data comparisons

The detailed clinical characteristics are presented in **Table 1** (see supplemental data for individual clinical and QSM data; **Supplementary Tables 1** and **2**). The percentage of males and age at acquisition of brain MRI did not differ among the patients with high and low NMS scores and controls. The patients with high and low NMS scores did not differ in the age at the onset of motor symptoms, disease duration, HY stage, MDS-UPDRS III score, and LEDDs.

Table 1. Clinical characteristics of the patients and controls

Characteristics	Patients with high NMS scores (n = 13)	Patients with low NMS scores (n = 16)	Controls (n = 19)	*P* value
Percentage of males (frequency)	23.1 (3)	31.3 (5)	26.3 (5)	0.922
Age at acquisition of brain MRI, yr	69.8 ± 11.2	71.4 ± 6.8	67.6 ± 8.0	0.433
Age at the onset of motor symptoms, yr	67.9 ± 11.4	70.4 ± 6.5		0.792
HY stage	2 (1, 2.5)	1.75 (1, 2)		0.200
LEDD	199.9 ± 230.7	150.0 ± 200.0		0.542
MDS-UPDRS part III	20.9 ± 10.9	19.7 ± 10.5		0.661
K-NMSS total score	32.1 ± 11.9	10.6 ± 4.2		< 0.001
Interval of K-NMSS and brain MRI, day	14.4 ± 10.3	13.4 ± 16.0		0.455

Data not otherwise specified are presented as mean ± standard deviation. Data are presented as percentage (frequency) or median (minimum, maximum).
NMS = non-motor symptom, MRI = magnetic resonance imaging, HY = Hoehn and Yahr, LEDD = levodopa-equivalent daily dose, MDS-UPDRS = Movement Disorder Society-sponsored Unified Parkinson's Disease Rating Scale, K-NMSS = Korean version of the Non-motor Symptoms Scale.

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Table 2. Detailed comparisons of the patients with high and low K-NMSS scores

Characteristics	Patients with high NMS scores (n = 13)	Patients with low NMS scores (n = 16)	*P* value
Domain 1 (cardiovascular)			
Dizziness	1.5 ± 1.7	0.3 ± 0.6	0.002
Falling due to fainting	0	0	1.000
Domain 2 (sleep/fatigue)			
Daytime sleep	0.7 ± 1.2	0.4 ± 0.6	0.747
Fatigue	1.3 ± 1.8	0.2 ± 0.4	0.027
Difficulty falling asleep	3.7 ± 2.7	0.8 ± 1.4	0.001
Restless legs	1.1 ± 2.5	0	0.019
Domain 3 (mood/cognition)			
Loss of interest in surroundings	1.1 ± 1.8	0.4 ± 0.7	0.337
Lack of motivation	0.8 ± 0.9	0.3 ± 0.7	0.081
Nervous feeling	1.5 ± 1.4	0.5 ± 0.6	0.035
Depression	1.4 ± 1.4	1.0 ± 1.7	0.313
Flat mood	1.3 ± 1.3	0.6 ± 1.0	0.052
Lack of pleasure	0.8 ± 1.3	0.1 ± 0.3	0.079
Domain 4 (perceptual/hallucination)			
Hallucinations	0.2 ± 0.4	0	0.110
Delusions	0	0	1.000
Double vision	0.3 ± 0.6	0.1 ± 0.3	0.187
Domain 5 (attention/memory)			
Poor concentration	1.2 ± 1.0	0.8 ± 0.9	0.165
Forgetfulness of past events	1.2 ± 1.1	0.9 ± 0.9	0.661
Forgetfulness for making plans	0.7 ± 1.0	0.4 ± 0.6	0.499
Domain 6 (gastrointestinal tract)			
Drooling	0.5 ± 0.9	0.4 ± 0.8	0.517
Dysphagia	0.3 ± 0.9	0	0.110
Constipation	2.5 ± 3.0	1.1 ± 1.2	0.400
Domain 7 (urinary)			
Urgency	2.1 ± 2.3	0.5 ± 0.8	0.038
Frequency	1.8 ± 2.0	0.3 ± 0.8	0.011
Nocturia	2.4 ± 1.5	1.3 ± 1.1	0.041
Domain 8 (sexual)			
Altered interest in sex	0.6 ± 1.7	0	0.110
Sexual problems	0.5 ± 1.6	0	0.110
Domain 9 (miscellaneous)			
Unexplained pain	0.5 ± 0.9	0.1 ± 0.3	0.082
Change in ability to taste or smell	0.9 ± 1.4	0.3 ± 0.6	0.181
Weight change	0.4 ± 0.7	0	0.019
Excessive sweating	0.8 ± 1.5	0.1 ± 0.3	0.165

Data not otherwise specified are presented as mean ± standard deviation.
K-NMSS = Korean version of the Non-motor Symptoms Scale, NMS = non-motor symptom.

The comparisons of the scores for each item on the K-NMSS between the patients with high and low NMS scores are presented in **Table 2**. Patients with high NMS scores had higher scores for dizziness, fatigue, difficulty falling asleep, restless legs, nervous feeling, urinary urgency, frequency, nocturia, and weight change.

ROI analysis results

The regional QSM values were compared among the patients with high and low NMS scores and controls (**Table 3**). There were no significant differences in the QSM values of all deep nuclei among the patients with high and low NMS scores and controls.

QSM and clinical data correlations

In the partial correlation analyses adjusting the age at acquisition of brain MRI, LEDD was correlated linearly with the QSM values of ipsilateral SNc (r = −0.426; $P = 0.024$) and

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Table 3. Regional differences in the susceptibility values in patients with PD and controls

Nucleus	Susceptibility value, ppm			
	Patients with high NMS scores (n = 13)	Patients with low NMS scores (n = 16)	Controls (n = 19)	*P* value[a]
RN				
Ipsilateral	0.104 ± 0.021	0.100 ± 0.040	0.092 ± 0.023	0.484
Contralateral	0.103 ± 0.017	0.104 ± 0.038		0.307
SNc				
Ipsilateral	0.128 ± 0.030	0.118 ± 0.048	0.121 ± 0.032	0.759
Contralateral	0.131 ± 0.024	0.133 ± 0.054		0.694
SNr				
Ipsilateral	0.123 ± 0.029	0.127 ± 0.048	0.115 ± 0.039	0.739
Contralateral	0.133 ± 0.028	0.128 ± 0.052		0.470
DN				
Ipsilateral	0.100 ± 0.037	0.096 ± 0.020	0.095 ± 0.023	0.828
Contralateral	0.101 ± 0.029	0.090 ± 0.018		0.423
GP				
Ipsilateral	0.141 ± 0.029	0.136 ± 0.040	0.134 ± 0.036	0.825
Contralateral	0.143 ± 0.028	0.140 ± 0.037		0.794
PU				
Ipsilateral	0.109 ± 0.042	0.103 ± 0.046	0.099 ± 0.035	0.851
Contralateral	0.111 ± 0.043	0.104 ± 0.045		0.781
HCN				
Ipsilateral	0.070 ± 0.021	0.064 ± 0.019	0.061 ± 0.022	0.497
Contralateral	0.068 ± 0.024	0.071 ± 0.025		0.401

Data are presented as mean ± standard deviation.
PD = Parkinson's disease, NMS = non-motor symptom, RN = red nucleus, SNc = substantia nigra pars compacta, SNr = substantia nigra pars reticulata, DN = dentate nucleus, GP = globus pallidus, PU = putamen, HCN = head of the caudate nucleus.
[a]The Kruskal-Wallis test with Bonferroni correction was used to test for statistical significance. Uncorrected *P* value was presented.

ipsilateral SNr (r = −0.518; *P* value = 0.005). Disease duration, HY stage, MDS-UPDRS part III score, and K-NMSS total score were not correlated with regional QSM values.

DISCUSSION

This study was the first study that evaluated the relationship between NMSs and regional iron accumulation in PD using a detailed NMS questionnaire and QSM. In contrast with our hypothesis, the QSM values in deep nuclei of the patients with PD, including SNc, were not different from the values of the controls. The QSM values of ipsilateral SNc and SNr were negatively correlated with LEDD.

Several possible reasons may explain these results. First, it can be assumed that different pathologies, which are not confined to the deep nuclei in this study, resulted in NMSs of patients with PD. This is consistent with a previous study that evaluated the relationship between the striatal dopamine level and NMS burden.[30] Second, most of the patients who participated in this study had a very early stage of PD, hence the NMS burden was not severe. As a result, the pathological changes in the brain associated with this NMS burden may not be evident. Finally, the K-NMSS is a collection of heterogeneous NMSs possibly originated from various brain regions. The definition of NMS burden in this study is a clinically meaningful distinction[27] but a simple sum without consideration of the heterogeneous nature of K-NMSS. Therefore, the association with the iron accumulation in a specific region of the brain may not be properly reflected. Neuroimaging studies on the brain pathology of NMSs in the future would be better to use specific and detailed clinical evaluation tools rather than K-NMSS.

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In contrast with previous studies, the QSM values in the SNc were not increased in patients with early PD.[11,13,16] The mean disease durations of the patients with high and low NMS scores in our study were 1.9 years and 1.0 years, respectively, which is much shorter than those reported in previous studies (mean 2.7–4.6 years).[11,13,16] Therefore, the patients selected in this study were a purer group of patients with very early stage of PD than those recruited in previous studies. On the other hands, the small number of participants in this study might have resulted in the inability to find statistical differences. For example, the QSM value in the contralateral SNc showed a tendency to be increased in the patients compared with the controls. Taken together, these observations suggest that the diagnostic accuracy of the QSM values in patients in the very early or prodromal stage of PD needs to be verified in large numbers of patients.

This study had several limitations. First, the generalization of our results is limited because inferential statistics were not used due to the small number of participants. Second, the ROIs were not drawn by independent investigators, and the reliability and reproducibility of the ROI analysis were not confirmed in this study. Third, the diagnosis of PD was based on clinical criteria without pathologic confirmation,[23] hence the QSM results would be influenced. Forth, the interval from K-NMSS to the acquisition of brain MRI was not consistent in patients, which may confound the correlation. Finally, the controls were not evaluated by a neurologist, and unknown neurodegenerative diseases could be present in the control group.

In conclusion, the study demonstrated that the NMS burdens are not associated with iron accumulation in the deep nuclei of patients with PD. These results suggest that future neuroimaging studies on the pathology of NMSs in PD should use more specific and detailed clinical tools and recruit PD patients with severe NMSs.

SUPPLEMENTARY MATERIALS

Supplementary Table 1

Individual data of the patients and controls

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Supplementary Table 2

Description of the variables in Supplementary Table 1

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