RESEARCH ARTICLE

Monitoring Substantia Nigra Degeneration Using Free Water Imaging across Prodromal and Clinical Parkinson's Disease

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ABSTRACT: Background: Substantia nigra (SN) free water has been suggested as a good surrogate marker in Parkinson's disease (PD). However, its usefulness for diagnosing prodromal PD (pPD) and monitoring disease progression warrants further validation.

Objective: The aim was to investigate SN free water values across prodromal and clinical stages of PD.

Methods: Four groups were enrolled in this study: 48 healthy controls (HC), 43 pPD patients, 50 de novo PD (dnPD) patients, and 49 medicated PD (mPD) patients. Based on diffusion tensor images, free water maps were calculated, and SN free water values were extracted from the anterior SN (ASN) and posterior SN (PSN). The SN free water values were compared among the four groups, and associations between free water and clinical symptoms were explored. The distinguishing power of PSN free water was evaluated using the receiver operating characteristic curve analysis. Follow-up was performed for 14 pPD patients.

Results: PSN free water in the pPD group was significantly higher than that in the HC group and significantly lower than that in the dnPD group. Surprisingly, the mPD group showed decreased PSN free water compared to the dnPD group. There was a positive correlation between motor symptoms and PSN free water in the pPD and dnPD groups. Longitudinal analysis showed a significant increase in PSN free water in pPD patients over time.

Conclusions: The PSN free water increased from prodromal to early clinical stages, but the trend might be reversed in late disease stages. This biphasic trend should be considered when applying this marker in future studies. © 2023 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: Parkinson's disease; prodromal Parkinson's disease; free water; posterior substantia nigra; biphasic

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by α-synuclein pathology and dopaminergic neuronal loss in the substantia nigra (SN).[1] The diagnosis of PD is mainly based on motor symptoms. However, a variety of nonmotor symptoms may appear years before clinical onset.[1] The latent phase of PD in which patients display motor and nonmotor clinical manifestations too subtle to be diagnosed is called prodromal PD (pPD)[2-4] and can last from 5 to more than 10 years. As pathological neurodegeneration has already begun,[5] initiating disease-modifying therapies in this period may provide an opportunity for slowing down disease progression.[6,7] Thus, there is an immediate need for reliable biomarkers to diagnose and predict pPD and to monitor nigrostriatal degeneration.

Diffusion tensor imaging (DTI), which can provide in vivo microstructural information, is widely used to study neurological disorders.[8] The free water diffusion model, which assumes a free diffusion compartment and a restricted tissue compartment, shows promising results for diagnosing PD. In particular, the free water content in the posterior SN (PSN) can reliably differentiate PD patients from healthy controls (HC) and patients with other parkinsonian syndromes[9-12] and increases with disease progression.[10,11] A previous study investigated the distinguishing power of free water in subjects with idiopathic rapid eye movement sleep behavior disorder (iRBD), who are considered to be at high risk of PD.[13] The authors found that PSN free water could differentiate between iRBD patients and HCs and increased over time.[14] In addition, a recent study[15] found increased free water in the PSN of asymptomatic LRRK2 G2019S mutation carriers compared to HCs. Thus, this method has potential applications for aiding early diagnosis of pPD. Nevertheless, the iRBD is only a specific type of pPD, and the LRRK2 mutations are relatively rare. The pathological development patterns of these subjects might be distinct from other types.[16-18] Thus, the value of SN free water should be validated in a mixed pPD population that meets the International Parkinson and Movement Disorder Society (MDS) research criteria for pPD (MDS-pPD). In addition, the characteristics of SN free water across the whole development course of PD need to be better understood to apply this method in patients at different disease stages.

In the present study, we investigated the characteristics of free water content in the anterior and posterior SN (ASN and PSN) in prodromal, de novo (dnPD), and medicated PD (mPD) patients. Follow-up data were available for a subset of pPD patients. The aims of this study were as follows: (1) to validate whether SN free water could differentiate MDS-pPD from HCs, (2) to investigate whether SN free water is related to disease severity, and (3) to test whether SN free water could track disease progression. Based on previous studies, we hypothesized that free water in the PSN can be used to distinguish pPD subjects from controls and monitor disease progression.

Patients and Methods

Participants

This study was approved by the Ethics Committee of the Affiliated Brain Hospital of Nanjing Medical University. Written informed consent was obtained from all participants at enrollment.

The pPD subjects were recruited as part of a community-based prospective study conducted at the Neurology Department of the Affiliated Brain Hospital with Nanjing Medical University. Participants completed a standardized structured questionnaire for screening pPD in the community. Demographic information and risk factors, including age, sex, occupational solvent exposure, coffee or tea use, regular pesticide exposure, family history, and nonsmoking status, were recorded. Additional prodromal markers included possible subthreshold parkinsonism, RBD (polysomnographic-proven idiopathic RBD or positive RBD screening questionnaire), abnormal hyperechogenicity of the SN, constipation, olfactory loss, urinary dysfunction, excessive daytime somnolence, symptomatic hypotension, severe erectile dysfunction, and depression/anxiety. Subjects with probable pPD were advised to visit the designated hospital to ensure whether the clinical assessment was performed and the diagnosis of pPD was made by two specialized neurologists according to the MDS-pPD criteria (posterior probability ≥80%).[19] Detailed steps are presented in the Supplementary Materials.

Patients with dnPD and mPD were recruited from the Movement Disorder Clinic at the Neurology Department of the Affiliated Brain Hospital of Nanjing Medical University between January 2013 and June 2021. All dnPD patients met the following criteria: (1) newly diagnosed with PD based on the United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria,[20] (2) currently untreated, (3) follow-up through the hospital for at least 1 year to confirm PD diagnosis, (4) a positive response to levodopa (L-dopa), and (5) early stage (modified Hoehn and Yahr scores [H&Y] <3). The inclusion criteria for mPD were as follows: (1) a diagnosis of PD based on the United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria,[20] (2) had received L-dopa therapy, and (3) responded well to L-dopa.

HCs were recruited from the community or among social workers at the Brain Hospital Affiliated to

Nanjing Medical University. They reported no symptoms associated with pPD and were clinically evaluated by two experienced neurologists.

All participants would be excluded due to (1) atypical or secondary parkinsonism; (2) clinically significant lesions found by brain magnetic resonance imaging (MRI); (3) severe chronic or psychiatric diseases such as heart failure and kidney failure; and (4) difficulty in cooperating with clinical assessments.

Clinical Evaluation

Both motor and nonmotor symptoms were assessed in all participants. Motor disability and PD severity were evaluated using the UPDRS III (Unified Parkinson's Disease Rating Scale, Part III) and modified H&Y stages.[21] The L-dopa equivalent daily dose was calculated based on a previously described method.[22] We assessed general nonmotor symptoms using the Non-Motor Symptoms Questionnaire (NMSQ). To assess cognitive impairments, we used the Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA). Emotional symptoms were assessed using the Hamilton Anxiety Scale (HAMA) and the Hamilton Depression Rating Scale (HAMD). Sleep quality was assessed using the Parkinson's Disease Sleep Scale and the Rapid Eye Movement Sleep Behavior Disorder Questionnaire-Hong Kong (RBDQ-HK). Patients with a total RBDQ-HK score higher than 18 were defined as having RBD.[23]

MRI Protocol

All participants were scanned with a 3-T Verio Siemens scanner (Siemens, Verio, Munich, Germany) in a supine position with their heads secured using foam pads to reduce movement. They were asked to remain as still as possible during the whole scan.

The 3D-T1 data were acquired using an MPRAGE sequence (repetition time/echo time [TR/TE] = 2530/3.34 ms, flip angle = 7°, field of view [FOV] = 256 × 256 mm, slice thickness/gap = 1.33/0.5 mm, matrix = 256 × 192, 128 slices covered the whole brain) for volumetric and image registration. DTI data were obtained using a spin–echo echo-planar imaging sequence in axial planes parallel to the anterior–posterior commissure line. The parameters were as follows: TR = 9000 ms, TE = 104 ms, flip angle = 7°, FOV = 230 × 230 mm, thickness = 2.5 mm with no gap between slices, matrix = 128 × 128, 49 slices covered the global brain, b-values = 0 and 1000 s/mm^2, and 64 diffusion gradient directions.

Free Water Analysis

DTI images were preprocessed using MRtrix3 (https://www.mrtrix.org/). The preprocessing steps included denoising, removing Gibbs artifacts, motion correction, and eddy current correction. The free water model was then estimated using the script provided by the MarkVCID projects (https://markvcid.partners.org/markvcid1-protocols-resources). Briefly, two compartments were estimated in this model: (1) a free water compartment that models isotropic diffusion to predominantly highlight water molecules in the extracellular space and (2) a tissue compartment that reflects tissue microstructure after removing the signal contributed by free water. The fractional volume of the free water compartment (ie, the free water measure) reflects the relative contribution of free water in each voxel, ranging from 0 to 1.

Regions of Interest and Extraction of Free Water Values

To make the placement of regions of interest (ROIs) more consistent, we first normalized b0 images into the MNI space through linear registration and then used the derived transformation matrices to transform free water maps into the MNI (Montreal Neurological Institute) space. ROIs were hand-drawn on the b0 images of each participant in MNI space by two experienced raters using ITK-SNAP (http://www.itksnap.org). Consistent with a previous study,[11] we placed four ROIs in bilateral ASN and PSN. Each ROI covered two consecutive slices comprising eight voxels (one voxel = 2 × 2 × 2 mm). Positioning of the ROIs involved the following steps. First, we identified the slice where the red nucleus was the largest on the b0 image. ROIs were then placed on axial slices, starting with the slice where the red nucleus began to fade away and including the next ventral slice where the red nucleus was no longer seen. The ASN and PSN ROIs were placed in the low-signal region corresponding to the SN. We assessed inter-rater reliability for ROI placement between the primary and secondary raters and ultimately applied the ROIs drawn by the primary rater. Finally, the mean free water values from bilateral ROIs were averaged.[9,10]

Statistical Analysis

All statistical analyses were conducted using IBM SPSS (v26.0). Continuous variables are reported as mean ± standard deviation, whereas categorical variables are reported as the number of cases (proportions). To compare data between four groups, one-way analysis of variance, Kruskal–Wallis W test, and χ^2 test were used for normally distributed continuous variables, nonnormally distributed continuous variables, and categorical variables, respectively. The intra-class correlation coefficient (ICC) was used to assess the inter-rater reliability of manual ROI delineation.

Free water values in the ASN and PSN were compared across the four groups using analysis of

covariance, adjusting for age, sex, and education. To understand whether MAO-B (monoamine oxidase inhibitor-B) treatment affects SN free water,[25] we divided the mPD group based on whether they had chronically used MAO-B and compared the two groups. The relationship between clinical outcomes and PSN free water values in the pPD, dnPD, and mPD groups was investigated using multivariable linear regression analysis, controlling for age, sex, and education in pPD patients and these three variables in addition to disease duration in dnPD and mPD patients. The discriminative power of free water in PSN was assessed using receiver operating characteristic (ROC) analysis. For longitudinal analyses involving pPD subjects, the paired-samples *t* test and the Wilcoxon test were used to test normal and nonnormal data, respectively. For all analyses, results with $P < 0.05$ were considered statistically significant.

Results

Demographic and Clinical Features

A total of 190 individuals participated in this study, comprising 48 HCs, 43 pPD, 50 dnPD, and 49 mPD patients. The demographics and clinical features of these participants at baseline are presented in Table 1. The four groups did not differ significantly in terms of age and sex. However, there were significant differences in education, MMSE, MoCA, HAMD, HAMA, and NMSQ scores. The pPD group had significantly higher HAMD, HAMA, and NMSQ scores than the HCs. Meanwhile, the pPD group had significantly lower MMSE and MoCA scores than the HC group. Moreover, the MoCA scores of the pPD group were significantly lower than those of the dnPD group. Twenty-seven (62.8%) of the pPD subjects were diagnosed with RBD based on the RBDQ-HK scale. Regarding medication in the mPD group, 2.0% of the patients received MAO-B inhibitor (selegiline, rasagiline) alone, 44.9% were treated with MAO-B as an adjunct to other antiparkinsonian medications (Madopar, pramipexole, ropinirole, entacapone, and amantadine), and the remaining 53.1% of patients were not treated with MAO-B inhibitors. The average duration of MAO-B treatment is 3.4 years.

Inter-Rater Reliability

ICC analyses showed good consistency for all SN ROIs. We obtained an ICC value of 0.836 for the left ASN ROI, 0.920 for the right ASN ROI, 0.786 for the left PSN ROI, and 0.742 for the right PSN ROI.

Free Water Values in the SN

As shown by the multivariable analysis (Fig. 1A), the free water values in the PSN ($P < 0.001$) differed significantly among the four groups. Post hoc tests showed that the PSN free water values in the pPD, dnPD, and mPD (0.156 ± 0.026, 0.180 ± 0.024, and 0.150 ± 0.028, respectively) groups were significantly higher than those in the HC (0.131 ± 0.023) group and that the dnPD group had significantly higher PSN free water values than the pPD and mPD groups. In contrast, the ASN free water values did not differ significantly among the four groups (Fig. 1A). In the mPD group, we found no differences in SN free water (ASN, $P = 0.356$; PSN, $P = 0.695$, respectively) between patients treated with and without MAO-B inhibitors.

Distinguishing Power of Free Water Values

ROC curves were drawn to evaluate the distinguishing power of free water values in the PSN (Fig. 1B). The area under the curve (AUC) for the pPD versus HC groups was 0.756 (95% confidence interval [CI], 0.674–0.839; sensitivity, 74.4%; specificity, 70.8%; Fig. 1B(a)). For the pPD versus dnPD groups, the AUC, 95% CI, sensitivity, and specificity were 0.742, 0.642 to 0.842, 78.0%, and 60.5%, respectively (Fig. 1B(c)), whereas for the HC versus dnPD groups, the AUC, 95% CI, sensitivity, and specificity were 0.940, 0.899 to 0.982, 98.0%, and 75.0%, respectively (Fig. 1B(b)).

Association between PSN Free Water and Clinical Symptoms

According to the multivariable regression analyses (Table 2), higher free water values in the PSN were significantly associated with worse UPDRS III scores in the pPD and dnPD groups ($\beta = 0.337$, 95% CI: 0.019–0.654, $P = 0.038$; $\beta = 0.338$, 95% CI: 0.033–0.529, $P = 0.031$, respectively) but not in the mPD group.

Longitudinal Free Water Analysis in pPD Patients

Of all pPD participants, 14 pPD participants completed longitudinal follow-up with a mean duration of 19.2 ± 5.3 months. Longitudinal changes in the clinical characteristics of these subjects are presented in Table S1. The MMSE, HAMD, HAMA, and NMSQ scores were not statistically different over time. At the end of follow-up, pPD subjects had a more severe motor impairment, worse MOCA performance, more severe depression, and worse sleep than at baseline. Among pPD subjects, the PSN free water values at follow-up (0.168 ± 0.040) were significantly higher than those at baseline (0.150 ± 0.023, $P = 0.027$), whereas there was no significant difference in ASN free water values between baseline and follow-up. The ASN and PSN free water values of the two groups are plotted in Figure 1C, which includes the changes in free

TABLE 1 *Demographic and clinical characteristics of subjects at baseline*

Variables	HC (n = 48)	pPD (n = 43)	dnPD (n = 50)	mPD (n = 49)	*P*
Male sex, n (%)	22 (45.8)	24 (55.8)	20 (40.0)	25 (51.0)	0.457[a]
Age (y)	59.4 ± 6.6	59.9 ± 11.2	60.1 ± 8.3	58.9 ± 5.4	0.878[b]
Education (y)	11.2 ± 4.6	10.6 ± 4.1	9.3 ± 3.1	10.2 ± 3.5	**0.009**[b]
Age at onset (y)	–	–	57.6 ± 8.3	53.2 ± 5.5	**0.002**[c]
Disease duration (y)	–	–	2.5 ± 2.2	5.7 ± 3.3	**<0.001**[c]
H&Y stage	–	–	1.7 ± 0.5	2.0 ± 0.7	**0.004**[c]
UPDRS III scores	–	8.0 ± 3.9	22.4 ± 3.1	28.8 ± 10.5	**<0.001**[b]
LEDD (mg/d)	–	–	–	400.7 ± 334.8	–
MMSE scores	28.7 ± 2.4	27.2 ± 3.2	27.1 ± 2.6	26.9 ± 2.4	**<0.001**[b]
MoCA scores	27.0 ± 2.5	23.2 ± 4.6	22.5 ± 3.6	22.7 ± 3.3	**<0.001**[b]
HAMD scores	1.6 ± 2.2	7.2 ± 5.6	11.6 ± 8.1	12.3 ± 8.5	**<0.001**[b]
HAMA scores	0.4 ± 0.8	5.5 ± 3.9	8.0 ± 5.3	12.7 ± 7.8	**<0.001**[b]
NMSQ scores	0.7 ± 1.0	8.3 ± 4.4	9.2 ± 4.5	11.4 ± 5.2	**<0.001**[b]
PDSS scores	–	132.9 ± 12.0	121.1 ± 25.2	100.3 ± 14.7	**<0.001**[b]
RBDQ-HK scores	–	23.8 ± 13.7	–	–	–
RBD subjects, n (%)	–	27 (62.8)	–	–	–

Note: Values are mean ± standard deviation unless otherwise stated. Statistically significant *P* values ($P < 0.05$) are indicated in bold.
[a]χ^2 test.
[b]Kruskal–Wallis W test.
[c]Mann-Whitney *U* test.
Abbreviations: HC, healthy control; pPD, prodromal Parkinson's disease; dnPD, de novo Parkinson's disease; mPD, mediated Parkinson's disease; H&Y, Hoehn and Yahr; UPDRS III, Unified Parkinson's Disease Rating Scale, Part III; LEDD, L-dopa equivalent daily dose; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; HAMD, Hamilton Depression Rating Scale; HAMA, Hamilton Anxiety Scale; NMSQ, Non-Motor Symptoms Questionnaire; PDSS, Parkinson's Disease Sleep Scale; RBDQ-HK, Rapid Eye Movement Sleep Behavior Disorder Questionnaire-Hong Kong.

water values for each patient and mean of the overall group.

Discussion

This study investigated changes in SN free water over the full development course of PD and tested the ability of free water as a marker for detecting and monitoring disease progression in pPD. Our study found that (1) free water values in the PSN of the pPD group were significantly higher than those in the HC group but significantly lower than those in dnPD patients; (2) the mPD group showed decreased PSN free water compared to the dnPD group; (3) higher UPDRS III scores (ie, more pronounced motor symptoms) were associated with increased free water in the pPD and dnPD groups; and (4) at longitudinal follow-up, there was a significant increase in PSN free water values in pPD subjects from baseline to follow-up.

Early diagnosis is crucial for providing potential disease-modifying therapies to PD patients. The use of nuclear imaging methods, such as single-photon emission computed tomography and positron emission tomography, is limited by high cost, low availability, and potential radiation damage.[26] Transcranial substantia nigra ultrasound is not suitable for all patients[27-29] due to the poor penetration of ultrasound through the bone window.[30] In comparison, MRI can be performed in large community cohorts for screening purposes and has demonstrated great potential.[6,26,31] Here, we show that free water in the PSN can be used to identify pPD and dnPD. Indeed, a previous study showed that dopamine active transporter availability is attributed to increased free water in the SN.[9] Notably, changes in free water may not be a direct reflection of dopaminergic neurons; rather, they may be attributed to various causes, including atrophy,[32] blood–brain barrier disruption,[33] and neuroinflammation,[34] all of which are associated with neurodegeneration.[35-38] We previously observed increased dilation of perivascular space in the midbrain of PD patients, suggesting that fluid stagnation might play an important role in this issue.[39] We also observed increased iron content in the SN of PD patients, which may induce oxidative stress and lipid peroxidation.[40,41] The increase in free water in the SN of PD patients could be a result of increased oxidative

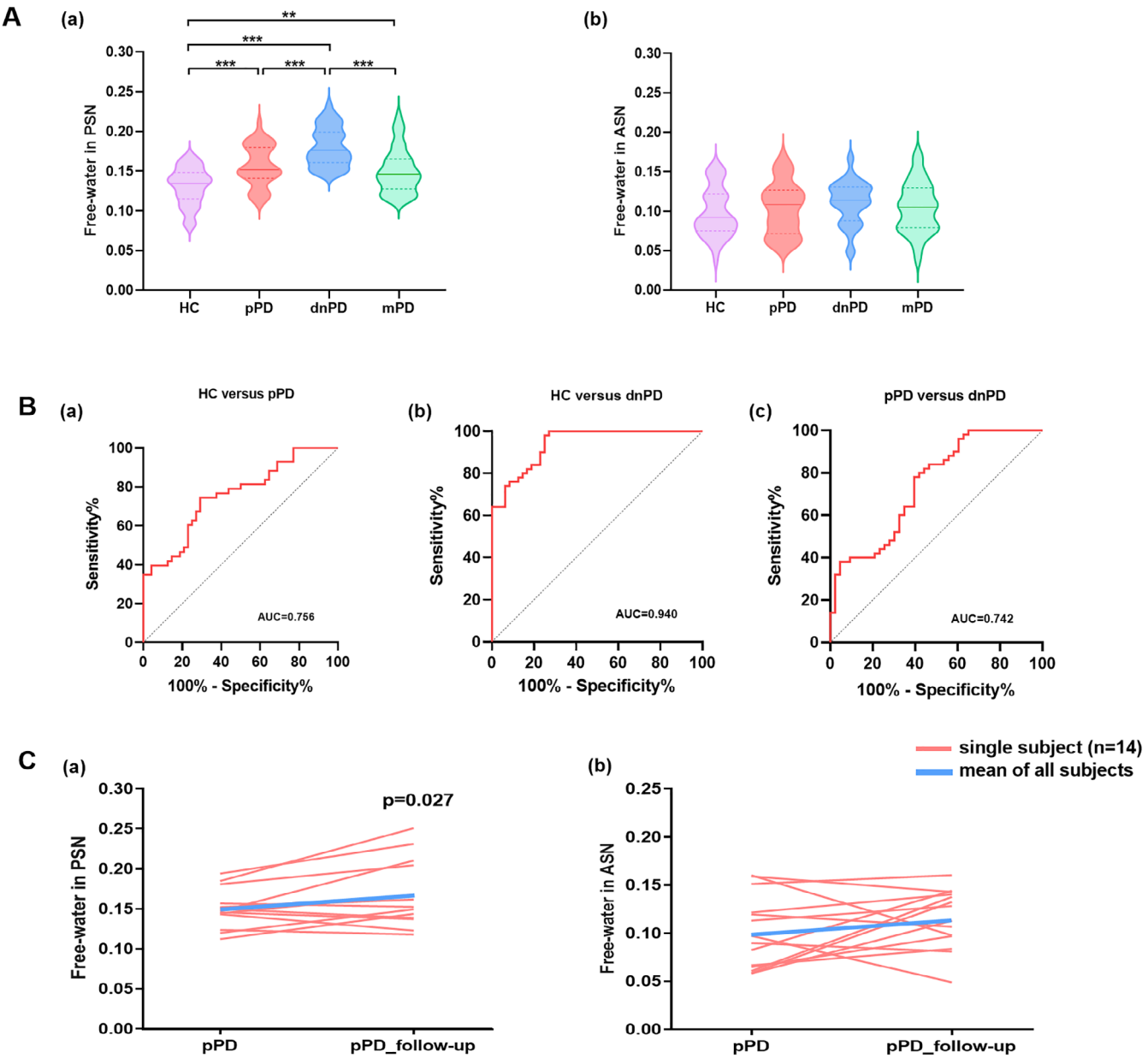

FIG. 1. Results of free water in the SN. (**A**) Free water values in the SN (substantia nigra) among the four groups. The mean free water values of the (a) PSN and (b) ASN for HCs and pPD, dnPD, and mPD patients. (**B**) ROC (receiver operating characteristic) curves showing the classification accuracy of the PSN free water values of (a) HC versus pPD, (b) HC versus dnPD and (c) pPD versus dnPD. (**C**) Longitudinal free water values of SN in pPD subjects. The single subject and mean free water values of all subjects of the (a) PSN and (b) ASN for pPD subjects at baseline and follow-up. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. ASN, anterior substantia nigra; dnPD, de novo Parkinson's disease; HC, healthy controls; mPD, medicated Parkinson's disease; PSN, posterior substantia nigra; pPD, prodromal Parkinson's disease; pPD_follow-up, prodromal Parkinson's disease at first follow-up. [Color figure can be viewed at wileyonlinelibrary.com]

stress.[9] Thus, increased free water may be mechanistically associated with PD pathology.

We found that free water values in the PSN significantly increased in pPD. This finding is consistent with the idea that nigrostriatal dopaminergic neuron loss has already occurred before the diagnosis of PD.[42-45] A previous study found that free water values in the PSN were significantly higher in patients with iRBD, who served as a representative population of pPD,[19] compared to HCs.[14] The results of the present study are consistent with these results. As we used the MDS-pPD criteria for determining inclusion, of which 62.8% of included patients had RBD, our cohort might be more representative of the general PD population. As some patients already had mild motor symptoms[46] (two or more UPDRS III items with a score of 1 or an item with a score of 2 or higher), they might represent a later stage of the prodromal phase.[47] Another notable

TABLE 2 *Multivariable linear regression analysis of free water in PSN and clinical information at baseline in patients*

	pPD (n = 57)		dnPD (n = 51)		mPD (n = 61)	
Variables[a]	**β**[b] **(95% CI)**	***P***	**β**[b] **(95% CI)**	***P***	**β**[b] **(95% CI)**	***P***
H&Y stage	–	–	0.247 (−0.035, 0.529)	0.084	0.071 (−0.269, 0.411)	0.675
UPDRS III scores	**0.337 (0.019, 0.654)**	**0.038**	**0.338 (0.033, 0.529)**	**0.031**	0.142 (−0.172, 0.456)	0.366
MoCA scores	−0.220 (−0.543, 0.103)	0.176	−0.236 (−0.513, 0.040)	0.092	−0.037 (−0.338, 0.264)	0.805

Note: Statistically significant *P* values ($P < 0.05$) are indicated in bold.
[a]Variables are clinical outcomes included in the regression model.
[b]β coefficient (and 95% CI) of free water values in PSN; multivariable linear regression adjusted for age, sex, and education in pPD patients but adjusted for age, sex, education, and disease duration in dnPD and mPD patients.
Abbreviations: PSN, posterior substantia nigra; pPD, prodromal Parkinson's disease; dnPD, de novo Parkinson's disease; mPD, medicated Parkinson's disease; CI, confidence interval; H&Y, Hoehn and Yahr; UPDRS III, Unified Parkinson's Disease Rating Scale, Part III; MoCA, Montreal Cognitive Assessment; –, not available.

finding was that the PSN free water values in the dnPD group were higher than those the pPD group, possibly due to aggravation of dopaminergic degeneration in the SN with disease progression.[4,12] Further, the ROC analysis showed that free water values in the PSN had good accuracy in discriminating dnPD patients from HCs and moderate accuracy for discriminating pPD subjects from HCs. In longitudinal analyses involving pPD subjects, we found that the PSN free water values increased at follow-up, consistent with two previous studies in iRBD and asymptomatic LRRK2 mutation carriers.[14] These results confirmed the potential of free water in detecting SN degeneration and monitoring disease progression at early disease stages.

Due to the complex pathologies underlying free water increase, the relationship between free water and PD development might not be linear. It is worth noting that the PSN free water values showed first an increasing and then a decreasing pattern, peaking at the dnPD group. Furthermore, free water in the PSN could distinguish dnPD from HCs with very high accuracy. Surprisingly, in contrast to two previous studies[10-12] reporting that free water in the PSN continued to increase during follow-up, we found that the PSN free water values in the mPD group were significantly lower than those in the dnPD group. A possible reason may be the effect of drug treatment. Dopamine treatment may influence systemic inflammation by inhibiting the inflammasome,[48] thus indirectly affecting changes in free water. In addition, rasagiline has been widely recognized for its neuroprotective effects[49]; a previous retrospective study showed that PD patients with a history of rasagiline use had lower free water values in the PSN than those who had not chronically taken this medication.[24] Another randomized controlled study[25] found a four times greater increase in pSN free water in PD randomized to placebo compared to PD randomized to rasagiline. However, we did not find any differences between patients treated with and without MAO-B inhibitors. Another possible reason for the discrepancy is disease duration. In this study, the average disease duration in the mPD group was 5.7 years, but the disease durations in previous studies were relatively short (less than 5 years). Although they found that PSN free water increased over time, the trend might have not peaked (probably about 5 years considering all the related evidence). The difference between our study and three previous studies[11,15,25] still needs to be investigated in future studies.

There was no significant difference in free water values in the ASN among the four groups. Meanwhile, the ASN free water values were not different between baseline and follow-up in pPD subjects. These results are consistent with previous imaging and pathology findings.[4,14] As SN degeneration mainly involves the PSN[4,42,50,51] (ie, the ventrolateral layer), it is reasonable that free water in this region is more sensitive to identifying early PD.[11,12]

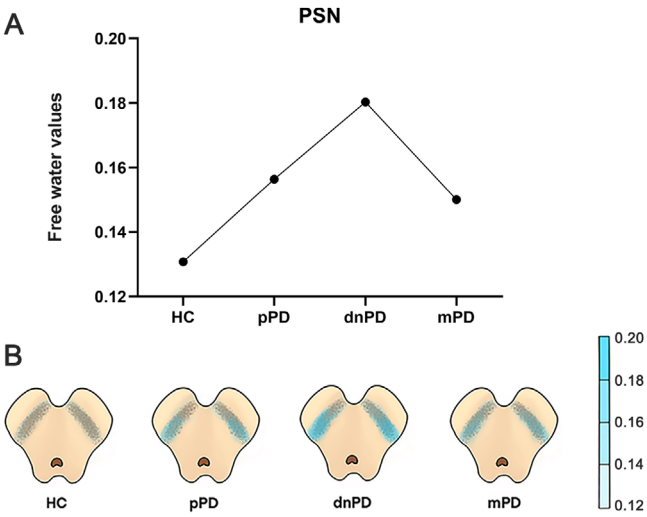

FIG. 2. The progression of SN (substantia nigra) free water in the whole development course of PD. The diagram shows the changes in SN with (**A**) a line chart and (**B**) a picture, respectively. Light blue represents the low free water values, and the deep color represents the high ones. dnPD, de novo Parkinson's disease; mPD, medicated Parkinson's disease; HC, healthy controls; PSN, posterior substantia nigra; pPD, prodromal Parkinson's disease. [Color figure can be viewed at wileyonlinelibrary.com]

To sum up, we described a biphasic changing trend of free water in the SN across the different stages of PD development. In particular, free water in the PSN gradually increased to its maximum from pPD to dnPD but decreased in mPD (Fig. 2). The association between motor symptoms and PSN free water also corresponded with this trend. We found that UPDRS III scores were positively associated with PSN free water values in the pPD and dnPD groups but not in the mPD group. This might suggest that the PSN free water values no longer reflect SN degeneration in later stages. Nonetheless, dopaminergic therapy may confound the associations because it is highly effective at reducing UPDRS III scores.[52,53]

The strength of this study includes studying pPD subjects based on the MDS-pPD research criteria and recruiting subjects at three different stages. Through this comprehensive analysis, we were able to demonstrate the complex changing pattern of PSN free water, providing important evidences for future applications. However, some limitations should be paid attention to. First, it is not clear whether these pPD subjects will eventually develop PD due to the short follow-up. This is a pitfall in all studies performed to date. Nevertheless, as defined by the MDS-pPD research criteria, the probability of transforming to PD is very high. Second, the follow-up analysis of pPD subjects was based on a small sample size and a relatively short follow-up duration. It is undeniable that recruitment of pPD subjects is difficult due to the prevalence of pPD in the elderly population being only 2%.[54] Further recruitment work and longer follow-up durations are needed in future studies.

In conclusion, the PSN free water increased from prodromal to early clinical stages, but the trend might be reversed in late disease stages. Further longitudinal studies are needed to verify this biphasic trend and to understand the underlying pathophysiology, which are crucial for using this marker in research and clinical settings. ●

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Data Availability Statement

The original data for this study can be obtained from the corresponding author via email upon reasonable request.

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.