

Brain proton magnetic resonance spectroscopy in patients with Parkinson's disease

Received: 09 Mar. 2022
Accepted: 06 May 2022

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Keywords

Parkinson Disease; Magnetic Resonance Spectroscopy; Basal Ganglia; Motor Cortex

Abstract

Background: The accuracy of current laboratory and imaging studies for diagnosis and monitoring of Parkinson's disease (PD) severity is low and diagnosis is mainly dependent on clinical examination. Proton magnetic resonance spectroscopy (MRS) is a non-invasive technique that can assess the chemical profile of the brain. In this study, we evaluated the utility of proton MRS in diagnosis of PD and determination of its severity.

Methods: Patients with PD and healthy age-matched controls were studied using proton MRS. The level of N-acetylaspartate (NAA), total creatine (Cr), and total choline (Cho), and their ratios were calculated in substantia nigra (SN), putamen (Pu), and motor cortex. PD severity was assessed by the Unified Parkinson's Disease Rating Scale (UPDRS) and the Hoehn and Yahr scale.

Results: Compared to 25 healthy controls (18 men, age: 59.00 ± 8.39 years), our 30 patients with PD (24 men, age: 63.80 ± 12.00 years, 29 under treatment) showed no significant difference in the metabolite ratios in SN, Pu, and motor cortex. Nigral level of NAA/Cr was significantly correlated with total UPDRS score in patients with PD ($r = -0.35$, $P = 0.08$). Moreover, patients with PD with Hoehn and Yahr scale score ≥ 2 had a lower NAA/Cr level in SN compared to patients with a lower stage.

Conclusion: This study shows that 1.5 tesla proton MRS is unable to detect metabolite abnormalities in patients with PD who are under treatment. However, the NAA/Cr ratio in the SN might be a useful imaging biomarker for evaluation of disease severity in these patients.

How to cite this article: Shoeibi A, Verdipour M, Hoseini A, Moshfegh M, Olfati N, Layegh P, et al. Brain proton magnetic resonance spectroscopy in patients with Parkinson's disease. *Curr J Neurol* 2022; 21(3): 156-61.

Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative brain disorders. From 1990 to 2015, the number of patients with PD has been almost doubled to reach over 6 million.¹ PD has had the most rapidly growing prevalence among all neurological disorders during this period of time and it is estimated that the prevalence will be doubled again by 2040.² Traditionally, PD is characterized by degeneration of the dopaminergic neurons in the substantia nigra (SN) pars compacta and widespread intracellular α -synuclein accumulation forming Lewy bodies.³ Currently, the diagnosis of PD is mainly based on clinical motor symptoms and relies on the presence of bradykinesia as the core clinical finding in association with tremor and/or rigidity, the absence of exclusionary criteria, and adjustment of red flags with supportive findings.⁴ However, PD is associated with a wide range of other motor and non-motor symptoms, some of them may present before the cardinal motor symptoms. In addition, it has been shown that PD has a preclinical phase in which neurodegeneration has commenced without any obvious motor or non-motor findings.⁵ Hence, clinical diagnosis takes place relatively late in the disease course. On the other hand, the accuracy of clinical diagnosis is only slightly above 80%.⁶ Furthermore, the lack of objective assessment tools creates ambiguity in monitoring the efficacy of treatment and following the disease course. These issues highlight the urgent need for discovery of imaging or other biomarkers to improve the accuracy of early diagnosis and also to increase quality of the disease progression monitoring.

Proton magnetic resonance spectroscopy (MRS) allows for a non-invasive assessment of the neurochemical profile of the brain by quantifying a range of metabolites including N-acetylaspartate (NAA) as a marker of neural integrity, choline (Cho) as an indicator of membrane turnover, and creatine (Cr) as the reference standard to normalize the spectral data for comparative purposes.⁷ A few studies have assessed the efficacy of MRS in the diagnosis of PD and the correlation of metabolic ratios with clinical indices such as disease duration and severity; however, they reported inconsistent results.⁸⁻¹⁰

In the present study, we aimed to investigate MRS changes in the SN, putamen (Pu), and primary motor cortex (MC) in the diagnosis and staging of PD. Previous studies using 1.5T

scanners, which are the most available equipment in clinical settings, have shown inconsistent results so far.^{8,11-13} Our data of a relatively larger sample of patients with PD will add to the previous knowledge of the topic.

Materials and Methods

Subjects: Thirty patients fulfilling the International Parkinson Disease and Movement Disorder Society Clinical Diagnostic Criteria for Parkinson's Disease (IPD-MDS-PD)⁴ and 25 age-matched healthy controls were recruited from our movement disorders clinic. Exclusion criteria included the inability to give informed consent, known contraindications of exposure to magnetic fields, diagnosis of a neurodegenerative disorder other than PD, history of a significant neurological illness such as stroke, tumor, and epilepsy, concomitant medical conditions, and substance abuse.^{14,15} Individuals with PD were evaluated using the Unified Parkinson's Disease Rating Scale (UPDRS) composing of 3 subscales: mentation, behavior, and mood (4 questions), activities of daily living (13 questions), and motor examination (14 questions). Each question is a 5-point ordinal scale scoring between 0 to 4 increasing with severity.¹⁶ Disease severity was also staged using the Hoehn and Yahr scale with a range of scores between 0 to 5, in which scores increase with severity of the disease.¹⁷ This study was approved by the Research Ethics Board of Mashhad University of Medical Sciences, Mashhad, Iran (code: IR.MUMS.fm.REC.1395.525) and each participant gave written informed consent before inclusion. Study was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

MRS technique: Proton MRS was performed using a 1.5T clinical scanner (MAGNETOM Avanto, Siemens, Germany). Eight-channel phased-array head coil was used for image obtaining. T2-weighted fluid-attenuated inversion recovery (T2-FLAIR) sequences parameters were as: repetition time (TR) = 10190 ms, echo time (TE) = 100 ms, inversion time (TI) = 2583 ms, matrix = 256 × 205, layer thickness = 5 mm, number of signal averages (NSA) = 1, field of view (FOV) = 230 mm, interval = 1 mm, and scan time = 134 seconds. Single-voxel MRS (point-resolved spectroscopy) was used. The scanning parameters were as: TR/TE, 1500/135 ms, voxel dimensions: 2 × 2 × 2 cm, and FOV: 230 mm. The voxel of interest was placed completely within the SN, Pu, and MC. In

patients with PD, measurements were performed on the side contralateral to the more severely affected limb. The metabolites, including NAA (2.0 ppm), Cr (3.0 ppm), and Cho (3.2 ppm) were calculated, and the following metabolite ratios were determined: NAA/Cr, NAA/Cho, and Cho/Cr.

For each parameter of interest, mean and standard deviation (SD) were computed. To ensure that there were no significant deviations from Gaussian distribution, Kolmogorov-Smirnov normality tests were performed. To test for differences in metabolite concentrations between the patients with PD and healthy controls, an independent samples t-test or Mann-Whitney U was used. Spearman rank correlation test was done to assess the relation of metabolite concentrations with the different clinical scales in the PD group. To investigate the level of metabolites in binary groups, the chi-square test was used. All statistical analyses were carried out using the SPSS software (version 16, SPSS Inc., Chicago, IL, USA) and P-values < 0.05 were considered as statistically significant.

Results

The demographic and clinical features of the patients with PD and healthy subjects are shown in table 1. Of all patients with PD, 24 (80%) were men. Mean age in PD group was 63.8 ± 11.9 and their mean clinical disease duration was 5.8 ± 4.9 years. Mean total UPDRS score was 34.0 ± 11.5 and mean Hoehn and Yahr stage was 1.8 ± 0.7 . Eight out of 30 patients were treated with levodopa alone, 20/30 with levodopa plus dopamine agonist, 1/30 with dopamine agonists alone, and 1/30 was not taking any PD medications.

Compared to the PD group, controls were not significantly different in terms of gender distribution (18/25 were men, $P = 0.20$) or age (mean age: 59.0 ± 8.3 , $P = 0.09$). Proton MRS was conducted in all participants. Due to movement artifacts, the metabolic profile for SN, Pu, and MC could be obtained in 27, 29, and 28 PD cases, respectively.

We found no significant difference between

patients with PD and the control group regarding NAA/Cr, NAA/Cho, and Cho/Cr ratios in the SN, Pu, and MC, although the NAA/Cr ratio in the SN showed borderline significance ($P = 0.06$) (Table 2). Correlations of the above-mentioned metabolic ratios with clinical scores are presented in table 3. Within the PD group, NAA/Cr and Cho/Cr ratios in the SN showed significant negative correlations with the activities of daily living subscale scores ($P = 0.03$ and $P < 0.01$, respectively).

Patients with a Hoehn and Yahr stage of ≥ 2 had a lower NAA/Cr level in the SN compared to those with a milder disease (2.62 ± 0.80 vs. 3.35 ± 0.80 , respectively, $P = 0.04$) (Figure 1). Patients with longer disease duration (more than 5 years) had significantly lower Cho/Cr levels in SN compared to those with a shorter disease course (0.96 ± 0.30 vs. 1.37 ± 0.50 , respectively, $P = 0.02$).

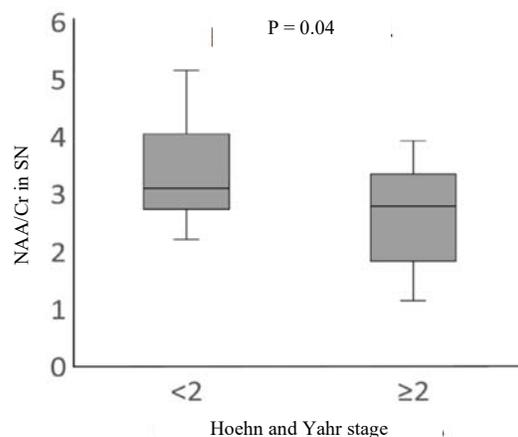


Figure 1. N-acetylaspartate/creatine (NAA/Cr) ratios in the substantia nigra (SN) with respect to Hoehn and Yahr stages

Discussion

In the present study, we measured proton MRS metabolite ratios in patients with PD compared to healthy controls and evaluated their association with the clinical disease severity.

Table 1. Demographic and clinical features

Variable	Parkinson's disease (n = 30)	Healthy control (n = 25)	P
Age (year)	63.80 ± 12.00	59.00 ± 8.39	0.09
Gender (men/women)	24/6	18/7	0.54
Disease duration (year)	5.84 ± 4.92	N/A	N/A
Age at onset (year)	57.96 ± 11.03	N/A	N/A
UPDRS total score	34.27 ± 11.52	N/A	N/A
Hoehn and Yahr stage	1.83 ± 0.72	N/A	N/A

Data are expressed in mean \pm standard deviation (SD) or number

UPDRS: Unified Parkinson's Disease Rating Scale; N/A: Not available

Table 2. Comparison of measured metabolic ratios between Parkinson's disease (PD) cases and healthy controls

Region	Parkinson's disease (n = 30)	Healthy controls (n = 25)	P
NAA/Cr			
SN	2.95 ± 0.93	3.65 ± 1.14	0.06
Pu	2.04 ± 0.70	2.12 ± 1.03	0.60
MC	1.79 ± 0.40	1.68 ± 0.39	0.20
NAA/Cho			
SN	2.92 ± 1.20	3.02 ± 0.80	0.15
Pu	2.52 ± 1.30	2.76 ± 0.85	0.08
MC	2.14 ± 0.60	1.93 ± 0.60	0.70
Cho/Cr			
SN	1.16 ± 0.40	1.26 ± 0.40	0.50
SN	0.98 ± 0.60	0.81 ± 0.48	0.10
Pu	0.87 ± 0.20	0.88 ± 0.10	0.50
MC	1.16 ± 0.40	1.26 ± 0.40	0.50

Data are presented as mean ± standard deviation (SD)

Cho: Choline; Cr: Creatine; MC: Motor cortex; NAA: N-acetylaspartate; SN: Substantia nigra; Pu: putamen

We observed no significant difference between PD and control subjects in any of the measured metabolite ratios in the SN, Pu, or the MC. However, we found that mean NAA/Cr ratio in the SN was lower in the PD group compared to healthy controls with borderline statistical significance. This indicates that significant results might be obtained in future studies evaluating larger samples. In addition, we found a negative correlation between the NAA/Cr level in SN and total UPDRS score in patients with PD which indicates that this metabolite ratio could be useful in evaluation of PD severity and progression.

In their study, Cao et al. used a 1.5T magnetic resonance imaging (MRI) scanner and found that patients with PD who were off their dopaminergic medications for over 12 hours had a significantly

lower NAA/Cr level in the SN compared to healthy controls.¹⁸ Moreover, Groger et al. found that rostral and caudal SN had clear differences in the biochemical profiles;¹⁹ thus, using more precise voxel placement may be reasonable in future studies.

While several studies have shown an altered metabolic status in untreated de novo patients compared with healthy controls, further investigations demonstrated that treatment of PD could restore the metabolic status.^{20,21} It has been shown that, compared to healthy controls, drug-naïve patients have significantly lower putaminal NAA/Cho level; however, the levodopa-treated group showed no difference.²² Similarly, Mazuel et al. in a crossover study demonstrated that an acute 200 mg levodopa dose could increase the level of NAA and Cr in the Pu.²³

Table 3. Correlation of metabolic ratios in various measured brain areas with scores of subscales of Unified Parkinson's Disease Rating Scale (UPDRS)

Region	UPDRS score		Part I: mentation, behaviour, and mood		Part II: activities of daily living		Part III: motor examination		Hoehn and Yahr stage	
	r	P	r	P	r	P	r	P	r	P
NAA/Cr										
SN	-0.35	0.08	-0.21	0.30	-0.41	0.03	-0.24	0.20	-0.28	0.10
Pu	-0.14	0.46	-0.23	0.20	-0.13	0.50	-0.04	0.80	0.05	0.79
MC	0.05	0.81	-0.14	0.40	-0.09	0.60	0.22	0.20	0.02	0.80
NAA/Cho										
SN	0.15	0.47	0.23	0.25	0.27	0.18	0.04	0.80	0.06	0.70
Pu	0.06	0.77	0.10	0.59	0.04	0.80	-0.03	0.80	-0.43	0.01
MC	0.06	0.76	-0.13	0.40	-0.10	0.60	0.27	0.10	-0.08	0.50
Cho/Cr										
SN	-0.39	0.05	-0.38	0.05	-0.58	< 0.01	-0.20	0.30	-0.27	0.16
Pu	-0.10	0.60	-0.20	0.30	-0.03	0.80	-0.07	0.70	0.35	0.06
MC	-0.04	0.83	0.04	0.80	0.01	0.90	-0.15	0.40	-0.04	0.80

UPDRS: Unified Parkinson's Disease Rating Scale; Cho: Choline; Cr: Creatine; MC: Motor cortex; NAA: N-acetylaspartate; SN: Substantia nigra; Pu: Putamen

The fact that 29/30 of PD cases in our study were under dopaminergic treatment might explain the lack of significant difference in metabolite ratios between PD group and healthy controls, especially in the SN and the Pu in our sample. This might also apply to our findings in MC. Our findings in the MC were similar to those of a study by Tedeschi et al.,¹¹ but not to other studies showing significantly lower NAA/Cr level in de novo patients.²⁴ On the other hand, Ciarleo et al. found that a 10-month course of ropinirole treatment was associated with an increase in NAA/Cr and NAA/Cho in MC, which were highly correlated with UPDRS motor scores.²⁰

Cho/Cr ratio in the SN was negatively correlated with the total UPDRS score in our study. This is in contrast to Cao et al. who found a positive correlation between these two variables.¹⁸ In addition to the technical differences, such as the volume of the voxels and features of the MRI devices, the fact that the patients in the Cao et al.'s study had overall higher UPDRS score may be responsible for this contradiction. Surprisingly, other studies have also shown contradictory results regarding the Cho/Cr ratio in MC.^{20,21} It might be possible that the Cho/Cr ratio can change in the course of PD in a non-linear pattern, highlighting the necessity of further longitudinal studies on these metabolites.

In the present study, we showed a negative correlation between NAA/Cr in SN and activities of daily living - as a subjective marker of PD progression. Moreover, the significantly lower levels of NAA/Cr in the SN in patients with Hoehn and Yahr stages greater than 2 compared to patients with PD with milder disorder were in agreement with previous studies.^{18,25,26} The fact that NAA/Cr ratio in patients with disease duration of longer than 5 years was significantly lower than those with shorter disease course might suggest that NAA/Cr ratio in the SN can serve as a marker for evaluation of disease progression.^{18,20,21}

Numerous factors have led to inconclusive results on the efficacy of MRS in diagnosing and grading of PD. While most studies have assessed the metabolic ratios, some researchers believe that the absolute quantity of metabolites is more

helpful.^{8,27} Furthermore, the newly introduced metabolites including myo-inositol, glutamate, and lactate have been shown as alternative candidates that may increase the diagnostic accuracy.^{23,28,29}

Our study suffers from a few limitations. First, we used a 1.5T MRI machine. Although the use of a 3T or 7T MRI machine will improve the quality of imaging, 1.5T device is the most routinely available equipment in most medical centers worldwide. Second, we measured SN as a single area, while the dopaminergic neurons of the nigrostriatal pathway are mainly located lateral to those of the mesolimbic pathway.³⁰ Finally, the number of drug-naïve patients was very low (one) in our study. Despite these limitations, we showed that the NAA/Cr ratio in the SN might be a useful imaging marker for evaluation of disease severity. Larger studies employing 3T or 7T MRI machines with more precise voxel placement are recommended to evaluate the value of proton MRS in the diagnosis of PD and monitoring of the disease severity. Proton MRS is a useful tool in the evaluation of clinical severity and progression of PD but not in its diagnosis in routine clinical settings using 1.5T scanners.

Conclusion

Our results showed that lower NAA/Cr level in the SN was associated with greater disease severity among patients with PD; however, we were unable to find any biochemical difference between patients with PD and healthy controls. These findings imply that PD disintegrates the neuronal network in advanced stages. Further research is needed to refine MRS techniques as biomarkers of neuronal injury in early PD stages.

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

We acknowledge Vice-Chancellor for Research of Mashhad University of Medical Sciences for funding this study (grant number: 950538) and wish to acknowledge all participants in this study.

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