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# In Vivo $^1$H MR Spectroscopic Study on Levodopa-Treated Parkinson's Disease

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***Abstract:*** Authors evaluated alterations of observable metabolite ratios between the cerebral lesion and the contralateral region related to the clinical symptomatic side in levodopa-treated Parkinson's disease (PD) and investigated correlation between age in patients with PD and metabolite ratios of the lesion. Patients with levodopa-treated PD (n = 54) and age-matched normal controls (n = 15) underwent magnetic resonance spectroscopy (MRS) examinations using a stimulated echo acquisition mode (STEAM) pulse sequence that provided 2x2x2 $cm^3$ volume of interest in the selected regions of substantia nigra (SN) and putamen-globus pallidus (PG). To evaluate dependence of metabolite ratios on age, we divided into two groups (i.e., younger and older age). We quantitatively measured N-acetylaspartate (NAA), creatine (Cr), choline-containing compounds (Cho), inositols (Ins), and the sum of glutamate (Glx) and GABA levels and obtained proton metabolite ratios relative to Cr using a Marquart algorithm. Compared with the contralateral region, a significant neuronal laterality of the NAA/Cr ratio in the lesion of SN related to the clinical symptomatic side was established (P = 0.01), but was not established in the lesion of PG (P = 0.24). Also, Cho/Cr ratio tended toward significance in the lesion of SN (P = 0.07) and was statistically significant in the lesion of PG (P = 0.01). Compared with that in the younger age group, NAA/Cr ratio in the older age was decreased in the lesion of SN (P = 0.02), while NAA/Cr ratio was not statistically significant in the lesion of PG (P = 0.21). Significant metabolic alterations of NAA/Cr and Cho/Cr ratios might be closely related with functional changes of neuropathological process in SN and PG of levodopa treated PD and could be a valuable finding for evaluation of the PD. A trend of NAA/Cr reduction, being statistically significant in older patients, could be indicative of more pronounced neuronal damage in the SN of the progressive PD.

**Key words**: Parkinson's disease; Putamen-Globus pallidus (PG); Substantia nigra (SN);levodopa; magnetic resonance spectroscopy (MRS)

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## INTRODUCTION

Parkinson's Disease (PD) is a progressive, neurodegenerative disease of the extrapyramidal system that is clinically characterized by akinesia, rigidity and tremor. Degeneration of the dopamanergic neurons of substantia nigra (SN) and their axon terminals in striatum (caudate nucleus and putamen), leading to a reduction of striatal dopamine is the pathological process of this disease. In PD, many of the cells of the SN, and a few cells in the striatum are commonly damaged. Deficiency of the neurotransmitter dopamine seems to be central in accounting for the attending motor symptoms. Other Parkinsonian syndromes include additional degenerative changes affecting other basal ganglia structures. Thus, neuronal changes within SN and striatum have important diagnostic and prognostic implications for patients with PD[1].

$^{1}$H Magnetic Resonance Spectroscopy (MRS) is a non-invasive technique that allows the concentration of a number of cerebral metabolites to be measured *in vivo*. $^{1}$H MRS at a short echo time (TE) is able to detect metabolites with short T2 relaxation times. The major metabolites detected are N-acetylaspartate (NAA), a marker for neuronal tissue, creatine/phosphocreatine (Cr), an indicator of energy status, and choline-containing compound (Cho), a metabolite involved in membrane synthesis and degradation[2,3]. There are also several other peaks present in the proton spectrum that can assigned to important metabolites, including myo-inositols (Ins), intracellular signal transduction, and GABA, glutamine and glutamate (Glx), the metabolism of neurotransmitters. Biochemical information may be obtained about local cellular metabolism by determining peak metabolite ratios of the neurochemicals detected in the spectra[4]. These metabolite ratios may have important implications regarding the neurologoical disorders: namely, tumors[5], stroke[6-8], multiple sclerosis[9,10], AIDS and HIV related disorders[11,12], and neurodegenerative disorders such as Huntington's disease and Alzheimer's disease [13,14].

Recently, *in vivo* $^{1}$H MRS studies have been also used in the study of Parkinson's disease localized the volume of interests involving the putamen, globus-pallidus, lentiform nucleus (putamen and globus-pallidus, PG), substantia nigra, and cerebellar cortex [15-17]. Using this technique, the present study evaluated the metabolite ratios concerning the neuronal changes of the local cerebral metabolism in potential locations in levodopa-treated PD. Especially, this study was based on the comparison of the metabolite ratios between the lesion and the contralateral of SN and PG. The aims of the study were to evaluate whether there are significant neuronal alterations of observable metabolite ratios between the cerebral lesion and the contralateral region related to the clinical symptomatic side in levodopa-treated PD and whether there is a correlation between age of patients and NAA/Cr ratio in the lesion of SN and PG.

## MATERIALS AND METHOD

### *Subjects*

During the period from August 1996 to March 1999, patients with Parkinson's disease were recruited from the Neurologic Clinics at Kangnam St. Mary's Hospital. Fifty-four patients with Parkinson's disease of mean age 56.8 years (26 males and 28 females; age range 41-73 years) and mean disease duration 4.7 years (range 1.3-12 years) that have treated with levodopa were included.

In order to evaluate an age dependence of metabolite ratios, the PD patients were classified into two age groups; the younger group (41-50 years; mean age 46.2 years, n = 17) and the older (51-73 years; mean age 60.4 years, n = 37).

Each patient was diagnosed with Parkinson's disease by neurologists using criteria of the United Kingdom Parkinson's Disease Society Brain Bank and completed a questionnaire detailing patient history, symptoms and medication. After complete description of the study to the subjects, written informed consent was obtained from patients and controls.

Fifteen age-matched controls with mean age 54.8 years (7males and 8 females; age range 39-64 years) were also examined.

### *$^1$H Magnetic Resonance Spectroscopy*

All $^1$H MRS studies were performed on a 1.5T MRI/MRS system (GE Signa Advantage, Version 4.8; GE Medical System, Milwaukee, WI) with a standard quadrature birdcage head coil. The localized single voxels (2x2x2 $cm^3$; 8mL) in the left and the right of SN and PG were selected using the T2-weighted MR images (TR 2500 ms; TE 90 ms). The stimulated-echo acquisition mode (STEAM)[18,19] was used with TR 2000 ms, TE 20 ms, data points of 2048, spectral bandwidth of 2500Hz, and acquisition averages of 128. $^1$H MR spectra were obtained both from the voxels in the lesion and the contralateral of the SN and the PG related to the clinical symptomatic side of each subject. It took approximately 1.5 hour per case, including the total acquisition time of $^1$H MR spectra. Raw data were transferred to a Sun SPARC station IPC (Sun Micro System, Mountains View, CA) and processed by using SAGE data analysis package (GE Medical System, Milwaukee, WI).

The shim procedure was performed to optimize the magnetic field homogeneity over the entire volume of interest detected by the receiver coil and focused on the water signal. After auto-prescan, typical line width (full width at half maximum; FWHM) was usually 3 to 4 Hz. After Fourier transformation and zero order phase correction, phased absorption spectra were obtained directly without baseline corrections or resolution enhancement. Peak areas were obtained from the phased spectra by employing the Marquardt algorithm to fit a series of Lorentzian lines[21]. Proton resonances in the spectra obtained from brain tissues were assigned on the basis of prior assignments[21]. Resonance peak assignments of major *in vivo* $^1$H MRS observable metabolites were $CH_3$ of NAA, 2.00 ppm; N-$CH_3$ of Cr, 3.00 ppm; N-$(CH_3)_3$ of Cho, 3.20 ppm; $\gamma$-$CH_2$ of Glu and GABA (Glx), 2.35, 2.25 ppm; $H_4$ and $H_6$ of Ins, 3.50 ppm. To obtain the relative metabolite ratios, Cr was used as a putative reference [22].

Results are expressed as mean ± SD of NAA/Cho, NAA/Cr, Cho/Cr, Glx/Cr, and Ins/Cr ratios.

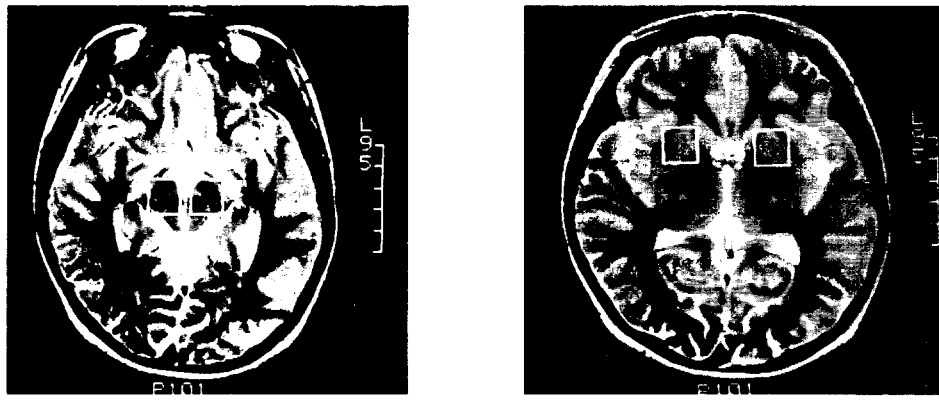
***Statistics***

Statistical analysis was performed by SPSS (Windows Version 6.0, SPSS Inc., Chicago, IL). The data were analyzed with independent samples *t*-test for comparison of two group data, PD patients and age-matched controls, and paired-samples *t*-tests for bilateral spectra within a group, where $P<0.05$ was considered significant to account for multiple comparisons. In particular, Pearson product-moment (bivariate) analysis performed a significance of metabolite ratios between the lesion and the contralateral of SN and PG of PD with levodopa treatment. A simple linear regression least-squares fit would provide the information of a correlation between age of patients and NAA /Cr ratio.

## RESULTS

Fig. 1 shows typical T2-weighted axial MR images in the levodopa-treated Parkinson's disease including the right and the left voxels centered on the SN and the PG. The typical spectra obtained from the volume of interest centered on the lesion and the contralateral voxels of SN and PG related to the clinical symptomatic side in PD patients with levodopa treatment are shown in Fig. 2.

Tables 1 and 2 show the results of the mean metabolite ratios for the levodopa-treated PD patients compared with age-matched controls. No significant difference between these two groups was statistically established. Distinct lactate signals could not be detected in levodopa-treated PD patients or age-matched controls.



(a) (b)

**Fig. 1.** Typical T2-weighted axial MR images in levodopa-treated Parkinson's disease including the right and the left voxels of substantia nigra (a) and putamen-globus pallidus (b) selected for localized *in vivo* $^{1}$H MRS.

***Substantia nigra***

Compared with the contralateral region, a significant neuronal laterality of NAA/Cr ratio was statistically observed in the cerebral lesion of SN related to the clinical symptomatic side in the levodopa-treated PD (1.63 ± 0.56 versus 1.41 ± 0.47, P = 0.01). Cho/Cr ratio tended toward to be significant in the lesion compared with the contralateral in SN (0.98 ± 0.38 versus 0.86 ± 0.33, P = 0.07). In particular, Ins/Cr ratio was significantly increased in the lesion of SN (0.91 ± 0.44 versus 0.78 ± 0.37, P = 0.03). Except for the NAA/Cr and Ins/Cr ratios, no significant laterality of other metabolite ratios such as NAA/Cho, Cho/Cr, and Glx/Cr ratios were established at short TE.

***Putamen-Globus pallidus***

NAA/Cr ratio was not significantly established in the lesion compared with the contralateral of PG related to the clinical symptomatic side in levodopa-treated PD (1.32 ± 0.42 versus 1.25 ± 0.27, P = 0.24). Compared with the contralateral, however, a significant neuronal laterality of Cho/Cr ratio was observed in the lesion of PG (0.79 ± 0.25 versus 0.68 ± 0.20, P = 0.01). Thus, except for the Cho/Cr ratio in PG, no significant laterality of other metabolite ratios such as NAA/Cho, NAA/Cr, Ins/Cr, and Glx/Cr ratios were established at short TE.

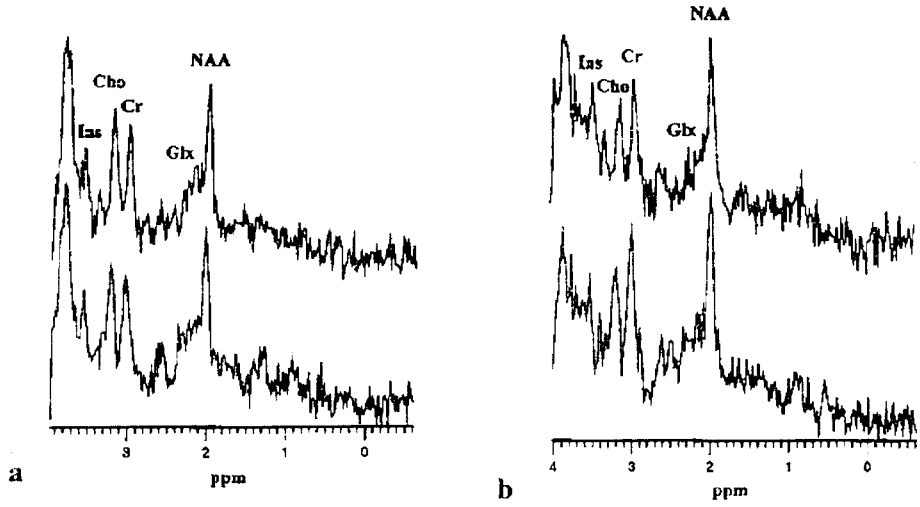



**Fig. 2.** Typical proton spectra obtained from the right and left voxels centered on the substantia nigra (a) and the putamen-globus pallidus (b) in a patient with levodopa-treated Parkinson's disease with related to the clinical symptomatic side.

*Age and NAA/Cr*

Fig. 3 shows correlation between age of patients and NAA/Cr ratio in the lesion of SN and PG related to the clinical symptomatic side in PD with levodopa treatment. A significant correlation was statistically appeared in the lesion of SN, but was not showed in PG ($r = -0.34$, $P = 0.01$; $r = -0.16$, $P = 0.25$). Compared with the younger group, a significant decrease of NAA/Cr ratio in the older group was observed in the lesion of SN ($1.84 \pm 0.69$ versus $1.48 \pm 0.41$, $P = 0.02$). However, there was no significant difference of NAA/Cr ratio in the lesion of PG ($1.44 \pm 0.39$ versus $1.30 \pm 0.34$, $P = 0.21$).

**Table 1.** Mean metabolite ratios for examining significant differences between the lesion and the contralateral of SN in levodopa-treated PD patients.

| | Controls (n = 15)* | Lesions (n = 54)* | Contralaterals (n = 54)* | P Value |
|---|---|---|---|---|
| NAA/Cho | 1.62 ± 0.57 | 1.83 ± 0.83 | 1.80 ± 0.58 | 0.91 |
| NAA/Cr | 1.50 ± 0.58 | 1.63 ± 0.56 | 1.41 ± 0.47 | 0.01† |
| Cho/Cr | 0.97 ± 0.41 | 0.98 ± 0.38 | 0.86 ± 0.33 | 0.07 |
| Ins/Cr | 0.74 ± 0.21 | 0.91 ± 0.44 | 0.78 ± 0.37 | 0.03† |
| Glx/Cr | 0.74 ± 0.21 | 0.71 ± 0.26 | 0.67 ± 0.27 | 0.42 |

Note. NAA = N-acetylaspartate, Cr = creatine, Cho = choline-containing compounds, Ins = inositols, Glx = the sum of glutamate and GABA, and SN = substantia nigra.

* Ratios are given as the mean ± SD.

† Statistical significance determined by using the paired-samples *t*-tests for bilateral spectra within PD patients, where $P<0.05$ was considered significant to account for multiple comparisons.

**Table 2.** Mean metabolite ratios for examining significant differences between the lesion and the contralateral of PG in levodopa-treated PD patients.

| | Controls (n = 15)* | Lesions (n = 54)* | Contralaterals (n = 54)* | P Value |
|---|---|---|---|---|
| NAA/Cho | 1.68 ± 0.41 | 1.83 ± 0.76 | 1.98 ± 0.73 | 0.24 |
| NAA/Cr | 1.30 ± 0.45 | 1.33 ± 0.42 | 1.25 ± 0.27 | 0.25 |
| Cho/Cr | 0.79 ± 0.25 | 0.79 ± 0.26 | 0.68 ± 0.20 | 0.01† |
| Ins/Cr | 0.69 ± 0.24 | 0.60 ± 0.24 | 0.61 ± 0.23 | 0.85 |
| Glx/Cr | 0.77 ± 0.34 | 0.72 ± 0.25 | 0.71 ± 0.21 | 0.77 |

Note. PG = putamen-globus pallidus (Lentiform nucleus).

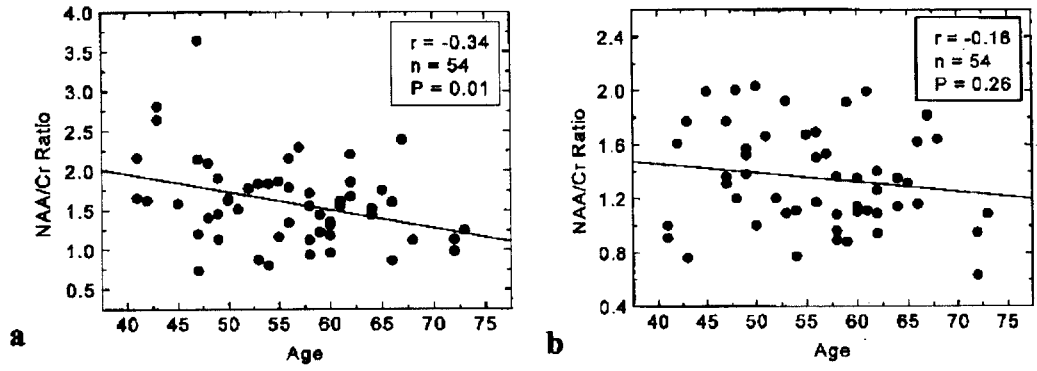



**Fig. 3.** Correlation between age of patients and NAA/Cr ratio observed from the lesion in substantia nigra (a) and putamen-globus pallidus (b) of Parkinson's disease patients with levodopa treatment (age range 41-73 years; mean age 56.8 years, mean disease duration 4.7 years).

## DISCUSSION AND CONCLUSIONS

*In vivo* $^{1}$H MRS is a noninvasive approach to measuring important proton metabolism in PD. Using this technique at a long TE, several investigations of the brain in levodopa-treated PD have been recently reported[15,16]. Holshouser et al (15) found no significant difference of NAA/Cho ratio between the left and the right centered on the globus pallidus in levodopa-treated PD. Ellis et al (16) reported that no difference of NAA/Cho ratio was found comparing with the ratios from the left and the right putamen in chronically levodopa-treated dyskinetic PD. Furthermore, they did not observe significant differences of other metabolite ratios such as NAA/Cr and Cho/Cr from both the left and the right in the regions of their interest. The present results indicated that NAA/Cho ratio between the left and the right in SN and PG was not statistically significant for levodopa-treated PD and were consistent with above other studies.

On the basis of NAA/Cr ratio between the lesion and the contralateral related to the clinical symptomatic side, we had previously reported the significant neuronal laterality in SN and PG of PD with unilateral symptoms[17]. We suggested that NAA/Cr ratio might be related to the clinical symptomatic side. Using the same protocol, we observed consistently a significant neuronal laterality of NAA/Cr ratio in the lesion of SN related to the clinical symptomatic side in levodopa-treated PD. These findings may indicate considerably increased NAA and unaffected Cr levels in the lesion of SN. NAA is an amino acid confined

in the brain only to neurons, and usually considered as a marker of neuronal integrity[23]. The Cr peak is relatively stable in interpreting many pathological MR spectra and has been used as an internal standard in previous study on basal ganglia[22]. Then, there might be remarkable neuronal laterality to produce significant increase in NAA level upon MRS examination, and that NAA may provide as a marker for neuronal change in levodopa-treated PD. Comparing with age-matched controls, we observed the neuronal loss in at least one of the two regions in SN. Slight neuronal accumulation (9%) was observed in the lesion in levodopa-treated PD, while slight neuronal loss (6%) was found in the contralateral. Moreover, a significant difference of the Ins/Cr ratio that has been found in Alzheimer's disease[24] was observed in the lesion of SN related to the clinical symptomatic side. Other metabolite ratios such as Cho/Cr and Glx/Cr did not show any significant neuronal laterality in the lesion of SN.

Compared with age-matched controls, Cho/Cr ratio was not significantly different in the lesion and the contralateral of SN and PG in levodopa-treated PD. However, A significant neuronal laterality of Cho/Cr ratio was observed from the lesion compared with the contralateral of PG, and were not established but tended to be significant in SN. These findings may indicate increased Cho and unaffected Cr levels in the lesion of PG related to the clinical symptomatic side. Changes of Cho in $^1$H MR spectrum reflect variations of the concentration and physiochemical state of choline-containing compounds, which are abundant in all cell synthesis and degradation. Thus, a significant neuronal laterality of the Cho/Cr ratio may indicate some membrane alteration or a possible functional change of the Cho metabolism in the PD with levodopa treatment. We suggest that the Cho may be closely correlated to the neuronal change in the lesion of PG related to the clinical symptomatic side. Except for the Cho/Cr ratio in PG, other metabolite ratios such as NAA/Cr, Ins/Cr and Glx/Cr ratios did not observed any significant neuronal laterality in the lesion of the levodopa-treated PD.

An additional analysis of our data in Fig. 3 showed correlation between the age of PD patients and the NAA/Cr ratio in the lesion of SN and PG related to the clinical symptomatic side. A significant correlation was appeared in the lesion of SN, which many of cells was commonly damaged in PD, but was not showed in the lesion of PG, which a few cells was damaged. We suggest that the age of PD patients with the levodopa treatment could be closely correlated with the neuronal change of NAA/Cr ratio in the lesion of SN related to the clinical symptomatic side. Classifying the PD patients into two age groups, we found a significant reduction of NAA/Cr ratio in the lesion of SN of the older age group. Therefore, advanced the age of PD might be associated with the more pronounced neuronal damage in the lesion of SN related to the clinical symptomatic side, as detected using *in vivo* $^1$H MRS.

In conclusion, using $^1$H MRS with the STEAM localization sequences at a short TE, the present study demonstrated the variations of the metabolic ratios in the SN and PG in PD with the levodopa treatment. Significant neuronal changes of NAA/Cr and Cho/Cr ratios

might be closely related with the alterations or the functional changes of metabolism in the SN and the PG of levodopa-treated PD, and may be a valuable criterion for evaluation of PD. A trend of NAA/Cr reduction, being statistically significant in the older patients, might be indicative of more pronounced neuronal damage in the SN of the progressive PD. Therefore, $^{1}$H MRS could be a useful modality to evaluate the diagnostic and prognostic implications for the PD with the levodopa-treatment.

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