Original Article

T2-weighted MRI in Parkinson's disease; Substantia nigra pars compacta hypointensity correlates with the clinical scores

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Background: Iron accumulation in substantia nigra pars compacta (SNpc) and related intensity and volumetric changes in patients with idiopathic Parkinson's disease (PD) has been reported previously. There are only a few studies evaluating the relation between neuroradiological findings and clinical scores, with contradictory results. **Aims:** In this study we aimed to measure the iron-rich brain areas of PD patients and healthy subjects with T2-weighted magnetic resonance imaging (MRI) and to evaluate the relation between the clinical scores of PD patients and these imaging results. **Methods and Materials:** T2-weighted MRI findings were studied in 20 patients with PD and 16 healthy controls. The width of SNpc, putamen volume, and the intensity of the basal ganglia were measured. Unified Parkinson's Disease Rating Scale (UPDRS) was used for evaluating the clinical status. **Statistical Analyses:** Mann Whitney U test for group comparisons, Wilcoxon sign rank test for comparisons within the patient group, and Spearman's rank correlation coefficient for analyses of correlations were used. **Results:** Mean SNpc and dentate nucleus intensities were lower in PD patients than healthy subjects. Mean SNpc width and putamen volumes were lower in patients. Decrease in the intensity of mean SNpc correlated with high UPDRS and rigidity scores. **Conclusion:** The results of our study reflect the increase in iron accumulation and oxidative stress in the SNpc in Parkinson's disease. The decrease in the intensity of SNpc correlates with poor clinical scores.

Key Words: Parkinson's disease, magnetic resonance imaging, susbstantia nigra, dentate nucleus, iron

Introduction

Idiopathic Parkinson's Disease (PD) is characterized by dopamine deficiency owing to the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) region of the midbrain. Neuronal loss has been attributed to the presence of excessive oxygen radicals[1] and iron has been blamed for the production of these radicals.[2,3] Iron concentration increases with age, in the brain.[4,5] In the human brain, iron shows an uneven distribution, with high levels in the basal ganglia (substantia nigra, putamen, caudate nucleus, and globus pallidus), red nucleus, and dentate nucleus.[6,7] In PD, iron accumulation in the globus pallidus and substantia nigra (SN) is more pronounced than in the liver and other organs.[8]

Iron deposits would create local inhomogenities in the magnetic field which in turn, would result in loss of the T2 signal. Magnetic resonance imaging (MRI) as a tool to measure regional iron concentrations should provide more information regarding the relationship between iron accumulation and parkinsonian symptoms. Iron distribution is clearly mapped as signal hypointensity (darkness) on T2-weighted image due to local-field heterogeneities produced by ferritin.[9] Heavily T2-weighted high-field MRI provides a unique opportunity for the evaluation of the extrapyramidal motor system. The increase in tissue iron is significant in magnitude, it is observable in postmortem parkinsonian brain,[10,11,12] and can be evaluated with imaging techniques in living subjects.[6,13,14,15] But some postmortem studies have not found increased iron levels in the SN of patients with PD compared to age-matched normal controls.[7,16] The results of the studies evaluating the availability of ferritin in the brain of PD patients are also conflicting, with indications that ferritin is both increased and decreased.[7,12,17,18]

In PD, iron is selectively increased only in the nigral region containing pigmented dopaminergic neurons called the SNpc.[11,14,19,20] Many studies measured the width of the SNpc in PD patients on T2-weighted MRI and reported a decrease in width.[21,22,23,24]

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In the literature there are only a few studies focusing on the correlation between MRI findings and clinical scores of the PD patients. Antonioni et al reported in their study that the hypointensity of the SN, and clinical findings of patients with PD were not correlated.[6] In another study the width of the SNpc was found to be correlated with motor performance in both healthy elderly and PD groups.[23] On the other hand there is also a study reporting no correlation between the width of SNpc and clinical scores in the Hoehn-Yahr scale.[21]

In this study, it was aimed to measure the iron-rich brain areas (SNpc, SN pars reticulata, putamen, caudate nucleus, globus pallidus, red nucleus, and dentate nucleus) of PD patients and healthy subjects with T2-weighted MRI. We compared the neuroradiological measurements and evaluated the relation between the clinical scores of PD patients and these imaging results.

Materials and Methods

In this prospectively designed study, 20 patients with PD (6 women, 14 men) from the movement disorders outpatient clinic of the Turkish Ministry of Health Ankara Research Hospital were included. Sixteen healthy subjects (5 men, 11 women) were taken as control group. The mean age of the patient group was 66.7± 8.5 and of the control group was 57.19± 9.46 PD patients had a mean disease duration of 6.25±3.31 years. The study was done between January 1999 and January 2000. As control group, 16 volunteers without previous neurological disorders, selected from amoung non-neurological patients visiting the outpatient department of the said hospital and some hospital employees were recruited. None of the control participants had any known relatives with Parkinson's disease and all were examined and questioned to rule out signs and symptoms suggestive of early Parkinson's disease. All subjects were native Turkish people and were told the purpose of the MR examination, and they were enrolled in the study with their consent. Ministry of Health Ankara Research Hospital's medical ethics committee approved the study protocol. Informed consent was obtained from each subject according to the Declaration of Helsinki[25] to participate in further examinations, including a thorough neurological examination, and MRI examination.

Clinical diagnosis of PD was made according to the published criteria.[26] Particular attention was paid to exclude patients with the "Parkinson's plus" syndrome or with a family history of movement disorders. Secondary causes of parkinsonism like intracranial hemorrhage, calcification, trauma, infarction and multi system atrophy (MSA), progressive supranuclear palsy (PSP) and Shy Drager syndrome were excluded by clinical examination and imaging techniques. PD severity was assessed using the Unified Parkinson's Disease Rating Scale (UPDRS). Accepted clinical scores (rigidity and finger tapping scores) were the sum of the right and left extremity scores. All patients scored in the normal range (24 or above) on the Mini-Mental State Examination (MMSE).

The same radiologist who was blind to the patients' diagnosis evaluated the MRI findings of all patients and controls. MR imaging was performed using a VISART system with a superconducting magnet at field strength of 1.5 Tesla (Toshiba, Japan). All patients were taking their anti-Parkinson's disease medication at the time of imaging. The head position in the scanner was stabilized during the scanning procedure using Velcro straps and foam head supports. A coronal pilot image was acquired and used to align the subsequent sagittal pilot images. The middle slice of the sagittal pilot images was aligned on the coronal pilot to obtain a true midsagittal image. After the sagittal pilot images were acquired, the midsagittal image was used to obtain thin axial slices. Whole brain T2-weighted 2D fast spin echo (flip angle = 90, TR/TE = 4000/120 ms, 1 avarage, 50 slices, slice thickness = 3.0 mm contiguous, FOV = 220x80 mm^2, matrix 256x256, run time = 4min 32 s) scan was conducted. T2-weighted axial image of a control object is presented in Figure 1. The purpose was to image the basal ganglia structures of interest for subsequent offline planimetric, volumetric, and densitometric analyses.

The examination and measurement of the putamen, globus pallidus, caudate nucleus, SNpc, SN pars reticulata, red nucleus and dentate nucleus were calculated bilaterally. Firstly, the contours of the caudate, putamen, globus pallidus were drawn manually by the rater using the gray/white matter contrast of the early echo (flip angle = 90, TR/TE = 3000/15 ms, 1 avarage, 50 slices, slice thickness = 3.0 mm ontiguous, FOV = 220x80 mm2, matrix 256x256, run time = 3 min 26 s) images, then T2-weighted 2D fast spin echo (as explained in the previous paragraph) was used for other measurements. For volumetric examinations, automatic freehand ROI technique that was present in the system of MRI was used. Putaminal plains were measured separately and were multiplied with slice thickness, by means of cm^3. The width of the SNpc was calculated by measuring the hyperintense band between the pars reticulata and nucleus ruber, as previously suggested by Duguid et al and Braffman et al.[21,27] To quantitatively analyze the substantia nigra, we selected an image that contained the superior colliculus and the largest low-intensity area of the red nucleus. On this image, the pars reticularis of the substantia nigra was also well visualized as an obvious low-intensity area, which was anteriorly merged into the cerebral peduncle with reduced signal intensities. There was a band with relatively high signal intensity between the red nucleus and pars reticularis, which included the pars compacta of the substantia nigra. T2-weighted axial midbrain image of a patient is presented in Figure 2. A point representing the half-height maximum intensity between the hypointense red nucleus and relatively hyperintense SNpc and an-

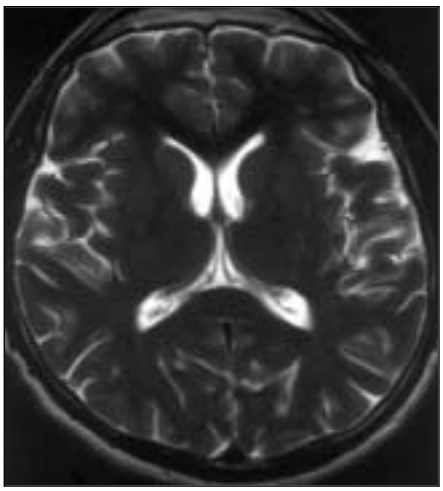

Figure 1: T2-weighted axial image of a control subject. (TR/TE = 4000/120 ms)

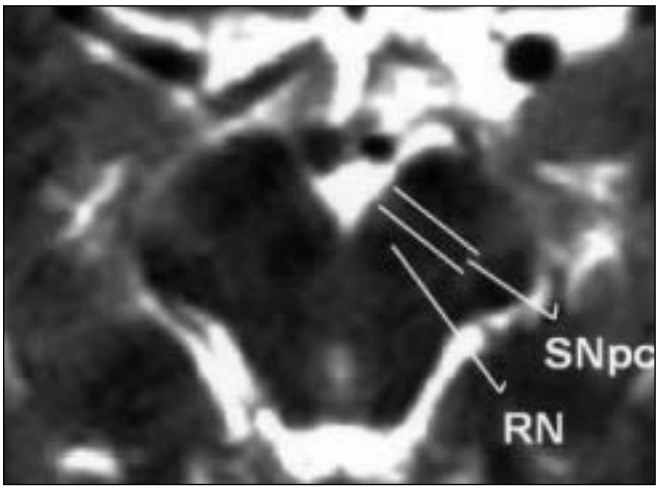

Figure 2: T2-weighted axial midbrain image of a patient. RN: Red nucleus, SNpc: Substantia nigra pars compacta with relatively high signal intensity between the red nucleus and pars reticularis

other point between the SNpc and the relatively hypointense substantia nigra pars reticulata were determined along a line perpendicular to the SNpc. The distance between these two points was defined as the width of the SNpc. For tentative contrast measurements in each subject, a square region of interest (ROI) was set to be divided into two equal parts by the boundary of the band and the red nucleus. The square ROI had sides two-fold longer than the breadth of the band to include the entire breadth of the band and to exclude the pars reticularis. Then the average intensity of the ROI was used as a cut-off level to discriminate between the low signals in the red nucleus and the pars reticularis and the relatively high signals in the band. When the display window width was set from 0 to the cut-off level, the software automatically traced the regions with the signal intensities, and calculated the areas. The intensity score of the basal ganglia was measured by the densitometer of the device, expressed in numbers. Increase in intensity score found by the device, represents the decrease in intensity.

For statistical analyses bilateral and separate means of left and right basal ganglia intensities, width of SNpc, and volume of putamen examination results were used. The measured basal ganglia intensities, SNpc witdh data and putamen volumetric examination data of patients were compared with the data from the healthy subjects. In the patients group the intensities of basal ganglia and the relation between clinical scores and mean volumes were also assessed.

Statistical analysis was performed by using the SPSS software package Version 10.0. We used non-parametric tests to compare the two groups. Group comparisons were assessed by the Mann Whitney *U* test. Comparisons of the mean left and right basal ganglia MRI values of the PD group was made using the Wilcoxon sign rank test. Non-parametric analysis of the correlation between the variables was assessed by Spearman's rank correlation coefficient. All tests were two-tailed, and the significance level was set at $P=0.05$.

Results

Mean total UPDRS, motor, daily activity, cognition, rigidity and finger tapping scores of the patients group are presented in Table 1. Mean SNpc width, putamen volume and intensity examination results of basal ganglia for both patients and control groups are presented in Table 2.

Table 1: Clinical scores of Parkinson's disease patients

Variables	Mean	Std. Dev	Min.	Max
Total UPDRS score	34,60	13,39	9	67
Total Motor score	21,25	9,27	6	45
Total daily activities score	10,50	5,06	1	21
Total cognition score	2,85	1,57	1	6
Right+left total rigidity score	2,00	1,52	0	5
Right+left total finger tapping score	2,55	1,23	0	4

A significant difference was found between the mean intensity of SNpc (Z=-2,23 p=0,026) and dentate nucleus (Z=-2,77 p=0,006) of PD and control groups, statistically. The intensities of these two areas were decreased in PD patients. There was no significant difference between the mean putamen, globus pallidum, pars reticulata and caudate nucleus intensity scores of the two groups.

There was a significant difference between the mean SNpc width measurement scores of the patients and controls (Z=-3.61 p<0.001). The width measurements of SNpc in PD patients were statistically lower. In volumetric examinations mean putamen volumes in PD patients were also significantly lower than the controls (Z=-1.97 p=0.049). In PD patients, the intensity of putamen was lower than globus pallidus (Z=-3.92 p<0.001).

All PD patients included in the study had bilateral presentations of PD at the time of MRI examination. Signs were predominant in the right extremities in 13 and in the left extremities in seven. When we compared the right and left basal ganglia intensity scores in the patients group, we found that the left SNpc intensity score was higher (564±123 vs 542±131, Z=-2.05 p=0.04), and, there was no difference between the intensity scores of other examined basal ganglia. There was also no difference between the right and left SNpc widths in the PD group. Mean left putaminal volumes seemed to be lower than right, but results didn't reach a significant level. When we compared the right and left intensity scores of the patients with controls, we found that left (553.7±107.4 vs 461.5±102.4 Z=-3.43 p<0,001) and right (531.6±103.7 vs 453.8±104.2 Z=-3.40 p<0,001) dentate nucleus intensity scores of patients were statistically higher. Although there was no difference between the right putamen volumes of these two groups, left putamen volume was significantly lower in the patient group (2.24±0.75 cm^3 vs 2.82±0.6 cm^3 Z=-2.48 p=0.012). Right (0.23±0,08 cm vs 0.33±0,05 cm Z=-3.58 p<0.001) and left (0.3±0.9 cm vs 0.3±0.5 cm Z=-3.7 p=0.01) SNpc witdth results of the patients were statistically lower than controls.

Mean SNpc intensity scores did not correlate with mean SNpc width and mean putamen volume scores. No correlation was found between mean SNpc width and mean putamen volume.

No correlation could be found between mean SNpc width

Table 2: Substantia nigra pars compacta width, putamen volume and intensity scores of the Parkinson's disease patients and control group found by the help of T2-weighted magnetic resonance imaging. Increase in intensity score found by the device, represents the decrease in intensity.

Variables	PD Group (n=20)		Control Group (n=16)	
	Mean	Std. Dev	Mean	Std. Dev
Mean Pars Compacta width cm*	0,23	0,08	0,33	0,5
Mean Putamen volume cm[3]†	2,36	0,77	2,86	0,62
Mean Pars Compacta intensity score‡	555,94	124,97	483,84	104,97
Mean Putamen intensity score	551,81	109,26	509,39	126,53
Mean Pars Reticulata intensity score	433,97	100,24	418,81	101,90
Mean Globus Pallidus intensity score	431,05	96,83	396,80	114,37
Mean Nucleus Caudatus intensity score	590,51	122,68	543,61	138,34
Mean Nucleus Dentatus intensity score§	542,67	104,88	457,63	102,83
Mean Red Nucleus intensity score	463,83	92,59	436,66	121,17

PD: Parkinson's disease, *(Z=-3,61 p<0,001) statisticaly significant between two groups, †(Z=-1,97 p=0.049) statisticaly significant between two groups, ‡(Z=-2,23 p=0,026) statisticaly significant between two groups, §(Z=-2,77 p=0,006) statisticaly significant between two groups

and clinical scores in PD patients. There was a correlation between mean intensity score of SNpc and total UPDRS scores (r=0.625 p= 0.003) and rigidity scores (r=0.503 p=0.024). Decrease in intensity was correlated with the increase in clinical scores. There was no correlation between mean intensity score of SNpc and other clinical scores. The mean intensity scores of the globus pallidus, nucleus ruber, putamen, pars reticulata and the caudate nucleus did not correlate with the clinical scores of PD patients. There was also no correlation between mean putamen volume scores and clinical scores.

Discussion

We report a brain MRI study of idiopathic Parkinson's disease, in which SNpc width, putamen volume, and T2 intensities of multiple basal ganglia structures were compared in 20 PD patients and 16 healthy controls. Within the patient sample, correlations of MR endpoints were also sought with clinical UPDRS scores. We found that SNpc and cerebellar dentate nuclear T2-intensities, as well as SNpc width and putaminal volumes, were significantly lower in PD patients than in controls. In PD patients, SNpc T2-intensity had a significant negative correlation with UPDRS and rigidity score. Right and left SNpc width results were significantly lower in patients. Our findings suggest that the neuron groups in the SNpc were selectively affected in PD. This selectively affected region may be seen with MRI by showing the intensity change. The intensity changes are correlated with the clinical scores of patients.

There are still controversial opinions about the above-discussed issues in the literature.[28,29] The moleculer pathophysiological changes in PD patients' brain (the role of Fe and the pathophysiological changes in SNpc) are under debate. We believe that we need to discuss the previously reported, same and opposite opinions related to our findings. In SNpc, hyperintense signals in the T2-weighted series should appear on MRI because of the relatively low iron concentration.[21,30] Previous studies reported that the mean width of the SNpc in PD patients was found lower than in the controls.[21,22] Although the hyperintense band between the pars reticulata and the nucleus ruber on the axial MRI of midbrain is widely used to measure the width of the SNpc, it is not universally recognized that this band on MRI necessarily corresponds precisely to the anatomic SNpc. In a recent study on parkinsonian substantia nigra using proton density-weighted spin-echo (SE) and fast short inversion-recovery (STIR) MR findings, the authors concluded that as substantia nigra is located mainly beneath the red nucleus, its location cannot be determined on the basis of T2-weighted imaging results but rather on the basis of proton density-weighted SE or fast STIR.[31]

Previous studies have reported that iron is selectively increased only in the SNpc, the nigral region containing pigmented dopaminergic neurons.[11,13,14,20] It has been reported that PD patients exhibit an increased echogenity of the SN on transcranial sonography.[32] Hirsch et al have showed that melanin-containing cells were easily damaged with respect to other cell types in SN in Parkinsonian patients.[33] In the study of Muthane et al, no significant loss of pigmented nigral neurons with age was reported, suggesting that the loss seen in PD is exclusively due to the disease process itself.[34] There are also contrary findings reported in the literature. First, the hypointensity may not be due exclusively to Fe accumulation, accumulation of other trace heavy metals may be involved. Some studies reported the decrease of Ferritin in PD brain,[18] in a few postmortem studies increased iron levels in the SN of patients with PD were found when compared with age-matched controls.[7,16] There is also another study detecting no increase in neuronal isoferritin when specific brain isoforms of ferritin were evaluated.[35] Mondino et al reported no change in the basal ganglias and SN of PD patients, compared with controls, in T2 relaxation time.[15] The intensity score measurement results that we found showed that intensity scores were higher in PD patients than controls (statistically significant only in SNpc and dentate nucleus) and the selective accumulation of iron in SNpc was in accord with clinical scores. However, in our study there is a controversial result similar to the previously reported ones, which is difficult to explain. The inten-

sity scores of globus pallidus and SN pars reticulata were found lower than the intensity scores of other anatomic structures in both the patient and control groups. This result is not in accord with the studies which explained that the hypointensity in the T2 series was due to the higher iron content in GP and especially in SN pars reticulata.[6,9] The cause of these differences may be the distribution of iron within the cell. From this point of view, serial MR imaging studies evaluating patients and controls with dynamic neuroimaging methods may be helpful for analyzing these contradictory results.

Another important subject that needs to be discussed is the correlation between MRI findings and clinical scores. We found a correlation between mean intensities of the SNpc and total UPDRS and rigidity scores in PD patients. However, Antonini et al did not demonstrate any correlation between hypointensity of the SN and clinical findings.[6] Pujol et al reported that PD patients exhibited a significant reduction of the width of the SNpc and the level of this reduction correlated strongly with the clinical status evaluated by the UPDRS.[23] In contrast, Duguid et al did not find any correlation between the width of the SNpc and clinical scores in the Hoehn-Yahr scale.[21] Although the width of the SNpc diminished in our patients, it did not correlate with the clinical scores. The correlation between clinical scores and neuroimaging findings has been analyzed by using different techniques described in the literature. Ma et al reported that the total number of pigmented neurons in the SNpc showed a significant negative correlation with the duration and the stage of disease.[36] Antonini et al reported that the FDOPA uptake in the putamen and caudate nucleus declined with increasing Hoehn and Yahr staging and bradykinesia and rigidity scoring.[37] In the study of Hutchinson et al the substantia nigra was imaged by a combination of two MR imaging inversion-recovery pulse sequences and the radiologic index was found to be highly correlated with the UPDRS score.[38] Our results seem to be in accord with these studies using new techniques.

We also found a significant difference in the mean dentate nucleus intensities of patients and healthy subjects. In the Hallervorden-Spatz syndrome, iron accumulation was reported in globus pallidus and SNpc, but not in the other iron-rich structures, such as the red nucleus and the dentate nucleus.[39] Waldvogel et al reported higher iron levels in the dentate nucleus in patients with Friedrich' ataxia than in controls.[40] To our knowledge this finding has not been reported before in PD patients. We believe that this result needs to be taken into account in future studies, and needs further research.

There are some limitations of our study. Firstly, the sample of the study group was relatively small, so the results may reflect an unusual sample of subjects. This study was cross-sectional and long-period observations may demonstrate some changes with normal aging and PD in individual cases. We don't have the postmortem verification of diagnosis in PD cases. In vivo T2 measurements have many sources of error and they can only be regarded as estimates. The gender distribution of cases was not balanced, peripheral iron levels, which are lower in females, may impact the results.

We tried to exclude atypical PD patients, while choosing the patient and control groups and used a rather simplistic way for assessing signal intensity. Decrease in intensity was assessed by densitometric display of the acquired images. This technique helps to measure the basal ganglia intensity changes with an easier, cheaper and rapid way. It can also be used easily in serial MRI examinations. We believe that this study contributes some help to the neurobiology of PD, which is still under debate.

In conclusion, the difference in SNpc intensities, measured by MRI in PD patients, suggests an increase in iron accumulation and oxidative stress in the SNpc in PD. The decrease in the intensity of SNpc correlates with poor clinical scores. We believe that prospective studies with long clinical follow-up and serial MRI are needed for the correlation between clinical scores and neuroradiological examinations.

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