Original Article / Özgün Araştırma

The Role of Ceruloplasmin in Neurodegeneration in Parkinson's Disease

İdiyopatik Parkinson Hastalığında Serüloplazminin Nörodejenerasyonda Rolü

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Summary

Objective: Oxidative stress has been implicated to play a major role in the neuronal cell death in idiopathic Parkinson's disease (IPD). Ceruloplasmin is a ferroxidase that oxidizes toxic ferrous iron to its nontoxic ferric form and thus helps prevent oxidative damage to proteins, lipids, and DNA. The aim of this study was to determine the serum ceruloplasmin levels in patients with IPD and evaluate its role in neurodegeneration using hydrogen proton magnetic resonance spectroscopic imaging (H-1 MRSI).

Materials and Methods: Twenty-three patients with the IPD and 12 healthy controls were studied using single-voxel H-1 MRSI of the bilateral putamen. The peak ratios of N-acetyl aspartate (NAA) to creatinine (Cr) and choline (Cho) were measured in both groups and serum ceruloplasmin levels were detected.

Results: Compared with the controls, the ratios of NAA/Cho and NAA/Cr in putamen contralateral (CL) to the symptomatic limbs were significantly lower in patients with IPD. Also in the IPD group, CL NAA/Cho and NAA/Cr ratios were significantly lower than the ipsilateral (IL) values (p<0.001). There was no significant difference between ratios of metabolites in putamen IL to the symptomatic limbs in the IPD group compared with the healthy controls. The mean serum ceruloplasmin level of IPD patients was significantly lower than that of the control group (p<0.001). In the IPD group, a significant direct correlation was found between levels of ceruloplasmin and CL putamen NAA/Cho ratio (p=0.011).

Conclusion: In our study the direct correlation between low levels of ceruloplasmin and CL putamen NAA/Cho ratio supports the hypothesis that ceruloplasmin deficiency may contribute to free radical-induced death of neuronal cells.

Keywords: Idiopathic Parkinson's disease, proton magnetic resonance spectroscopy, ceruloplasmin

Öz

Amaç: İdiyopatik Parkinson hastalığında (İPH) oksidatif stres nöronal hücre ölümü üzerinde majör role sahiptir. Serüloplazmin ferrooksidaz aktivitesi ile ferröz demiri ferrik forma okside eder ve böylece hücrenin DNA, lipid ve protein bileşenlerini oksidatif hasardan korur. Bizim çalışmamızda proton manyetik rezonans spektroskopi (H-1 MRS) ile serüloplazmin eksikliğinin nörodejenerasyon üzerindeki etkisi araştırılmıştır.

Gereç ve Yöntem: İPH tanılı 23 hasta ve 12 sağlıklı kontrol olguda iki yanlı putamen tek voksel H-1 MRS yöntemiyle incelendi. Her iki grupta N-asetil aspartat (NAA), kreatin (Cr) ve kolin (Cho) pik değerleri ve serum serüloplazmin düzeyleri belirlendi.

Bulgular: İPH hastalarında semptomatik ekstremitenin kontralateral (KL) putaminal bölge NAA/Cho ve NAA/Cr oranı kontrol grubuna kıyasla anlamlı derecede düşüktü. Ayrıca İPH grubunda KL NAA/Cho ve NAA/Cr oranları ipsilateraline (İL) göre anlamlı derecede düşüktü (p<0,001). Semptomatik ekstremitenin İL putaminal bölgeye ait metabolit oranları karşılaştırıldığında sağlıklı kontrol ile İPH grubu arasında anlamlı fark saptanmadı. Serum serüloplazmin seviyeleri kontrol grubu ile karşılaştırıldığında İPH grubunda anlamlı derecede düşük saptandı (p<0,001). İPH grubunda KL putaminal NAA/Cho oranı ile serüloplazmin seviyeleri arasında anlamlı korelasyon saptandı (p=0,011).

Sonuç: Çalışmamızda saptanan serum serüloplazmin düşüklüğü ile KL putamen NAA/Cho oranı arasındaki bu korelasyon serbest radikal aracılı nöronal hücre ölümünde serüloplazmin yetersizliğinin katkıda bulunabileceği hipotezini desteklemektedir.

Anahtar Kelimeler: İdiyopatik Parkinson hastalığı, proton manyetik rezonans spektroskopi, serüloplazmin

Introduction

Idiopathic Parkinson's disease (IPD) is a neurodegenerative disease characterized by progressive neuronal degeneration of specific parts of the central nervous system (1,2). There are data to suggest that excitotoxic mechanisms, oxidative stress, inflammation, impairment of mitochondrial energy metabolism, and intracellular calcium balance could be involved in the pathogenesis of neurodegenerative diseases. Recent studies have shown clues that formation of free radicals might also play an important role in the pathogenesis of neurodegenerative diseases (3,4,5).

Degeneration and loss of dopaminergic neurons in the substantia nigra are pathognomonic for IPD (6,7). During degeneration of dopaminergic neurons in the substantia nigra, levels of intracellular iron progressively increase. Most of the degenerated neurons involve an amyloid formation called "Lewy body", which binds iron. The center of Lewy bodies involve proteins called " -synuclein." Their function is not well known but it is suggested that they take part in deposition and storing of intracellular iron, which can result in conformational change and aggregation (8,9,10,11). Postmortem and in vivo studies showed that deposition of iron in the substantia nigra had effects on selective dopaminergic degeneration (8,9,12,13,14). In addition to the accelerative effect on cellular aerobic metabolism, iron plays important roles in various biologic processes including signaling, and synthesis of neurotransmitters and DNA (15,10). Iron deposition in brain causes change in the ferrous iron to ferric iron equation. This change causes production of toxic hydroxyl radicals, which result in neuronal damage (16). Increased iron concentration causes increased lipid peroxidation in the brain. Also, enzyme activities of complex 1 and 4, which are involved in the mitochondrial Krebs cycle, are decreased by 43-50% in basal ganglia (17).

Many potential factors that can trigger neurodegeneration in IPD have been suggested and increased iron deposition in the substantia nigra has been shown to be one of these factors in postmortem and in vivo studies (8,16). Rossi et al. (18) showed iron deposition in the anterior globus pallidus, caudate nucleus, and substantia nigra pars compacta in their magnetic resonance imaging (MRI)-based study.

Ceruloplasmin was shown to be an effective antioxidant in the central nervous system and protected neural cells against oxidative stress in in vivo studies (19). Its role in copper transport has been known for a long time. Ceruloplasmin' s role in iron metabolism has also been shown (20). Ceruloplasmin helps iron to dock its transporter protein "transferrin" by its ferroxidase activity (oxidation of ferrous iron). In this way, free radical reactions caused by free ferrous iron are prevented (21,22,23,24). Hereditary aceruloplasminemia, which is characterized by nonexistence of ceruloplasmin in serum, is caused by a mutation in the ceruloplasmin gene (25). It was shown that deposition of iron in basal ganglia and retina led to neurodegeneration and retinal degeneration, it was not caused by copper toxicity (26,27). Patel et al. (28) showed iron deposition and increased free radicals were associated with damage in ceruloplasmin mice because of aceruloplasminemia. These data show that ceruloplasmin deficiency causes neuronal cell damage by lipid peroxidation, decreased mitochondrial energy production, and iron-mediated increase in free radicals.

Hydrogen protons (H-1) are more sensitive compared with other protons with magnetic forces, and these are used in magnetic resonance spectroscopy (MRS), which is a noninvasive technique. 1.5 Tesla MRI can measure levels of metabolites in the brain including N-acetylaspartate (NAA), creatinine (Cr), and choline (Cho). NAA is a neuron-specific marker and it represents the main peak in the proton spectrum. A loss in the NAA peak shows neuronal damage. Cr is an important metabolite in energy metabolism. It is used as an internal standard in spectroscopic studies. Cr decreases in situations in which oxidative phosphorylation and anaerobic glycolysis cannot be sustained and adenosine triphosphate cannot be produced. Cho is found in the cell membrane. It reflects membrane metabolism and phospholipid turnover. Increased Cho indicates increased cell production or membrane breakdown, which can suggest presence of brain tumor (29). Iron deposition in the substantia nigra in the pathogenesis of IPD is well understood and has been studied before, but the risk factors that triggers iron deposition are not known. In our study, the effect of ceruloplasmin deficiency on this chain shown using MRS and laboratory techniques was investigated.

Materials and Methods

Patients with a definite diagnosis of IPD following referral to the movement disorders polyclinic of Göztepe Training and Research Hospital between January 1st, 2005, and May 1st, 2005, were included in the study. The control group comprised healthy volunteers. Written informed consent was obtained from all participants and the study was approved by the Ethics Committee of Göztepe Training and Research Hospital.

A neurologic examination and general laboratory tests were performed for all participants. The IPD group met the United Kingdom Parkinson's Disease Society Brain Bank Clinical Diagnostic criteria (30). The Unified Parkinson's Disease Rating Scale (UPDRS) and Hoehn and Yahr staging of Parkinson's disease were used to evaluate the patients (31). The first symptoms and current clinical findings, duration of treatment, and dosages were noted. Patients with severe arterial hypertension, diabetes mellitus, cerebrovascular disease, cardiovascular system disease, hyperlipidemia, other psychiatric diseases, liver and renal failure, and alcohol and drug users were excluded. Serum levels of ceruloplasmin were measured in the biochemistry laboratory of İstanbul University, İstanbul Faculty of Medicine. MRI and H-1 MRS were performed for all participants.

A radiologist who was unaware of the patients' clinical findings evaluated the MRSs and MRIs. The patients' treatments were stopped 12 hours before neuroimaging. There are several MRS sequences available; we used Point Resolved spectroscopy (PRESS) sequences, which can provide wider tissue samples (3-27 cm^3) with lesser stimulus and has a better signal/noise ratio. Proton MRS (General Electric Signa HiSpeed 1,5 Tesla), using PRESS sequences echo times (TE) (TE 144 ms, 64 acquisition, 2x2x2 'voxel size', TR 1500 ms with axial or coronal T2 sequences) and "single voxel spectroscopy" was performed to all patients. Sequences were acquired by automatically shimming (2-6 Hz) and 99% water suppression. Acquired spectra were evaluated in terms of quality and automatically quantified. NAA, Cho, and Cr values were acquired by logarithmic calculation of the area under peak

curves semi-quantitatively. NAA, Cho, and Cr values acquired by TE 144 sequence from the bilateral putamen where dopamine loss was marked were used for study. The Cr value was used as control because it does not change in many diseases. New parameters were acquired by estimating values: NAA/Cho and NAA/Cr. These ratios were compared between groups.

GraPHpad Prism V3 pocket was used for statistical analysis. Definitive statistics (mean, standard deviation) were used to evaluate data. Independent t-test was used to compare dichotomous groups. Two-sample t-test was used to compare patients and controls, and right and left sides. The Mann-Whitney U test was used to compare small dichotomous groups and the Chi-square test was used to compare qualitative data. A p value of <0.05 was considered statistically significant.

Results

Twenty-three patients (19 women, 82.6%; 4 men, 17.4%) with IPD and 12 healthy subjects (7 women, 58.3%; 5 men, 41.7%) were included in the study. The mean age was 67±8.8 years (range, 50-81 years) in the IPD group and 63.67±9.32 years (range, 52-76 years) in the control group. The mean disease duration was 6.6±5.33 years (range, 0-18 years); the mean age at disease onset was 60.4±10.77 years (range, 42-80 years); the mean UPDRS was 33.43 (range, 11-62); and the mean H&Y scale was 1.87 (range, 1-3). There was no statistically significant difference between the IPD and control groups in terms of mean age (t=1.04; p=0.305) and distribution of sex (χ^2=2.43; p=0.119) (Table 1).

The mean ages, ages at disease onset, duration of disease, duration of L-Dopa use, serum levels of ceruloplasmin, and MRS values were investigated in the IPD group (Table 2).

H-1 MRS features of groups were compared. The mean NAA/Cho and NAA/Cr ratio values in the contralateral (CL) putamen were significantly lower compared with the ipsilateral (IL) putamen. There was no significant difference between the right and left mean NAA/Cho and NAA/Cr values of the control group (Table 3). The CL mean NAA/Cho and NAA/Cr values were significantly lower in the IPD group compared with the control group. There was no difference in IL mean NAA/Cho and NAA/Cr values between the groups. Serum levels of ceruloplasmin were significantly lower in the IPD group compared with the control group. There was no correlation between levels of ceruloplasmin and mean ages and durations of disease (Table 4).

The neurologic examination findings, UPDRS, and H&Y scores were evaluated in the IPD group. There were no correlations between UPDRS, H&Y scores, and CL and IL NAA/Cho and NAA/Cr values, and levels of serum ceruloplasmin. Also, there were no correlations between the dominant clinical findings and H-1 MRS values and levels of ceruloplasmin. There were

no correlations between ages disease onset, durations of disease, durations of L-Dopa use, and levels of serum ceruloplasmin in the IPD group. However, there were correlations between ages of disease onset, durations of disease, durations of L-Dopa use and CL mean NAA/Cr values. There were correlations between CL putamen mean NAA/Cho values and levels of ceruloplasmin in the IPD group. We found that when ceruloplasmin decreased, the ratio of CL NAA/Cho also decreased (Table 4).

Discussion

It is difficult to investigate the neurochemical and metabolic changes of the striatum because of methodologic difficulties. There are few data about in vivo striatal changes in IPD. Iron deposition in the brain has been shown in pathogenesis of many neurodegenerative diseases, especially IPD (9,14).

The nonexistence or deficiency of serum ceruloplasmin can lead to neurodegeneration by causing iron deposition and increased free radical-mediated damage (25,26,27,28). Few studies in the literature have addressed the role of ceruloplasmin deficiency-mediated iron deposition in the pathogenesis of IPD. Hochstrasser et al. (33) showed increased iron deposition in the substantia nigra using ultrasonography in patients with IPD who had a ceruloplasmin gene mutation. The same research group showed in 2005 that iron metabolism is affected in patients with IPD who had ceruloplasmin gene mutations (34). Lirong et al. (35) showed in 2009 that regardless of the existence of the ATP7B mutation, a decrease in serum levels of ceruloplasmin was associated with movement disorders including IPD. Ceruloplasmin has been shown to protect the central nervous system against oxidative stress (28). A decrease in serum levels of ceruloplasmin may indicate decreased ceruloplasmin synthesis in the brain, which could cause increased oxidative stress in the substantia nigra in IPD (36). Jin et al. (36) showed marked iron deposition in the substantia nigra in patients with IPD using susceptibility-weighted imaging. The authors also showed that there was a correlation between low levels of ceruloplasmin and mean phase values of the bilateral substantia nigra. However, there were no correlations between mean phase values of the bilateral substantia nigra and other areas of the brain in patients with IPD who had normal serum levels of ceruloplasmin. Their study showed that a deterioration of ceruloplasmin metabolism could play a role in the pathogenesis of IPD, which also supports our findings.

Jin et al. (36) showed (2011) correlations between phase of disease, severity of disease, and serum levels of ceruloplasmin, and mean nigral phase values. In contrast, we found no correlations between severity of disease and CL and IP NAA/Cho, NAA/Cr, and serum levels of ceruloplasmin. Jin et al. (37) found no correlations between nigral phase values and serum levels of ceruloplasmin and motor symptoms in IPD patients in their other study (2012). Before symptoms of disease occur, over fifty percent of nigral dopaminergic neurons have already degenerated (38). We did not make a subgroup analysis but these findings suggest this question: Does the deterioration of ceruloplasmin metabolism begin before symptoms occur?

Different from other studies, we planned to simultaneously show the decrease of serum ceruloplasmin levels and metabolic changes in areas specific for IPD with H-1 MRS. We chose H-1 MRS

Table 1. Age and sex distributions of groups		IPD group	Control group	
Age		67±8.8	63.67±9.32	t=1.04 p=0.305
Sex	Male	4 (%17.4)	5 (%41.7)	χ^2=2.43 p=0.119
	Female	19 (%82.6)	7 (%58.3)	
IPD: Idiopathic Parkinson's disease				

because it is a non-invasive technique that is used to investigate cerebral metabolites. It gives better information compared with conventional MRI about metabolic changes in lesions or selected areas and the relation between evolution of disease and metabolic changes (39).

There are studies about the role of NAA on cerebral metabolism (40,41). NAA is believed to be an indicator of normal neuronal tissue. Studies on brain tumor tissues and brain cell cultures showed that NAA was only found in neuronal tissues (42,43,44,45). Also, lesions caused by specific neurotoxic agents were shown to contain decreased NAA (46). Decreased NAA in proton MRS due to neuronal damage has been shown in cerebrovascular diseases (47), Wilson's disease (48), AIDS (49), multiple sclerosis (50,51), demyelinating diseases (44), and diabetes mellitus. There are well-

Table 2. Comparison of groups in term of study parameters

IPD group n=23 min-max (mean)		Control group n=12 min-max (mean)	
Age	50-81 (67±8.8)	Age	52-76 (63.67±9.32)
Ceruloplasmin	14.9-30.0 (22.35±3.66)	Ceruloplasmin	22.0-30.1 (25.83±2.26)
Duration of disease	0-18 (6.6±5.33)		
Age when disease started	42-80 (60.4±10.77)		
Duration of L-Dopa use (years)	0-12 (4.39±3.8)		
NAA/Cho (CL)	1.01-1.71 (1.312±0.177)	NAA/Cho right	1.16-2.24 (1.62±0.283)
NAA/Cho (IL)	1.24-1.93 (1.641±0.218)	NAA/Cho left	1.22-2.5 (1.693±0.427)
NAA/Cr (CL)	1.12-1.71 (1.454±0.174)	NAA/Cho right-left	1.16-2.5 (1.657±0.356)
NAA/Cr (IL)	1.02-2.2 (1.69±0.307)	NAA/Cr right	1.4-1.87 (1.678±0.164)
		NAA/Cr left	1.37-2.2 (1.723±0.226)
		NAA/Cr right-left	1.37-2.2 (1.701±0.194)

IPD: Idiopathic Parkinson's disease, min: Minimum, max: Maximum, NAA: N-acetylaspartate, Cho: Creatinine, Cr: Choline, IL: Ipsilateral, CL: Contralateral

Table 3. Compare of groups in term of hydrogen proton magnetic resonance spectroscopic imaging features

	IPD group				Control group			
	CL	IL	t	p	Right	Left	t	p
NAA/Cho	1.312±0.177	1.641±0.218	-8.24	0.0001***	1.62±0.283	1.693±0.427	-0.46	0.651
NAA/Cr	1.454±0.174	1.69±0.307	-4.79	0.0001***	1.678±0.164	1.723±0.226	-0.76	

***p<0.001, very high statistical significance, IPD: Idiopathic Parkinson's disease, NAA: N-acetylaspartate, Cho: Creatinine, Cr: Choline, IL: Ipsilateral, CL: Contralateral

Table 4. Compare of clinical findings, hydrogen proton magnetic resonance spectroscopy values and serum levels of ceruloplasmin of the groups

		NAA/Cho (CL)	NAA/Cho (IL)	NAA/Cr (CL)	NAA/Cr (IL)	Ceruloplasmin
H-1 MRS	IPD group (n=23)	1.312±0.177	1.641±0.218	1.454±0.174	1.69±0.307	22.35±3.66
	Control group (n=12)	1.657±0.356	1.657±0.356	1.701±0.194	1.701±0.194	25.83±2.26
	t;p	-4.17; 0.0001***	-0.18; 0.858	-4.57; 0.0001***	-0.14; 0.89	-3.00; 0.005**
IPD group	BC	1.3±0.099	1.563±0.138	1.403±0.225	1.745±0.313	21.6±4.494
	TR	1.316±0.199	1.669±0.237	1.472±0.156	1.671±0.313	22.612±3.436
	MWU; p	48.5; 0.861	32; 0.183	40.5; 0.461	45.5; 0.7	39.5; 0.421
	Age (r;p)					0.108; 0.625
	Duration of disease (r;p)					-0.072; 0.743
	Ceruloplasmin (r;p)	0.424; 0.011*	0.178; 0.307	0.103; 0.555	-0.018; 0.917	
	UPDRS (r;p)	-0.01; 0.963	0.127; 0.565	0.188; 0.389	0.413; 0.053	0.134; 0.543
	H&Y (r;p)	0.007; 0.973	0.149; 0.496	0.306; 0.156	0.299; 0.166	0.057; 0.797
	Duration of disease (r;p)	-0.12; 0.584	-0.133; 0.546	0.457; 0.028*	0.026; 0.905	-0.072; 0.743
	Age when disease started (r;p)	-0.098; 0.655	0.097; 0.66	-0.516; 0.012*	-0.173; 0.43	0.124; 0.573
	Duration of L-Dopa use (r;p)	-0.053; 0.809	-0.086; 0.697	0.56; 0.006**	0.219; 0.315	-0.165; 0.452

BC: Bradykinesia, TR: Tremor, MWU: Mann-Whitney U test, UPDRS: Unified Parkinson's disease rating scale, H&Y: Hoehn and Yahr, NAA: N-acetylaspartate, Cho: Creatinine, Cr: Choline, IPD: Idiopathic Parkinson's disease, IL: Ipsilateral, CL: Contralateral, *0.01 <=p<0.05; statistical significance, **0.001 <=p<0.01; high statistical significance, ***p<0.001; very high statistical significance

attended studies showing neuronal degeneration related decreased NAA in temporal lobe epilepsy (52), AIDS (53), and Alzheimer disease (54).

Studies using H-1 MRS on patients with IPH showed reduced NAA/Cho values in IPH specific areas (55,56,57,58). Clarke et al. (59) found no difference between Cho levels of patients with IPH and healthy subjects. Chaudhuri (60) observed no difference between groups in terms of Cho/(Cr+polymerase chain reaction) ratios. The same study also showed that this change in NAA/Cho rate was due to the differences in levels of NAA and differences between patients who took L-dopa treatment and those who did not, with same average ages, which meant that differences in NAA/Cho ratios between groups were not age dependent. There were no correlations between duration of disease, severity of disease, asymmetry of disease, and values. H-1 MRS findings can be different in different phases of the disease. Weiduschat et al. (61) showed no differences between H-1 MRS findings and UPDRS, ages of patients, durations of disease, and they also reported that H-1 MRS findings did not differ from the normal

population. The authors suggested that their results showed that clinical heterogeneity plays an important role in IPH.

It is known that when clinical findings occur and disease is diagnosed, more than 50% of nigral dopaminergic neurons have already degenerated (38,62). Zhou et al. (63) (2014) reported that NAA/Cr, NAA/Cho and NAA/(Cho+Cr) mean values were significantly lower in CL substantia nigra compared with those of IS in line with the presentation of the affected extremity. The authors also showed that H-1 MRS could be used in the early diagnosis of IPH and clinical course of disease. We showed that CL NAA/Cho and CL NAA/Cr mean values in IPD were significantly lower compared with the control group, as documented in the literature. Studies in patients with IPD using proton MRS, including ours, show metabolic changes are indicators of neurodegeneration.

We found that serum levels of ceruloplasmin were significantly lower in the IPD group compared with healthy subjects. We also found a positive correlation between decreased CL NAA/Cho and decreased serum levels of ceruloplasmin in the IPD group (Figure 1). These findings suggest that a deficiency of ceruloplasmin
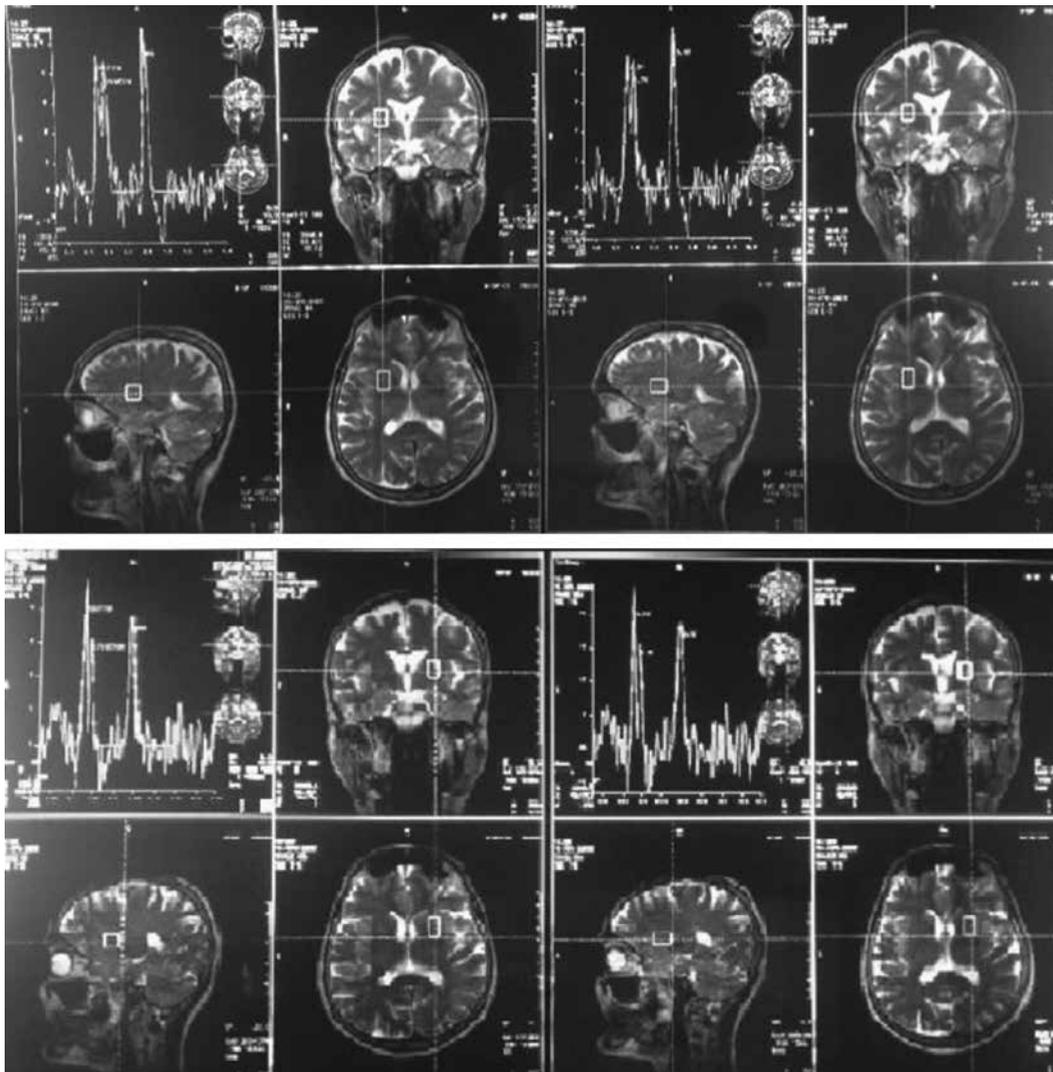

Figure 1. Contralateral magnetic resonance spectroscopic imaging of the Idiopathic Parkinson's disease group

might play a role in neurodegeneration in the pathogenesis of IPD. Correlations between CL NAA/Cr values years of L-dopa use, durations of disease, and age of onset suggest H-1 MRS can be used as indicators for neuronal and axonal degeneration, as shown in the literature (64,65).

Conclusion

The benefits of current neuroprotective treatments in IPD are yet to be proven. Determining risk factors that could be effective in pathogenesis of IPD might lead to neuroprotective treatments. Our study showed that a deficiency of ceruloplasmin could contribute to neurodegeneration in IPD. These findings may light the way for developing new neuroprotective agents that could affect ceruloplasmin metabolism for the treatment of IPD.

Ethics

Ethics Committee Approval: The study were approved by Göztepe Education and Research Hospital Ethics Committee, Informed Consent: Consent form was filled out by all participants, Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Reyhan Gürer, Concept: Reyhan Gürer, Nihal Işık, Design: Reyhan Gürer, Nihal Işık, Tunahan Ayaz, Data Collection or Processing: Reyhan Gürer, Dilvin Gökçe, Analysis or Interpretation: Reyhan Gürer, Şenay Aydın, Literature Search: Reyhan Gürer, Dilvin Gökçe, Writing: Reyhan Gürer, Şenay Aydın.

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