Neuroscience Letters 757 (2021) 135975

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Cerebrospinal fluid levels of oxidative stress measured using diacron-reactive oxygen metabolites and biological antioxidant potential in patients with Parkinson's disease and progressive supranuclear palsy

Kenta Takahashi, Kazuhiro Iwaoka, Kai Takahashi, Yoshio Suzuki, Keita Taguchi, Kanako Yamahara, Tetsuya Maeda *

Division of Neurology and Gerontology, Department of Internal Medicine, School of Medicine, Iwate Medical University, 2-1-1 Idai-dori, Yahaba-cho, Shiwa-gun, Iwate 028-3694, Japan

ARTICLE INFO

Keywords:
Oxidative stress
Antioxidant
Parkinson's disease
d-ROM
BAP

ABSTRACT

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by motor and non-motor symptoms. Because no curative therapy is available for PD, elucidation of its pathophysiology is important to establish more effective treatments. Oxidative stress (OS) has gained attention and been investigated as one of the candidates involved in the pathogenesis of PD. This study aimed to evaluate OS in the cerebrospinal fluid (CSF) of patients with PD and progressive supranuclear palsy (PSP) using diacron-reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP) tests, which can easily assess OS in liquid samples. Results were compared to the clinical background of patients and with those of the normal control (NC) group. CSF samples were obtained from 69 patients with PD, 14 patients with PSP, and 22 individuals in the NC group. OS levels and antioxidant capacity were measured using d-ROMs and BAP tests, respectively. CSF d-ROM levels were extremely low (<10 U.CARR) in all 3 groups than the plasma d-ROM levels. Antioxidant capacity was significantly higher in patients with PSP (1074 ± 79 μM) than in patients with PD (918 ± 350 μM) ($p = 0.019$). In the PD group, antioxidant capacity was significantly lower in patients with tremor (858 ± 269 μM) than in those without tremor (1132 ± 505 μM) ($p = 0.004$). Our study suggests that the CSF level of OS is under homeostatic control of antioxidative mechanisms in healthy individuals as well as those with neurodegenerative diseases, and increased antioxidant capacity can indicate the CSF level of OS. The lower CSF level of OS in the tremor dominant subtype of PD may be the reason for the benign clinical course.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by tremor, rigidity, and bradykinesia. These symptoms are associated with Lewy bodies and loss of dopaminergic neurons in the substantia nigra [1]. However, the exact etiology of PD remains unknown. Despite the current evolution of treatment options, no curative therapy is available for PD. Hence, elucidating the pathogenesis of PD is crucial to overcome this intractable disease. Progressive supranuclear palsy (PSP) is also a neurodegenerative movement disorder, which shows atypical parkinsonism and comorbid symptoms such as supranuclear gaze palsy, nuchal rigidity, dementia. Its pathological background, known as tau pathology, is also different from PD. No treatments specific to PSP have been developed [2].

Recently, oxidative stress (OS) has been reported to play an important role in the pathological processes in neuromelanin-pigmented cell bodies [3], which can lead to PD onset. Typical OS substances generated in humans are superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH·). These three substances are called reactive oxygen species (ROS). On the other hand, superoxide dismutase and

Abbreviations: PD, Parkinson's disease; PSP, progressive supranuclear palsy; NC, normal control; CSF, cerebrospinal fluid; CNS, central nervous system; OS, oxidative stress; ROS, reactive oxygen species; L-dopa, levodopa; DA, dopamine agonist; H&Y stage, Hoehn & Yahr stage; UPDRS, Unified Parkinson's Disease Rating Scale; d-ROMs, diacron-reactive oxygen metabolites; BAP, biological antioxidant potential; SOD, superoxide dismutase; TD, tremor-dominant.

* Corresponding author.
E-mail addresses: kentat@iwate-med.ac.jp (K. Takahashi), kiwaoka@iwate-med.ac.jp (K. Iwaoka), sea.t.0926@gmail.com (K. Takahashi), 345def@gmail.com (Y. Suzuki), k.t.dwkisr415@gmail.com (K. Taguchi), kkonno@iwate-med.ac.jp (K. Yamahara), maeda@iwate-med.ac.jp (T. Maeda).

https://doi.org/10.1016/j.neulet.2021.135975
Received 8 February 2021; Received in revised form 17 May 2021; Accepted 17 May 2021
Available online 21 May 2021

hydrogen peroxide scavenging enzymes (catalase, glutathione, peroxidase, etc.) scavenge active oxygen and act as antioxidants. To date, few studies have investigated overall OS level and antioxidant capacity in the CSF of patients with PD in a large cohort and never in PSP. In dopaminergic neurons, dopamine can breakdown in the presence of iron or can be catalyzed by monoamine oxidase. This process can generate hydrogen peroxide and can be cytotoxic to neuronal cells [4]. DJ-1, also known as parkinsonism-associated deglycase, is encoded by the *PARK7* gene, and it plays a neuroprotective role, apart from transcriptional regulation. This enzyme functions as a chaperone and protease, and it regulates mitochondrial functions [5,6]. Many substances have been shown to possess antioxidant effects including uric acid, glutathione, coenzyme Q10, superoxide dismutase, and catalase [7–9].

Measuring the exact amount of ROS and antioxidant substances can elucidate the pathophysiology. However, most ROS are chemically labile and very reactive. For example, the half-life of hydroxyl radicals is no longer than several seconds. Detecting these substances requires specific methods [10]. Numerous substances can be used to measure antioxidant capacity, but measuring them separately is not reasonable. With regards to the specimen, plasma is easy to obtain from patients in clinical settings. However, OS level in the blood can be influenced by infection, metabolic disorder, dehydration, and many other clinical conditions. This can change the examination results, which no longer reflect the condition of the central nervous system (CNS). For evaluating OS in the CNS, cerebrospinal fluid (CSF) may reflect redox balance more accurately.

Recently, the Fenton reaction has been applied for measurement of OS. The diacron-reactive oxygen metabolites (d-ROMs) test can measure the total hydroperoxide in liquid samples, and the biological antioxidant potential (BAP) test can quantify the total antioxidant capacity. In this study, we aimed to evaluate OS in the CSF of patients with PD and PSP using these tests and comparing their levels to the clinical background.

2. Materials and methods

2.1. Participants

All participants enrolled in the study signed a written informed consent form. This research was conducted in accordance with the Declaration of Helsinki, and the medical ethics committee of our university reviewed and approved the study protocol. All patients were recruited from our hospital between November 2011 and October 2020. All participants aged 20 years and older and met the following criteria. The PD group met the PD diagnostic criteria of the Ministry of Health, Labor and Welfare's Intractable Disease and Neurodegenerative Disease Research Group [11]. The PSP group met the National Institute of Neurological Disorders and Stroke and the Society for Supranuclear Palsy criteria [12]. The normal control (NC) group consisted of patients with no history of CNS diseases and negative neurological disorders based on neurological examination and neuroimaging findings.

2.2. Data acquisition and sample collection

By referring to electronic medical records, the clinical backgrounds of each participant, including age, sex, medical history, alcohol consumption, and smoking, were collected. In the PD and PSP groups, disease duration, age at onset, symptoms, imaging abnormality, and dose of medications were also assessed. Disease severity and clinical features were evaluated using the Hoehn and Yahr scale (H-Y scale) and the International Parkinson and Movement Disorder Society version of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III. CSF was collected by conventional lumbar puncture. CSF samples from each subject were immediately divided into 1 mL tubes and stored at −80 °C until use.

2.3. Measurement method

CSF samples were thawed at 20 °C only once before examination and centrifuged at 2000 g. Tests were conducted using REDOXLIBRA (Diacron International, Grosseto, Italy), according to the Fenton reaction. Hydrogen peroxide (H_2O_2) interacts with the cations of transition metals, such as iron ($Fe^{2+} + H^2O^2 \rightarrow \cdot OH + OH^-$) [13]. In the d-ROMs test, free radicals generate alkoxyl and peroxyl radicals in the presence of iron. These radicals oxidize an alkyl-substituted aromatic amine and transform it into a pink-colored derivative. The change in color is photometrically quantified to calculate the OS level. This test is used to measure total hydroperoxide in liquid samples, and the results are expressed in Carratelli units (U.CARR). In the BAP test, ferric (Fe^{3+}) ions are reduced to ferrous (Fe^{2+}) ions by the antioxidant substances in the samples, which can also be photometrically quantified to calculate antioxidant capacity. The results of the test are expressed in μM [14].

2.3.1. d-ROMs

The CSF sample (20 μL) was placed in a cuvette filled with a pH 4.8-buffer that had been warmed in an incubator for at least 15 min. The cuvette was then gently overturned and mixed, and N,N-diethyl-p-phenylenediamine (20 μL) was placed in the cuvette as a color-developing chromogen substrate. The cuvette was again overturned and mixed, and then placed in a spectrophotometer to measure the optical density at 505 nm [15].

2.3.2. BAP

A 50 μl of chromogenic reagent (containing Fe^{3+}) was added to a cuvette to induce red coloration. The cuvette was overturned and mixed, and spectrophotometric measurement was then performed. Subsequently, 10 μL of CSF sample was placed into the cuvette, mixed, and placed in a spectrophotometer to measure the optical density at 505 nm [15].

2.4. Statistical analysis

Statistical tests were conducted using IBM SPSS Statistics for Windows, version 25 (IBM Corp. Armonk, NY, USA). Data are reported as the mean ± standard deviation. Normal distribution of data was tested using the Shapiro-Wilk test and was analyzed with parametric or non-parametric test. The chi-square test was used to assess the sex ratio between groups. The Kruskal-Wallis test was performed to evaluate the difference between three or more groups, and post hoc Bonferroni correction and the Dunnett T3 test were utilized. The Mann-Whitney U-test was used to evaluate significance between two groups. Spearman's rho coefficient correlation was used to assess the strength of the relationship between the result of measurement and age of onset, disease duration, H-Y stage, score of MDS-UPDRS part III, duration from onset to appearance of motor fluctuations, score of Hasegawa dementia scale-revised, and dose of drugs. A covariate-adjusted regression model was used to adjust for age and sex. We considered a p-value < 0.05 to be significant.

3. Results

3.1. Participant characteristics

The demographic and clinical characteristics of the participants are shown in Table 1. In total, 69 patients with PD and 14 patients with PSP were included in this study. A total of 22 individuals without CNS abnormalities were also recruited as the NC group. The ages of the PD and PSP groups were significantly higher than those of the NC group. Between these three groups, sex ratio, medical history, and rate of drinking were not significantly different (data not shown). Between the PD and PSP groups, age of onset and disease duration were not significantly different. Similarly, score of MDS-UPDRS part III and H-Y stage were not

Table 1
Clinical characteristics.

	PD	PSP	NC
Age, y	64.2 ± 11.2 (31 – 84)	69.4 ± 7.6 (55 – 79)	32.1 ± 15.8 (13 – 65)*
Sex, F/M	31/38	4/10	11/11
Age of onset, y	57.9 ± 11.3 (27 – 79)	66.5 ± 7.2 (50 – 69)	–
Disease duration, y	6.7 ± 5.9 (1 – 23)	3.8 ± 3.1 (1 – 7)	–
LED, mg	429 ± 591 (0 – 2748)	57 ± 165 (0 – 600)	–

PD: Parkinson's disease, PSP: progressive supranuclear palsy, NC: normal control, LED: levodopa equivalent dose. All values except for sex are expressed as mean ± standard deviation (range). *: p value < 0.05 against both the PD and PSP groups.

significantly different. L-dopa dose and L-dopa equivalent dose of the PD group were significantly higher than those of the PSP group (data not shown).

3.2. CSF levels of d-ROMs and BAP

Fig. 1 shows CSF OS levels and antioxidant capacity measured using d-ROMs and BAP, respectively, in each group. CSF d-ROM levels in all groups were significantly lower than those observed in the plasma levels

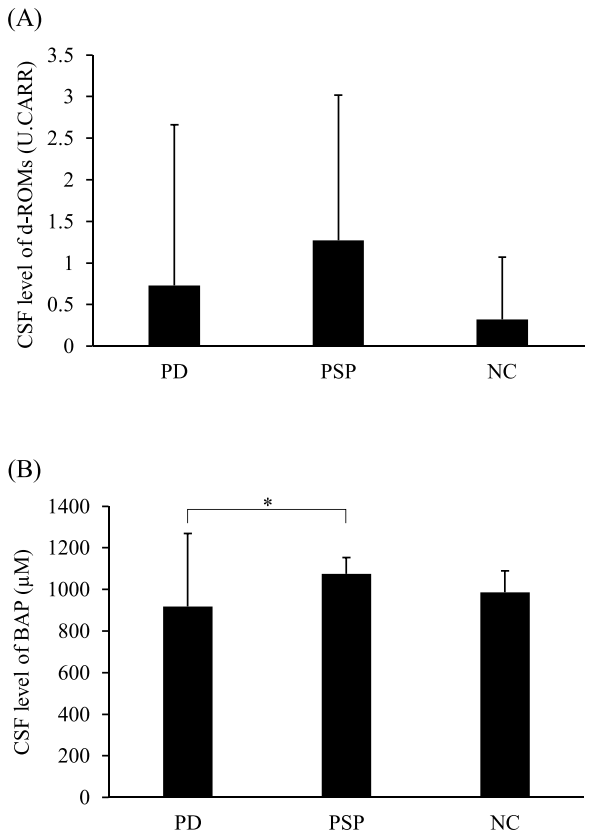

Fig. 1. CSF levels of d-ROMs (A) and BAP (B) in PD, PSP and NC.
(A) Adjusted CSF levels of d-ROMs are shown. All values were extremely low. (B) Adjusted CSF levels of BAP are shown. There was no significant difference between the NC group and the PD group or the PSP group, whereas the PSP group showed significantly higher levels than the PD group. The error bars show the standard deviations. *: p value < 0.05. CSF: cerebrospinal fluid; d-ROMs: diacron-reactive oxygen metabolites; BAP: biological antioxidant potential; PD: Parkinson's disease; PSP: progressive supranuclear palsy; NC: normal control.

of healthy adults (approximately 250–300 U.CARR [16]). The BAP of the PSP group (1074 ± 79 μM) was significantly higher than those of the PD (918 ± 350 μM) and NC (987 ± 103 μM) groups. After adjusting for age and sex, the significance between the PSP and PD groups remained.

3.3. Statistical analysis of CSF BAP levels in PD

Table 2 shows the correlation of BAP and dosage of anti-PD drugs in the PD group. No significant correlation was found between these two variables. In addition, in the PD group, BAP had no significant correlations with age of onset, disease duration, H-Y stage, score of UPDRS part III, duration from disease onset to motor fluctuation, and Hasegawa dementia scale-revised scores. Similarly, in the PSP group, there was no significant correlation between BAP and the variables assessed.

Fig. 2 shows the BAP of the PD group with or without tremor separately. In the PD group, the BAP of patients with tremor was significantly lower than that of patients without tremor, even after adjusting for age and sex. Other PD symptoms (both motor and non-motor), comorbidity, drinking, smoking, disease severity (H-Y stage ≥ 3 or not), and clinical subtypes (tremor-dominant (TD) or postural instability/gait difficulty [17]) showed no relationship with BAP.

3.4. Receiver operating characteristics analysis

We analyzed the accuracy of CSF levels of BAP in diagnosing PD and PSP using receiver operating characteristic analysis (Fig. 3). The result showed an area under the curve of 0.770. The sensitivity and specificity of BAP in distinguishing PSP from PD patients were 0.846 and 0.676, respectively (cutoff value: 1042 μM).

4. Discussion

To the best of our knowledge, this case-control study is the first to evaluate OS in the CSF of patients with PD and PSP in a large cohort by using d-ROMs and BAP tests simultaneously. Our study showed the characteristics of OS in the CSF of patients with PD or PSP and the NC group. d-ROMs and BAP tests are simple and easy. They enable researchers and medical doctors to understand the redox balance of patients with neurologically abnormal conditions in the CNS using prepared CSF samples easily.

In this study, we revealed that d-ROM levels were extremely low in the CSF of the NC group and patients with PD or PSP. These findings suggest that the CSF level of OS measured using d-ROMs test is under homeostatic antioxidative control even in some neurodegenerative conditions. This study is the first to report d-ROM levels in the CSF of neurodegenerative diseases. In the case of other neurological diseases, children with neuroinfectious disease show high levels of d-ROMs, as reported in previous studies [18,19]. Kawashima et al. reported that the

Table 2
Correlation between CSF levels of BAP and dosage of antiparkinsonian agents in PD.

Agent	coefficient of correlation	p value
L-dopa	−0.145	0.239
ROP	−0.232	0.056
PPX	−0.082	0.508
ROT	−0.185	0.130
ENC	−0.085	0.492
ZNS	−0.228	0.062
SGL	−0.167	0.135
AMD	−0.018	0.887
ISF	−0.125	0.310
LED	−0.228	0.062

BAP: biological antioxidant potential; CSF: cerebrospinal fluid; L-dopa: levodopa; ROP: ropinirole; PPX: pramipexole; ROT: rotigotine; ENC: entacapone; ZNS: zonisamide; SGL: selegiline; AMD: amantadine; ISF: istradefylline; LED: Levodopa-equivalent dose; PD: Parkinson's disease.

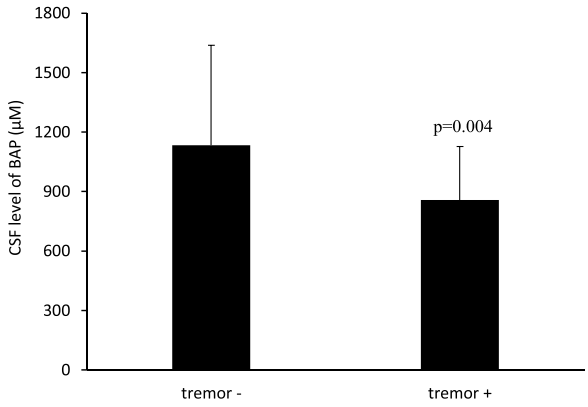

Fig. 2. CSF levels of BAP in PD with or without tremor.
Adjusted CSF levels of BAP in PD with tremor is significantly lower than without tremor ($p = 0.004$). The error bars show the standard deviations. CSF: cerebrospinal fluid; BAP: biological antioxidant potential; PD: Parkinson's disease.

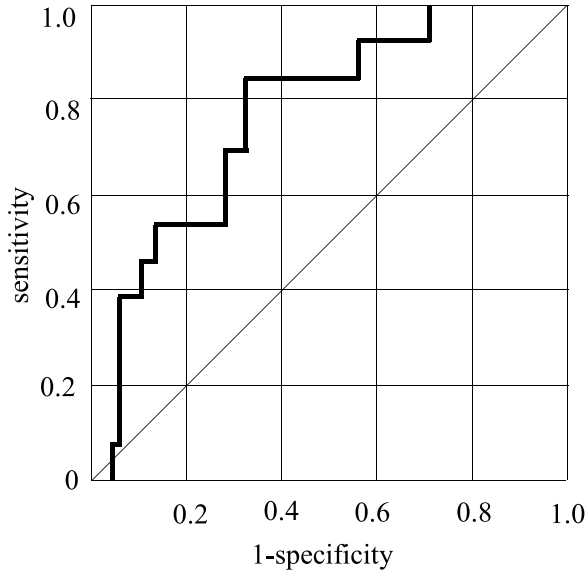

Fig. 3. ROC curve of CSF BAP level of PSP and PD.
When we set the cutoff value of BAP to 1042 μM, its sensitivity and specificity in distinguishing PSP from PD were 0.846 and 0.676, respectively. ROC: receiver operating characteristic; CSF: cerebrospinal fluid; BAP: biological antioxidant potential; PD: Parkinson's disease; PSP: progressive supranuclear palsy.

CSF level of d-ROMs in pediatric patients with enteroviral infection was elevated (39 ± 24.1 U.CARR) [18] Yamanaka et al. reported that CSF d-ROM levels in children with influenza-associated encephalopathy and influenza virus-associated febrile seizure are significantly higher than those of patients with febrile convulsion [19]. Whether neuroinfectious disease can universally increase CSF d-ROM levels remains to be clarified because this was not a case-control study and targeted children. In a meta-analysis of CSF biomarkers in PD, interleukin-6, interleukin-1β, and α-synuclein (αS) and its oligomeric and phosphorylated forms are reliable CSF biomarkers. However, fewer CSF biomarkers of PD associated with OS have been reported [20]. This evidence may also support our results in OS level in the CSF of patients with PD.

In our study, CSF levels of BAP were detectable in NC, PD, or PSP. Because there are several types of OS in the CNS, we considered an increase in CSF levels of BAP as a result of the variable extent of OS. The CSF levels of BAP in both PD and PSP were not different from those in NC. The results suggest that the CSF levels of OS in patients with PD and PSP are also under antioxidative control, and the CSF levels of BAP can reflect the CSF levels of OS. The CSF levels of BAP were relatively higher in PSP than in NC, but the difference was not statistically significant. The concentration of Cu/Mn-superoxide dismutase is known to be elevated in the cerebellum, substantia nigra pars reticulata, caudate nucleus, pallidum, calcarine cortex, frontal cortex, and CSF in PSP. The antioxidant effect of this enzyme may contribute to the redox processes in the CNS [21,22]. Antiparkinson agents, such as DAs [23,24], monoamine oxidase B inhibitors [25,26], and zonisamide [27,28], were reported to have some redox effects in in vitro and in vivo studies. Dopamine can auto-oxidize at normal pH into toxic species, superoxide radicals, and hydrogen peroxides, increasing the OS level [29]. Therefore, pharmacotherapy may be one of the reasons why no difference in antioxidant levels was found between NC and PD. In this study, we could not restrict PD recruitment in *de novo* patients.

PSP has significantly higher CSF levels of antioxidation than PD. This result was a novel finding of this study. The differences in the neuropathological findings may be one of the factors underlying the significant difference in CSF levels of OS. Neuropathologically, PD and PSP can be distinguished by the accumulated proteins and the distribution pattern of iron. In terms of the accumulated proteins, PD is characterized by Lewy bodies, mainly consisting of phosphorylated αS, while PSP is characterized by tuft-shaped astrocytes, mainly consisting of phosphorylated tau protein. In terms of the distribution pattern of iron, typical neuropathological findings in PSP are detected in the pons, substantia nigra, subthalamic nucleus, and pallidum [30], where accumulation of iron is remarkable. It has been reported that iron deposition increases in the brains of patients with PSP and MSA, but not PD [31]. Deposition of this transition metal can affect the metabolism of OS in the brain.

In PD, CSF levels of BAP were significantly lower in patients exhibiting tremor than in patients without tremor. This remained statistically significant after adjusting for age and sex, as well as UPDRS part III scores. This is another novel finding of this study. A previous report had shown that the plasma level of BAP and d-ROMs exhibit a positive correlation [32], whereas in CSF, the levels of BAP can change depending on the degree of OS without correlation with the levels of d-ROMs. These results may suggest that patients with tremor demonstrate OS inhibitory mechanism in the brain. However, we could not clarify these mechanisms in this study. From the clinical point of view, PD can be classified into the two subtypes of TD and postural instability/gait difficulty [33]. The TD type generally exhibits a relatively benign clinical course. Lower CSF levels of BAP may be associated with this clinical picture.

Our study has several limitations. First, drug administration could disturb the redox balance of the CSF. We recognize that this limitation might have affected the result, but could not restrict PD as *de novo* patients because CSF samples are not easy to access and valuable. Further studies should involve *de novo* patients with PD. As our OS analyzing system had the lowest limit of measurable d-ROM levels (17 U.CARR) [16], further improvement of measurement sensitivity is expected without losing the advantages of practical convenience, ease of installation, and portability. We could not analyze the plasma level of d-ROM because of insufficient sample conservation and management. We selected PSP as disease control against PD because they have different pathological backgrounds, but are both neurodegenerative diseases. Numerous types of movement disorders with neurodegenerative processes should be considered as research candidates for OS studies. Finally, we were unable to match the groups in terms of age and sex. Because lumber puncture is an invasive procedure, it is extremely difficult to perform on healthy adults and collect CSF samples. We believe that this is one of the reasons why our results exhibited a statistical tendency but remained non-significant in the CSF levels of BAP between normal controls and patients with PD or PSP.

5. Conclusions

This study provides novel evidence that the CSF levels of OS measured using d-ROMs are under strict homeostatic control in PD or PSP to the same extent as NC and that CSF levels of OS are exhibited as BAP levels, which increase its capability to reflect the magnitude of OS in the CSF. We suggest that the CSF levels of OS are higher in patients with PSP than in those with PD, and that PD patients with tremor are exposed to lower OS and have a benign clinical course.

CRediT authorship contribution statement

Kenta Takahashi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Writing - original draft. **Kazuhiro Iwaoka:** Investigation, Resources. **Kai Takahashi:** Investigation, Resources. **Yoshio Suzuki:** Investigation, Resources. **Keita Taguchi:** Investigation, Resources. **Kanako Yamahara:** Investigation, Resources. **Tetsuya Maeda:** Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors have no conflicts of interest to report.

References

[1] L.V. Kalia, A.E. Lang, Parkinson's disease, Lancet 386 (2015) 896–912, https://doi.org/10.1016/S0140-6736(14)61393-3.

[2] A.L. Boxer, J. Yu, L.I. Golbe, I. Litvan, A.E. Lang, S. Francisco, Q.M. Hospital, N. Brunswick, S. Diego, M. Cluster, New diagnostics and therapeutics for progressive supranuclear palsy, Lancet Neurol. 16 (2018) 552–563, https://doi.org/10.1016/S1474-4422(17)30157-6.New.

[3] X.M. Shen, B. Xia, M.Z. Wrona, G. Dryhurst, Synthesis, redox properties, in vivo formation, and neurobehavioral effects of N-acetylcysteinyl conjugates of dopamine: Possible metabolites of relevance to Parkinson's disease, Chem. Res. Toxicol. 9 (1996) 1117–1126, https://doi.org/10.1021/tx960052v.

[4] H. Yuan, J.C. Zheng, P. Liu, S.F. Zhang, J.Y. Xu, L.M. Bai, Pathogenesis of Parkinson's disease: oxidative stress, environmental impact factors and inflammatory processes, Neurosci. Bull. 23 (2007) 125–130, https://doi.org/10.1007/s12264-007-0018-x.

[5] Y. Saito, Oxidized DJ-1 as a possible biomarker of Parkinson's disease, J. Clin. Biochem. Nutr. 54 (2014) 138–144, https://doi.org/10.3164/jcbn.13-108.

[6] H. Ariga, K. Takahashi-Niki, I. Kato, H. Maita, T. Niki, S.M.M. Iguchi-Ariga, Neuroprotective function of DJ-1 in Parkinson's disease, Oxid. Med. Cell. Longev. 2013 (2013), 683920, https://doi.org/10.1155/2013/683920.

[7] L.K. Mischley, L.J. Standish, N.S. Weiss, J.M. Padowski, T.J. Kavanagh, C.C. White, M.E. Rosenfeld, Glutathione as a biomarker in Parkinson's disease: associations with aging and disease severity, Oxid. Med. Cell. Longev. 2016 (2016), 9409363, https://doi.org/10.1155/2016/9409363.

[8] L.K. Mischley, J. Allen, R. Bradley, Coenzyme Q10 deficiency in patients with Parkinson's disease, J. Neurol. Sci. 318 (2012) 72–75, https://doi.org/10.1016/j.jns.2012.03.023.

[9] K.H. Chang, C.M. Chen, The role of oxidative stress in Parkinson's disease, Antioxidants 9 (2020) 1–32, https://doi.org/10.3390/antiox9070597.

[10] R.P. Mason, Imaging free radicals in organelles, cells, tissue, and in vivo with immuno-spin trapping, Redox Biol. 8 (2016) 422–429, https://doi.org/10.1016/j.redox.2016.04.003.

[11] N. Yanagisawa, Annual Report of the Research Committee of CNS Degenerative Disease, The Ministry of Health and Welfare of Japan, 1996, pp. 22–27.

[12] S. Gilman, P.A. Low, N. Quinn, A. Albanese, Y. Ben-Shlomo, C.J. Fowler, H. Kaufmann, T. Klockgether, A.E. Lang, P.L. Lantos, I. Litvan, C.J. Mathias, E. Oliver, D. Robertson, I. Schatz, G.K. Wenning, Consensus statement on the diagnosis of multiple system atrophy, J. Auton. Nerv. Syst. 74 (1998) 189–192, https://doi.org/10.1055/s-0028-1086654.

[13] X. Qian, J. Zhang, Z. Gu, Y. Chen, Nanocatalysts-augmented Fenton chemical reaction for nanocatalytic tumor therapy, Biomaterials 211 (2019) 1–13, https://doi.org/10.1016/j.biomaterials.2019.04.023.

[14] D. Hu, D. Li, M. Shigeta, Y. Ochi, T. Okauchi, H. Neyama, S. Kabayama, Y. Watanabe, Y. Cui, Alleviation of the chronic stress response attributed to the antioxidant and anti-inflammatory effects of electrolyzed hydrogen water, Biochem. Biophys. Res. Commun. 535 (2021) 1–5, https://doi.org/10.1016/j.bbrc.2020.12.035.

[15] T. Kitaoka, M. Morimoto, T. Hashimoto, Y. Tsuda, T. Nakatsu, S. Kyotani, Evaluation of the efficacy of drug treatment based on measurement of the oxidative stress, using reactive oxygen metabolites and biological antioxidant potential, in children with autism spectrum disorder and attention deficit hyperactivity disorder, J. Pharm, Health Care Sci. 6 (2020) 8, https://doi.org/10.1186/s40780-020-00164-w.

[16] E.L. Iorio, The Measurement of Oxidative Stress, 2007, pp. 3–34.

[17] G.T. Stebbins, C.G. Goetz, D.J. Burn, J. Jankovic, T.K. Khoo, B.C. Tilley, How to identify tremor dominant and postural instability/gait difficulty groups with the movement disorder society unified Parkinson's disease rating scale: comparison with the unified Parkinson's disease rating scale, Mov. Disord. 28 (2013) 668–670, https://doi.org/10.1002/mds.25383.

[18] H. Kawashima, H. Ioi, C. Ishii, Y. Hasegawa, M. Amaha, Y. Kashiwagi, K. Takekuma, A. Hoshika, Y. Watanabe, Viral loads of cerebrospinal fluid in infants with enterovirus meningitis, J. Clin. Lab. Anal. 22 (2008) 216–219, https://doi.org/10.1002/jcla.20244.

[19] G. Yamanaka, H. Kawashima, Y. Suganami, C. Watanabe, Y. Watanabe, T. Miyajima, K. Takekuma, S. Oguchi, A. Hoshika, Diagnostic and predictive value of CSF d-ROM level in influenza virus-associated encephalopathy, J. Neurol. Sci. 243 (2006) 71–75, https://doi.org/10.1016/j.jns.2005.11.029.

[20] T. Katayama, J. Sawada, K. Takahashi, O. Yahara, Cerebrospinal fluid biomarkers in Parkinson's disease: a critical overview of the literature and meta-analyses, Brain Sci. 10 (2020) 1–14, https://doi.org/10.3390/brainsci10070466.

[21] K. Aoyama, K. Matsubara, S. Kobayashi, Aging and oxidative stress in progressive supranuclear palsy, Eur. J. Neurol. 13 (2006) 89–92, https://doi.org/10.1111/j.1468-1331.2006.01139.x.

[22] I. Cantuti-Castelvetri, C.E. Keller-Mcgandy, D.S. Albers, M.F. Beal, J.P. Vonsattel, D.G. Standaert, S.J. Augood, Expression and activity of antioxidants in the brain in progressive supranuclear palsy, Brain Res. 930 (2002) 170–181, https://doi.org/10.1016/s0006-8993(02)02244-8.

[23] C. Deng, J. Zhu, J. Yuan, Y. Xiang, L. Dai, Pramipexole Inhibits MPP+-Induced Neurotoxicity by miR-494-3p/BDNF, Neurochem. Res. 45 (2020) 268–277, https://doi.org/10.1007/s11064-019-02910-5.

[24] N. Isooka, I. Miyazaki, R. Kikuoka, K. Wada, E. Nakayama, K. Shin, D. Yamamoto, Y. Kitamura, M. Asanuma, Dopaminergic neuroprotective effects of rotigotine via 5-HT1A receptors: Possibly involvement of metallothionein expression in astrocytes, Neurochem. Int. 132 (2020) 104608, https://doi.org/10.1016/j.neuint.2019.104608.

[25] V. Dror, M. Rehavi, I.E. Biton, S. Eliash, Rasagiline prevents neurodegeneration in thiamine deficient rats - A longitudinal MRI study, Brain Res. 1557 (2014) 43–54, https://doi.org/10.1016/j.brainres.2013.12.030.

[26] M. Naoi, W. Maruyama, M. Shamoto-Nagai, Rasagiline and selegiline modulate mitochondrial homeostasis, intervene apoptosis system and mitigate α-synuclein cytotoxicity in disease-modifying therapy for Parkinson's disease, J. Neural Transm. 127 (2020) 131–147, https://doi.org/10.1007/s00702-020-02150-w.

[27] M. Asanuma, I. Miyazaki, F.J. Diaz-Corrales, N. Kimoto, Y. Kikkawa, M. Takeshima, K. Miyoshi, M. Murata, Neuroprotective effects of zonisamide target astrocyte, Ann. Neurol. 67 (2010) 239–249, https://doi.org/10.1002/ana.21885.

[28] V.A. Yürekli, S. Gürler, M. Nazıroğlu, A.C. Uğuz, H.R. Koyuncuoğlu, Zonisamide attenuates MPP(+)-induced oxidative toxicity through modulation of Ca2+ signaling and caspase-3 activity in neuronal PC12 cells, Cell. Mol. Neurobiol. 33 (2013) 205–212, https://doi.org/10.1007/s10571-012-9886-3.

[29] J. Lotharius, P. Brundin, Pathogenesis of Parkinson's disease: Dopamine, vesicles and α-synuclein, Nat. Rev. Neurosci. 3 (2002) 932–942, https://doi.org/10.1038/nrn983.

[30] D.W. Dickson, R. Rademakers, M.L. Hutton, Progressive supranuclear palsy: Pathology and genetics, Brain Pathol. 17 (2007) 74–82, https://doi.org/10.1111/j.1750-3639.2007.00054.x.

[31] D.T. Dexter, A. Carayon, F. Jacoy-Agid, Y. Agid, F.R. Wells, S.E. Daniel, A.J. Lees, P. Jenner, C.D. Marsden, Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting basal ganglia, Brain 114 (1991) 1953–1975, https://doi.org/10.1093/brain/114.4.1953.

[32] S. Fukuda, J. Nojima, Y. Motoki, K. Yamaguti, Y. Nakatomi, N. Okawa, K. Fujiwara, Y. Watanabe, H. Kuratsune, A potential biomarker for fatigue: oxidative stress and anti-oxidative activity, Biol. Psychol. 118 (2016) 88–93, https://doi.org/10.1016/j.biopsycho.2016.05.005. Get rights and content.

[33] C. Eggers, D.J. Pedrosa, D. Kahraman, F. Maier, C.J. Lewis, G.R. Fink, M. Schmidt, L. Timmermann, Parkinson subtypes progress differently in clinical course and imaging pattern, PLoS One 7 (2012), e46813, https://doi.org/10.1371/journal.pone.0046813.