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ORIGINAL RESEARCH

Clinical Characteristics, Iron Metabolism and Neuroinflammation: New Insight into Excessive Daytime Sleepiness in Parkinson's Disease

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Background: To investigate the clinical characteristics, iron metabolism and neuroinflammation in Parkinson's disease (PD) patients with excessive daytime sleepiness (EDS).

Methods: We studied 379 patients with PD and 30 age-matched controls. All subjects were evaluated by Epworth sleepiness scale (ESS) and a series of rating scales and were divided into PD-EDS and PD-NEDS groups according to ESS score. The concentrations of iron and iron-related proteins and inflammatory cytokines in both cerebrospinal fluid (CSF) and serum were examined.

Results: 1. The occurrence rate of EDS in total PD patients was 16.09%. 2. PD-EDS group had significantly severer disease stages, more severe motor and non-motor features of the disease. 3. In CSF, the concentrations of iron and IL-1β in the PD-EDS group were significantly higher and ferritin concentration was prominently lower when compared with the PD-NEDS group and the control group; ESS score was significantly associated with high concentrations of iron and IL-1β and low concentration of ferritin in the PD group. Iron concentration was positively correlated with IL-1β concentration in the PD-EDS group. 4. In serum, no changes were observed in iron and iron-related proteins and inflammatory cytokines among the three groups.

Conclusion: EDS was a common symptom in PD patients. PD patients with EDS had more severe motor and some non-motor symptoms. Overloaded iron-relevant inflammation in the brain might be an underlying mechanism of PD-EDS.

Keywords: Parkinson disease, excessive daytime sleepiness, clinical features, iron metabolism, inflammation

Background

Excessive daytime sleepiness (EDS) is very common in Parkinson's disease (PD). Studies report that the prevalence of EDS in PD patients ranges from 16% to 74%.[1,2] Dopaminergic treatment, severity stages, other concomitant sleep disturbances, dysautonomia, anxiety, and depression have been reported to be potential risk factors for EDS in PD.[3–6] One study demonstrated that EDS was different from poor sleep quality and fatigue.[7] A few studies concentrated on apathy, rapid eye movement sleep behavior disorder (RBD) and restless leg symptoms in PD with EDS (PD-EDS) patients. In short, the correlation of EDS with disease stage, motor symptoms, motor complications and non-motor symptoms remains controversial.

Magnetic resonance imaging at 3.0 Tesla suggests that the iron concentrations in substantia nigra (SN) and other nuclei are increased and linked to the severity of

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motor symptoms in PD.[8] Iron-related neurodegenerative disorders can result from both iron accumulation in specific brain regions or defects in its metabolism. Iron-exerted toxicity in the presence of unbound or free iron, and excessive free iron caused damaging effects on many cellular processes and induced neurodegeneration.[9] Compared with normal subjects, transferrin concentration was remarkably elevated in PD brains,[10] suggesting that iron metabolism disruption in the central nervous system (CNS) participated in the pathogenesis of PD. However, previous studies reported that the concentrations of iron and ferritin in serum or cerebrospinal fluid (CSF) from PD patients were not different from healthy controls.[11,12] To date, no research has paid attention to the association between EDS and iron metabolism disruption in both CNS and peripheral systems of PD patients.

The role of inflammation in PD has been suggested by increasing evidence showing microglial activation and inflammatory cytokines production from *in vivo* and post-mortem studies.[13,14] Inflammation in the SN served as a driving force for dopaminergic cell death and played a pivotal role on PD progression.[15] The dead neurons released iron into the extracellular domain and provoked neuroinflammation by way of activating microglia,[16] aggravating the deterioration of PD motor symptoms. Recent investigations showed that inflammation besides SN has a relationship with non-motor symptoms of PD.[17] For example, patients with EDS had higher concentrations of C-reactive protein.[18] Meanwhile, peripheral inflammation potentially participated in the pathogenesis of non-motor symptoms, such as fatigue and cognitive impairment.[18] However, the relationship among EDS, iron metabolism disruption and inflammation in PD remains unclear.

In this study, we firstly assessed EDS, motor symptoms and non-motor symptoms in PD patients recruited by using the Epworth sleepiness scale (ESS) and related rating scales. Additionally, we detected concentrations of iron and iron-related proteins and inflammatory cytokines in both CSF and serum. Finally, we analyzed the relationships among ESS score and the concentrations of the above factors in both CSF and serum.

Methods

Subjects

PD Patients

We consecutively recruited 379 PD patients from the Department of Geriatrics and the Department of Neurology, Beijing Tiantan Hospital, Capital Medical University. We diagnosed patients with PD according to Movement Disorder Society Clinical Diagnostic Criteria for Parkinson Disease.[19] Clinical information and levodopa equivalent daily dose (LEDD) of PD patients were recorded when they were recruited in this study. Approximately 42.48% of the recruited PD patients in this study are drug-naive and newly diagnosed.

Exclusion criteria were a diagnosis of anemia, hepatopathy, heart failure, pulmonary diseases and chronic renal disease and a history of blood donation. Female patients who had not been through menopause were excluded in this study. Patients who had taken iron supplements were excluded.

Control Subjects

A total of 30 normal control subjects were consecutively recruited according to the following criteria: (1) no neurological symptom or signs; (2) no essential tremor, PD and related disorders; (3) magnetic resonance imaging of the head was normal; (4) no other diseases influencing patients' sleep; (5) no systemic infectious diseases or autoimmune diseases; (6) no hallucination or other neuropsychiatric symptoms; (7) no heavy drinking or drugs abuse; (8) no iron supplement taking; and (9) no sleep disturbance.

The exclusion criteria were also applied to the control group.

Clinical Features and Assessments

EDS

EDS of PD patients was identified and quantified by ESS. This rating scale is a 24-point scale containing 8 questions with each score ranging from 0 to 3. ESS ≥10 was regarded as EDS.[20]

Demographic Variables, Motor Symptoms and Non-Motor Symptoms

We recorded PD patients' demographics, including age, age at disease onset, gender, disease duration and LEDD. We assessed motor symptoms using the Unified Parkinson's Disease Rating Scale (UPDRS) III. The component scores for motor symptoms were as follows: (1) tremor score: the sum of UPDRS items 20 and 21; (2) rigidity score: UPDRS item 22; (3) bradykinesia score: the sum of UPDRS items 23, 24, 25, 26 and 31; and (4) postural and gait abnormalities score: the sum of UPDRS items 27, 28, 29 and 30. The wearing-off phenomenon was evaluated using the Wearing-

off Questionnaire-9 (WOQ-9), and dyskinesia was evaluated using the sum of UPDRS items 32, 33, 34 and 35.

Motor phenotype was determined as either TD phenotype or PIGD phenotype following the classification algorithm.[21] According to the original classification methods, the ratio of the mean UPDRS tremor scores (8 items) to the mean UPDRS PIGD scores (5 items) was used to define TD phenotype (ratio ≥1.5), PIGD phenotype (ratio ≤ 1), and indeterminate phenotype (ratios > 1.0 and < 1.5).

The following scales were used to evaluate non-motor symptoms: Pittsburgh Sleep Quality index (PSQI), Rapid Eye Movement (REM) Sleep Behavior Disorder (RBD) Screening Questionnaire (RBDSQ), Scale For Outcomes in PD For Autonomic Symptoms (SCOPA-AUT), Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), Hamilton Depression (HAMD) Scale −24 items, Hamilton Anxiety (HAMA) Scale −14 items, Fatigue Severity Scale (FSS), Modified Apathy Evaluation Scale (MAES) and Restless Leg Syndrome Rating Scale (RLSRS).

This study has been approved by the Institutional Review Board (IRB) in Beijing Tiantan Hospital, Capital Medical University (KY2013-003-03). This study met the guidelines of the Capital Medical University, which abide by the Helsinki Declaration on ethical principles for medical research involving human subjects. Written informed consent has been provided by the participants. The ethical statements cover all of these requirements.

CSF and Serum Samples Collection

Drug withdrawal was made for 12–14 hours if patients' condition permitted. Lumbar puncture (3 mL CSF) and blood draw (2 mL) were performed between 7.00 and 10.00 a.m. under fasting condition. Testing samples were centrifuged immediately at 3000 rpm for 10 minutes. An average of 0.5 mL CSF and serum were respectively aliquoted and stored at −80°C. Disposable blood collecting needle was from Becton Dickinson (Franklin Lakes, New Jersey, USA), Lumbar puncture package was from SPETCH (Foshan, China).

Assay for the Concentrations of Iron and Iron-Related Proteins in CSF and Serum

Enzyme Linked Immunosorbent Assay method was applied to determine the concentrations of iron and iron-related proteins, including transferrin, ferritin and lactoferrin in CSF and serum. Iron: kit Ab83366 (Abcam Company, Cambridge, UK), transferrin: kit Ab108911 (Abcam Company, Cambridge, UK), ferritin: kit Ab108837 (Abcam Company, Cambridge, UK), lactoferrin: kit E01L0224 (Shanghai Lanji Biological Limited Company, Shanghai, China).

Assay for the Concentrations of Inflammatory Cytokines in CSF and Serum

Chemical colorimetric method was used to measure the concentrations of NO and H_2O_2. NO: kit A012, H_2O_2: kit A064 (Nanjing Jiancheng Biological Engineering Research Institute, Nanjing, China).

Enzyme Linked Immunosorbent Assay was applied to measure the concentrations of inflammatory cytokines, including IL-1β, TNF-α and PGE_2 both in serum and CSF. IL-1β: kit 1R040 (RB Company, Shanghai, China), TNF-α: kit1R350 (RB Company, Shanghai, China), PGE_2: kit CSB-E07965h (CUSABIO Company, Wuhan, China).

Data Analyses

SPSS Statistics 20.0 (IBM Corporation, New York, USA) was used for statistical calculation. Continuous variables, if they were normally distributed, were reported as mean± SD values, and were compared by two-tailed *t*-test. If they were not normally distributed, they were reported as mean (range interquartile), and were compared by non-parametric test. Discrete variables were compared by Chi square test.

Pearson correlation was performed between ESS score and the concentrations of iron and iron-related proteins and the concentrations of inflammatory cytokines in CSF and serum, between the concentrations of iron and iron-related proteins and inflammatory cytokines in CSF and serum in PD-EDS group. To further explore significant correlations between ESS score and motor and non-motor symptoms, and the concentrations of iron, ferritin and IL-1β in CSF, logistic regression model was carried out. $P < 0.05$ was considered significant.

Results

The Frequency of PD with EDS

A total of 379 PD patients completed the evaluation of motor and non-motor symptoms. Sixty-one out of 379 PD patients (16.095%) had EDS (ESS score ≥ 10), while 318 PD patients (83.905%) did not have EDS (NEDS) (ESS score < 10). The average ESS scores of PD-EDS and PD-NEDS groups were 11.803 ± 2.372 and 3.327 ± 2.460, respectively.

Demographic Variables, Motor Symptoms, Non-Motor Symptoms and Dopaminergic Medication Usage of PD-NEDS and PD-EDS Groups

The PD-EDS group had dramatically more advanced Hoehn-Yahr (H-Y) stage, larger numbers of wearing-off, and scored higher for dyskinesia than the PD-NEDS group (Table 1).

The PD-EDS group had significantly higher scores on the scales of SCOPA-AUT, HAMD, HAMA, FSS and RLSRS than the PD-NEDS group, indicating that the PD-EDS group had significantly severer sleep disorders, autonomic dysfunctions, depression, anxiety, fatigue, and restless leg symptoms than the PD-NEDS group (Table 1). The median score of HAMD and HAMA in our PD patients is 12.00 (6.00~18.00) and 10.00 (5.00~17.00).

Table 1 Clinical Variables of PD-NEDS and PD-EDS Groups

Variables	PD-NEDS Group (318 Cases)	PD-EDS Group (61 Cases)	P value
Gender (male/total, %)	167/318 (52.516%)	31/61 (50.820%)	0.522
Age (years, mean ± SD)	60.290 ± 10.543	60.800 ± 9.704	0.710
Age at onset (years, mean ± SD)	56.679 ± 12.232	56.619 ± 10.110	0.969
Disease duration [years, median (quartile)]	2.000 (1.000~5.000)	3.000 (1.000~6.000)	0.485
LEDD (mg, mean ± SD)	384.116 ± 114.563	318.771 ± 121.659	0.139
PD severity			
Hoehn-Yahr stage [stage, median (quartile)]	2.000 (1.000~2.500)	2.500 (1.500~3.000)	0.046*
Motor symptoms			
UPDRS III (scores, mean ± SD)	25.280 ± 12.880	30.400 ± 16.236	0.023*
Tremor [scores, median (quartile)]	4.000 (2.000~8.750)	4.000 (2.000~7.000)	0.311
Rigidity [scores, median (quartile)]	5.000 (2.000~7.000)	4.000 (2.000~6.00 0)	0.793
Bradykinesia [scores, median (quartile)]	10.000 (5.000~15.000)	9.000 (5.000~14.000)	0.466
Postural and gait abnormalities [scores, median (quartile)]	4.000 (2.000~6.000)	3.000 (2.000~5.000)	0.512
Motor complications			
Numbers of wearing-off [numbers, median (quartile)]	0.000 (0.000~0.000)	0.000 (0.000~9.000)	0.042*
Score of dyskinesia [scores, median (quartile)]	0.000 (0.000~1.000)	2.000 (0.000~4.750)	0.016*
Non-motor symptoms			
PQSI [scores, median (quartile)]	6.000 (4.000~9.000)	6.500 (5.000~10.000)	0.365
RBDSQ [scores, median (quartile)]	2.000 (0.250~5.000)	5.000 (1.000~7.000)	0.069
SCOPA-AUT [scores, median (quartile)]	34.000 (30.000~40.000)	39.500 (35.750~45.500)	0.003**
MMSE [scores, median (quartile)]	28.000 (26.000~30.000)	28.000 (26.000~30.000)	0.516
HAMD [scores, median (quartile)]	11.000 (5.000~18.000)	15.000 (8.000~19.000)	0.045*
HAMA [scores, median (quartile)]	9.000 (5.000~16.000)	13.000 (8.250~19.000)	0.003**
FSS [scores, median (quartile)]	41.000 (25.000~52.000)	49.000 (42.000~60.000)	0.001**
MAES [scores, mean± SD]	16.480 ± 9.000	15.850 ± 9.111	0.752
RLSRS [scores, median (quartile)]	8.500 (0.000~18.000)	15.000 (5.750~24.250)	0.028*
MoCA (scores, mean± SD)	22.00±5.56	21.22±5.68	0.766
Dopaminergic Medication Usage			0.055
No L-dopa and no DA	145/318 (45.597%)	16/61 (26.230%)	
L-dopa mono	90/318 (28.302%)	25/61(40.984%)	
L-dopa + DA	39/318 (12.264%)	10/61 (16.393%)	
L-dopa + multiple DA	2/318 (0.629%)	0/61 (0.000%)	
DA mono	42/318 (13.208%)	10/61 (16.393%)	

Notes: **P<0.01, *P<0.05.
Abbreviations: DA, dopamine agonist; L-dopa; levodopa; LEDD, levodopa equivalent daily dose; mono, monotherapy.

PD patients who have higher scores of HAMD and HAMA in our study mostly had mild depression and anxiety, and were firstly informed to maintain good mood, intensify communication with others and take no medicine.

The majority of our patients were drug-naive patients with neither L-dopa nor a dopamine agonist (42.480%), about 30.343% were on L-dopa monotherapy, about 13.720% on a dopamine agonist monotherapy, about 12.929% were receiving L-dopa and a single dopamine agonist; and 0.528% received multiple dopamine agonists in combination with L-dopa. There were no significant differences for the dopaminergic medication usage in the PD-EDS and PD-NEDS groups (Table 1). In this study, the dopamine receptor agonists that PD patients used were pramipexole and piribedil. For statistical comparison of the prevalence of EDS in patients with different dopamine agonists therapy, the patients with one dopamine agonist in combination with L-dopa and patients with a dopamine agonist monotherapy were considered. However, there were no significant differences for the prevalence of EDS in the usage of pramipexole and piribedil (Table 2).

The Relationship Among PD-EDS, Iron Metabolism and Inflammation in CNS

In this study, a total of 69 PD patients (PD-EDS group: 16 cases, PD-NEDS group: 53 cases) and 30 controls had CSF samples collected and completed the tests. The concentrations of iron and iron-related proteins in CSF were compared among control, PD-NEDS and PD-EDS groups (Table 3). We found that the concentration of iron in CSF in the PD-EDS group was significantly elevated compared with both control and PD-NEDS groups. Further analyses indicated that ESS score was increased as iron concentration was elevated in CSF in the PD group. The concentration of ferritin in CSF in the PD-EDS group was significantly reduced compared with that in PD-NEDS and control groups. Further analyses revealed that ESS score was enhanced as ferritin concentration in CSF was reduced in the PD group.

Table 2 The Prevalence of EDS in Different Dopamine Agonist Therapy

	L-Dopa + One DA		P value
	L-Dopa + Pramipexole	**L-Dopa + Piribedil**	
PD-EDS	6/20(30.000%)	4/27(14.815%)	0.211
PD-NEDS	14/20(70.000%)	23/27(85.185%)	
	One DA		
	Pramipexole	Piribedil	
PD-EDS	4/21(19.048%)	6/30(20.000%)	0.933
PD-NEDS	17/21(80.952%)	24/30(80.000%)	

Abbreviations: DA, dopamine agonist; L-dopa, levodopa.

The concentrations of inflammatory cytokines in CSF were compared among the control, PD-NEDS and PD-EDS groups (Table 3). It was observed that IL-1β concentration in CSF in the PD-EDS group was significantly increased compared with that in PD-NEDS and control groups. TNF-α concentrations in CSF in PD-NEDS and PD-EDS groups were all significantly reduced when compared with controls. Further analyses showed that ESS scored higher as IL-1β concentration in CSF elevated in patients with PD.

The correlations of the concentration of iron and iron-related proteins with inflammatory cytokines in CSF were analyzed. Data showed that the concentration of iron increased as IL-1β concentration in CSF elevated in the PD-EDS group ($r = 0.914$, $P = 0.004$) (Table 4). The iron concentration did not correlate significantly with IL-β concentration in CSF in the PD-NEDS group.

Influencing Factors of ESS for PD Patients

Logistic regression model was carried out, we put ESS score as a dependent variable, whereas concentrations of iron, ferritin and IL-1β in CSF, motor subtype, H-Y stage and the score of MoCA were independent variables.

Data showed that the concentrations of iron, ferritin and IL-1β in CSF, Hoehn-Yahr stage and TD phenotype were the influencing factors of ESS in PD patients (regression coefficient = 2.787, −0.347, 0.080, $P < 0.05$) (Table 5).

The Relationship Among PD-EDS, Iron Metabolism and Inflammatory Cytokines in Peripheral System

In this study, a total of 166 PD patients (PD-EDS group: 27 cases, PD-NEDS group: 139 cases) and 30 controls had blood samples collected and completed the tests. First of all, the relationship between PD-EDS and the concentrations of iron and transferrin, ferritin and lactoferrin in serum was studied (Table 3). The results showed that iron and transferrin

Table 3 The Concentrations of Iron and Iron-Related Proteins and Inflammatory Cytokines in CSF or Serum from Control, PD-NEDS and PD-EDS Groups

Concentrations	Control Group (30 Cases)	PD-NEDS Group (53 Cases)	PD-EDS Group (16 Cases)	P^1	P^2	P^3
Iron and iron-related proteins in CSF						
Iron (nmol/mL, mean ± SD)	0.355 ± 0.149	0.518 ± 0.278	0.704 ± 0.410	0.005**	0.003**	0.042*
Transferrin [ug/mL, median (quartile)]	0.153 (0.117 ~ 0.172)	0.146 (0.114 ~ 0.182)	0.119 (0.091 ~ 0. 130)	0.676	0.021*	0.095
Lactoferrin [ug/mL, median (quartile)]	126.078 (64.874 ~ 207.359)	146.045 (85.426 ~ 215.085)	113.471 (65.735 ~ 168.905)	0.480	0.567	0.109
Ferritin [ng/mL, median (quartile)]	13.127 (8.804 ~ 18.586)	11.082 (5.837 ~ 17.023)	3.062 (1.588 ~ 8.522)	0.293	0.000**	0.003**
Inflammatory cytokines in CSF						
NO [mmol/L, median (quartile)]	51.825 (27.007 ~ 61.719)	61.327 (31.774 ~ 92.928)	49.193 (34.746 ~ 66.632)	0.075	0.814	0.419
H_2O_2 [mmol/L, median (quartile)]	1.973 (1.184~4.004)	2.314 (1.850~3.028)	2.012 (1.717~3.978)	0.509	0.556	0.88
IL-1β (pg/mL, mean ± SD)	15.489 ± 4.749	17.268 ± 8.699	23.910 ± 10.540	0.238	0.009**	0.038*
PGE_2 [pg/mL, median (quartile)]	6.253 (5.711 ~ 11.212)	9.016 (5.466 ~ 15.868)	13.184 (5.540 ~ 16.375)	0.573	0.221	0.458
TNF-α [pg/mL, median (quartile)]	139.227 (78.938 ~ 168.261)	47.059 (25.682 ~ 147.452)	39.771 (24.802 ~ 131.730)	0.001**	0.007**	0.646
	Control Group (30 Cases)	**PD-NEDS Group (139 Cases)**	**PD-EDS Group (27 Cases)**	**P^1**	**P^2**	**P^3**
Iron and iron-related proteins in serum						
Iron [(nmol/mL, median (quartile)]	4.521 (3.620 ~ 4.845)	2.55 (1.600 ~ 3.699)	2.642 (1.813 ~ 3.746)	0.000**	0.000**	0.699
Transferrin [ug/mL, median (quartile)]	0.159 (0.153 ~ 0.179)	0.121 (0.097~ 0.161)	0.126 (0.107 ~ 0.146)	0.000**	0.000**	0.833
Lactoferrin [ug/mL, median (quartile)]	37.695 (30.204 ~ 79.990)	91.855 (47.896 ~ 147.413)	74.216 (39.057 ~ 150.162)	0.000**	0.005**	0.457
Ferritin [ng/mL, median (quartile)]	8.4 (5.487 ~ 12.302)	15.399 (8.006 ~ 37.026)	13.521 (7.158 ~ 26.679)	0.000**	0.025*	0.496
Inflammatory cytokines in serum						
NO [mmol/L, median (quartile)]	42.336 (23.723~52.190)	48.365 (29.878~84.565)	60.993 (36.496~94.326)	0.346	0.108	0.397
H_2O_2 [mmol/L, median (quartile)]	27.167 (23.306~37.033)	27.486 (21.733~33.273)	29.636 (17.730~34.316)	0.701	0.949	0.694
IL-1β [pg/mL, median (quartile)]	16.026 (14.330~18.320)	16.64 (12.103 ~ 19.302)	18.264 (13.428~ 20.818)	0.890	0.191	0.176
PGE_2 [pg/mL, median (quartile)]	9.45 (6.548 ~ 17.091)	7.196 (4.315~12.938)	9.624 (5.770~ 12.417)	0.018**	0.226	0.342
TNF-α (pg/mL, mean ± SD)	68.394 ± 4.462	61.334 ± 22.841	63.932 ± 24.953	0.094	0.359	0.589

Notes: P^1: PD-NEDS group vs Control group; P^2: PD-EDS group vs Control group, P^3: PD-EDS group vs PD-NEDS group. **P<0.01, *P<0.05.

Table 4 Correlation Between Iron Level in CSF and IL-1β Level in CSF from PD-EDS Group

	R	P value
IL-1β level in CSF (pg/mL)	0.914	0.004**

Note: **P<0.01.

Table 5 Influencing Factors for EDS in PD Group

Variable	B	Univariate	
		OR(95% CI)	P value
Hoehn-Yahr stage (stage)	1.337	1.320~10.988	0.013*
Motor subtype			
Indeterminate phenotype	Reference	-	-
TD phenotype	−22.414	0.000~	0.999
PIGD phenotype	−21.164	0.000~	0.999
MoCA	0.003	1.320~10.988	0.958
Iron level in CSF (nmol/mL)	2.787	1.25~210.601	0.033*
Constant	15.851	-	0.999
Hoehn-Yahr stage (stage)	0.818	0.600~8.549	0.228
Motor subtype			
Indeterminate phenotype	Reference	-	-
TD phenotype	−4.509	0.000~0.340	0.010*
PIGD phenotype	−1.918	0.009~2.407	0.179
MoCA	0.023	0.891~1.175	0.747
Ferritin level in CSF (ng/mL)	−0.347	0.544~0.919	0.010*
Constant	−0.896	-	0.754
Hoehn-Yahr stage (stage)	1.017	1.046~7.307	0.040*
Motor subtype			
Indeterminate phenotype	Reference	-	-
TD phenotype	−3.532	0.001~0.735	0.032*
PIGD phenotype	−0.993	0.027~5.077	0.457
MoCA	0.093	0.965~1.247	0.157
IL-1β level in CSF (pg/mL)	0.080	1.002~1.171	0.043*
Constant	−5.411	-	0.004**

Notes: *P<0.05, **P<0.01.

concentrations in serum in PD-NEDS and PD-EDS groups were all prominently decreased when compared with the control group. The concentrations of lactoferrin and ferritin of serum in PD-EDS and PD-NEDS groups were all strikingly elevated when compared with the control group. The differences in serum concentrations of iron, transferrin, lactoferrin and ferritin between PD-NEDS and PD-EDS groups have no statistical significance. Further study showed no significant correlation between ESS scores and the concentrations of iron and iron-related proteins in PD patients (P > 0.05).

Next, analyses of the correlations between EDS scores and the serum concentrations of NO, H_2O_2, IL-1β, PGE_2 and TNF-α in PD patients were performed. The serum concentrations of the inflammatory cytokines were compared among the control, PD-NEDS and PD-EDS groups (Table 3). It was found that serum concentration of PGE_2 in the PD-NEDS group was strikingly decreased when compared with the control group.

Finally, correlation of the concentrations of iron and iron-related proteins with inflammatory cytokines in serum were analyzed and no correlation was found (P > 0.05).

Discussion

As we observed, the occurrence rate of EDS in PD patients was 16.09%, which was consistent with the result from a previous study,[22] demonstrating that EDS was very common in PD patients. In this investigation, compares with the PD-NEDS group, the PD-EDS group has more advanced H-Y stage and more severe motor symptoms, suggesting that PD-EDS patients exhibited a mode of more widespread neurodegenerative progression in SN.[23] Motor complications were common in advanced PD patients. Previous studies reported that the cumulative levodopa dose, female sex and younger age of onset were associated with the development of motor complications.[24] This study found that PD-EDS group had significantly more numbers of wearing-off and higher score of dyskinesia than PD-NEDS group. One prospective study recruited 21 drug-naive PD patients at baseline and followed up for a mean of 2.6 ± 1.3 years, and found that the median time to development of motor complications after initiation of levodopa therapy was 6 months. It revealed that the incidence of motor complications after initiating levodopa was independent of the initial treatment, it was associated with levodopa daily dose and disease progression, but not with the duration of levodopa therapy.[25] In this study, we found no significance for LEDD between the PD-EDS and PD-NEDS groups, but more advanced H-Y stage in the PD-EDS group, implying that disease progression might play an important role in the motor complications in PD-EDS patients. In logistic regression, this study found that EDS was significantly and negatively related to TD-phenotype, and was not associated with PIGD-phenotype. A previous study found that EDS was associated with higher PIGD score[26] because of prediction of more rapidly progressive disability in PD. Yet, our study differed from that study. Tremor in PD appeared at rest and disappeared

during sleep. It might be that patients in our study had lighter symptoms and were in a relatively early stage.

In this study, PD-EDS patients had significantly more severe non-motor symptoms, indicated by higher scores of SCOPA-AUT, HAMA, HAMD, FSS and RLSRS. The autonomic system controls alertness via a mechanism relating to circadian rhythm,[27] and thus autonomic symptoms were a line of clinical variables significantly associated with EDS.[28] It implied that the Braak's stage involved structures related to both dysautonomia and EDS were severely and simultaneously compromised in PD-EDS patients. A previous study showed that the serotoninergic system in raphe nucleus and noradrenergic system in locus ceruleus were involved in the control of mood.[28] Interestingly, another study reported that EDS in PD was commonly associated with the dysfunction of raphe nuclei.[29] Thus, there might be a biological substrate for the close association between EDS and anxiety and depression in PD patients. Our previous investigation revealed that decrease of serotonin concentration in CSF was associated with fatigue.[30] Since 5-hydroxytryptamine governed sleep-wake behavior, an imbalance was correlated to both EDS and fatigue.[31] Therefore, these findings suggested that EDS and fatigue in PD might share a common dysfunction in pathophysiology and serotoninergic systems. RLS was considered to be related to dysfunction of the central dopaminergic system.[32] We recently found that the CSF concentration of dopamine in PD with RLS was much lower than in PD patients with no RLS.[33] It was also reported that the depletion of dopamine caused EDS.[34] Therefore, EDS and RLS might both be related to the low brain dopamine concentration.

Further, we investigate the mechanism of EDS in PD. Abnormal iron accumulation in the brains of PD patients has been regularly reported since 1922. In the brains of PD patients, elevated iron concentrations in nigra and lateral globus pallidus were observed,[35] and excessive free iron enhanced α-synuclein aggregation and thus promoted the formation of Lewy bodies.[36] There are a few studies that investigated the iron level in CSF or serum and they have no definite conclusions. One study reported that increased CSF iron concentration was correlated with oxidative stress in PD patients.[37,38] Another meta-analysis illustrated that there is no difference in CSF iron concentration between PD patients and controls.[39] However, no study has established the relationship between CSF iron level and EDS in PD. This study found that CSF iron concentration was strikingly increased and CSF ferritin concentration in the PD-EDS group was obviously decreased compared with the PD-NEDS group. Logistic regression showed that CSF iron concentration was positively and CSF ferritin concentration was negatively related to EDS. Ferritin was the main iron storage cellular protein, and iron bound to ferritin was considered nontoxic. However, lower ferritin level was incapable of bearing the excessive iron in the brain, resulting to iron accumulation and neuronal death. Therefore, excessive iron accumulation and iron metabolism disruption in brain might take part in the development of EDS in PD patients.

Iron and its metabolism participate in the progression of PD pathology.[40] Serum iron was transported into the brain across the blood–brain barrier (BBB). The widely known mechanism accounting for excessive iron penetration into brain involves the binding of iron-loaded large molecules, mainly transferrin, to its receptor and its translocation to the intracellular compartment.[41] In a similar manner, lactoferrin-bound iron could bind to lactoferrin receptors, and contribute to iron transport through the plasma membrane. Meanwhile, a broken or leaky BBB might provide the possibility allowing elevated iron into the brain[42] via lactoferrin receptors. In the current study, compared with the control group, both the PD-NEDS and PD-EDS groups had significantly decreased concentrations of iron and transferrin, and prominently increased concentrations of lactoferrin and ferritin in serum. A previous study has reported that serum ferritin was upregulated in the circulation of PD patients.[43] Another study has suggested a progressive partitioning of iron to the SN pars compacta of the brain from the peripheral system in PD patients,[44] suggesting that there was a disruption of the normal homeostasis between peripheral system and brain iron, in favor of an accumulation of iron in the brain.

In the brain, inflammation played a crucial part in the development of PD, and it was involved in several nonmotor symptoms, such as pure apathy[45] and cognitive impairment.[46] One study demonstrated elevated IL-1β concentration in hypothalamus mediated sleep disorders in rats with rotenone-induced parkinsonism.[47]

In the study, the correlation of EDS and inflammation in PD patients was investigated. We found that the PD-EDS group had significantly increased CSF IL-1β concentration when compared with the PD-NEDS and the control groups, and ESS score was significantly correlated to CSF IL-1β concentration in PD patients. Furthermore, it was demonstrated that IL-1β concentration in CSF was the influencing factor of ESS for PD patients by using

logistic regression. Thus, inflammation might play a pivotal role on EDS in PD patients. PD-EDS and PD-NEDS groups showed no difference in other inflammatory cytokines, implying that IL-1β might be a potential inflammatory indicator for EDS of PD. We also found that the TNF-α concentration in CSF in the PD-NEDS and PD-EDS groups were significantly reduced, but there was no difference between the two groups. The decreased CSF concentration of TNF-α in the PD-EDS and PD-NEDS groups might be related to the increased usage of TNF-α in the brain or because TNF-α was bound to the brain. This hypothesis was sustained by another study which found higher brain TNF-α concentration in PD patients than in controls.[48]

A previous study found that microglial activation was correlated with elevated iron concentration in SN.[49] Meanwhile, excessive neuroinflammatory cytokines generated by microglia led to dysregulation of iron.[50] In this study, CSF iron concentration was positively related to IL-1β concentration in the PD-EDS group. We hypothesize the elevated brain iron concentration might promote microglial activation, robustly produce IL-1β, and evoke neuronal death in EDS-associated regions, leading to the occurrence of EDS in PD patients.

The relationship of inflammatory cytokines between peripheral system and brain is still unclear. Several peripheral blood leukocyte adhesion molecules have been reported as contributing to a connection between systemic inflammation and neuroinflammation in PD, including macrophage antigen complex-1, lymphocyte function-associated antigen 1, E-selectin, and P-selectin.[51] Another study reported that the origin of inflammatory biomarkers in the peripheral system could be by entering the body via gut dysbiosis and translocation.[52]

The evaluation of EDS on the basis of a subjective scale is one of the limitations in the study, another is the lack of assessment of sleep apnea, which can cause daytime sleepiness and is associated with inflammation.

Conclusions

In summary, EDS was common in PD patients. PD-EDS patients showed more advanced disease stage, and more severe motor and some non-motor symptoms. Iron metabolism disruption in CNS might be associated with PD-EDS through inflammation. This investigation may cast a new light in terms of clinical features and pathogenesis of PD-EDS involving disturbed iron metabolism and related inflammation in the brain.

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Disclosure

The authors report no conflicts of interest in this work.

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