Serotonergic dysfunctions and abnormal iron metabolism: Relevant to mental fatigue of Parkinson disease

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Abstract

Fatigue is a very common non-motor symptom in Parkinson disease (PD) patients. But its potential mechanisms involving serotonergic dysfunction and abnormal iron metabolism in the brain and peripheral system in patients with mental fatigue are still unknown. In this study, we evaluated the fatigue symptoms by fatigue scales, classified into fatigue group and non-fatigue group, and detected the levels of serotonin, iron and related proteins in CSF and serum. In CSF, the level of 5-HT in fatigue group is decreased and the levels of iron and transferrin in fatigue group are increased. Mental fatigue score is negatively correlated with the level of 5-HT and positively correlated with the levels of iron and transferrin in PD group. Transferrin level is negatively correlated with 5-HT level in CSF in PD group；In serum, the levels of 5-HT and transferrin are decreased in fatigue group; Mental fatigue score exhibits a negative correlation with 5-HT level in PD group. Thus serotonin dysfunction in central and peripheral systems may be correlated with PD mental fatigue through abnormal iron metabolism.

Key words：Parkinson disease, mental fatigue, cerebral spinal fluid, serotonin, transferrin

Introduction

Fatigue is one of the most common and disabling symptoms in Parkinson's disease (PD) with high prevalence of 58.1% [1]. As Kluger proposes criteria for diagnosis of PD-related fatigue, patients must report significantly diminished energy levels or increased perceptions of effort that are disproportionate to attempted activities or general activity level. Symptoms must be present for most of the day every day or nearly every day during the previous month, adding other 4 or more additional symptoms[2]. Above 50% PD patients consider fatigue as one of top three disabling symptoms[3]. Fatigue in PD can be divided into mental fatigue and physical fatigue, which can be identified by Fatigue scale-14 (FS-14). Mental fatigue occurs after sustained intellectual activity or emotional tension[4]. Physical fatigue is a sense of exhaustion caused by repeated muscular contraction or continuous physical activity[5]. Study has showed that mental fatigue and physical fatigue had different mechanisms, and fatigue in PD mainly manifested mental fatigue [6]. So

mental fatigue may present the main characteristic of fatigue in PD. Yet, there are few studies investigating the potential mechanisms about fatigue in PD. Moreover, there is no study exploring the underlying mechanism about mental fatigue in PD.

[N,N-dimethyl-2-(2-amino-4-cyanophenylthio) benzylamine] (^{11}C-DASB) PET reveals that fatigue in PD patients is related to striatal and limbic serotonergic (5-HT) dysfunction [7] , however, few studies directly investigated the level of 5-hydroxytryptamine (5-HT) both in CSF and serum in PD patients with fatigue. Furthermore, the role of 5-HT dysfunction in brain and peripheral system on mental fatigue in PD patients remains unclear.

Several autopsy reports show iron deposition in substantia nigra (SN) in PD patients. Studies showed PD patients have hyperechogenicity in SN by transcranial sonography（TCS) [8] and iron deposition mainly in SN pars compacta (SNpc） by susceptibility weighted imaging（SWI） [9]. Iron-related neurodegeneration can be attributed for the defects in its metabolism and/or homeostasis and subsequent accumulation in the specific brain regions. For example, the level of transferrin, an iron metabolism-related protein, in brains of PD subjects is remarkably increased comparing with normal control subjects[10]. Studies imply that mutations in metabolism-related proteins genes, such as transferrin[11], and ferritin[12] are related to PD incidence, indicating that abnormal iron related proteins in brain participate in the pathogenesis of PD. However, no study detects the levels of iron and related proteins in CSF and serum in PD patients with mental fatigue, and no investigation focuses on the correlation between mental fatigue and iron metabolism in CSF and serum in PD patients. Moreover, the relationship between 5-HT and iron and related proteins in PD with mental fatigue is unknown.

In this study, in PD patients, we assessed mental fatigue by FS-14, detected the levels of 5-HT, iron and related proteins, including transferrin, lactoferrin and ferritin in CSF and serum, and analyzed the correlations among mental fatigue score and the levels of above factors, and attempt to figure out the underlying mechanisms of PD with mental fatigue relating 5-HT and iron metabolism.

Methods

Subjects

Patients with PD. PD patients were recruited from the neurodegenerative outpatient clinics in the Department of Geriatrics and Neurology, Beijing Tiantan Hospital, Capital Medical University. Demographic information including age, sex, disease severity and disease duration as well as levodopa equivalent daily doses was recorded. Patients were diagnosed with PD according to Movement Disorder Society Clinical Diagnostic Criteria for Parkinson's Disease [13]. PD patients with blood donation histories, systemic diseases including anemia, heart failure, pulmonary disorders, hepatosis, chronic liver/renal failure, severe hypothyroidism and diabetes were excluded. Female patients who had not been through menopause were not included in this study. PD patients with an Epworth Sleepiness Scale score of >6[14] or an Apathy Scale score of $\geqslant$14 were excluded[15]. This study consecutively recruited 530 PD patients. Of 530 PD patients, 4 patients with pulmonary disorders, 5 patients with severe hypothyroidism and 3 patients with heart failure were also excluded. Finally, a total of 518 PD patients were recruited in this study.

Control subjects. Total 29 age-matched controls from Beijing Tiantan Hospital were selected based on the following criteria: 1) no neurological symptoms and signs; 2) no histories of blood donation; 3) no intracranial diseases; 4) no systemic diseases affecting sleep or fatigue, such as hypertension, anemia, hepatosis, heart failure, pulmonary disorders, chronic liver/renal failure, severe hypothyroidism, diabetes, or epilepsy history; 5) no essential tremor, PD, secondary parkinsonism, or Parkinson-plus syndrome; 6) no obvious apathy, cognitive impairment, or psychiatric symptoms; 7) no dysarthria or mental illness that affect expression; 8) no alcohol or drug abuse. Female controls who had not been through menopause were not included in this study. The controls were also patients, but their diseases were not related to and did not influence the results of this investigation, such as peripheral neuropathy and headache caused by high intracranial pressure.

Assessment of PD

Assessment of fatigue

The Fatigue Severity Scale (FSS) satisfies the criteria of a ''recommended'' fatigue scale in PD (both for screening and severity rating) because it has been shown to have good psychometric properties (including discrimination between fatigued and non-fatigued patients) in PD patients and has been used by some studies[16]. It is a self-administered 9-item fatigue rating scale, and

encompasses several aspects of fatigue and their impact on patients' daily functioning. Patients were asked to rate how each item described their fatigue level from 1 (strongly disagree) to 7 (strongly agree). Total FSS score was obtained by dividing the sum of all item scores by 9. Patients with total FSS score＞4 points and ≤4 points were classified into the fatigue group and non-fatigue group, respectively[16].

FS-14 is a reliable and valid self-rating scale with14-items for fatigue evaluation. Item 1-8 and 9-14 of FS-14 reflect physical fatigue and mental fatigue, respectively. Higher total score of FS-14 indicates severer fatigue[17]. The sensitivity and specificity of FS-14 are75.5 % and 74.5%, respectively.

This study has been approved by Beijing Tiantan Hospital review board (KY2013-003-03). Written informed consent was obtained from all participating subjects. This study was performed according to the guidelines of Capital Medical University, which abides by the Helsinki Declaration on ethical principles for medical research involving human subjects.

Clinical assessments of motor symptoms and non-motor symptoms

The severity of PD was assessed based on the Hoehn and Yahr (H-Y) stage. Motor symptoms were evaluated by Unified Parkinson's Disease Rating Scale (UPDRS) III, in which items 20 and 21 were for tremor, item 22 was for rigidity, items 23–26 and 31 were for bradykinesia, and items 27–30 were for postural and gait abnormalities. The score for each motor symptom was calculated by summing up the score for the relevant items in UPDRS III. Non-motor symptoms were evaluated by using the following scales: Hamilton Depression Scale (HAMD) (24 items) for depression, Hamilton Anxiety Scale (HAMA) (14 items) for anxiety, Mini-Mental State Examination (MMSE) for cognitive function, Pittsburgh Sleep Quality Index (PSQI) for sleep disorders.

CSF and serum sample collection

Anti-parkinsonian drugs were withheld for 12-14 hours if patients' condition allowed. Total 3 ml CSF was taken in a polypropylene tube between 7 a.m. and 10 a.m. under fasting condition through lumbar puncture. Total 2 ml venous whole blood was collected. Approximately 0.5 ml volume of CSF and serum were aliquotted into separate Nunc cryotubes and kept frozen at -80℃ until ready for assay. Each aliquot dedicated for each measure to avoid freeze-thawing and potential degradation of protein.

Detection of the levels of 5-HT in CSF and serum

The levels of 5-HT in CSF and serum from PD patients were measured by high performance liquid chromatography (HPLC). Henomenex 150*2mm,150*3mm chromatographic columns and LC-MS-MS 6410 chromatographic instrument were from Agilent Company (USA), and standard sample was from Sigma Company (USA).

Detection of the levels of iron and related proteins in CSF and serum

The levels of iron and its metabolism-related proteins, including iron, ferritin, transferrin and lactoferrin, in CSF and serum from PD patients are detected by Enzyme Linked Immunosorbent Assay (ELISA). Ab83366 kit for iron, Ab108911 kit for transferrin, and Ab108837 kit for ferritin were from Abcam Company (Cambridge, United Kindom). CSB-E08831h kit for lactoferrin was from Wuhan Huamei Biological Limited Company (Wuhan, China).

Data analyses

Statistical analyses were performed with SPSS Statistics 20.0 (IBM Corporation, New York, USA). P value was statistically significant when it was less than 0.05.

Demographics information, motor symptoms, depression and anxiety were compared between fatigue and non-fatigue groups. The levels of 5-HT，iron and related proteins in CSF and serum were compared among control, fatigue and non-fatigue groups.

Continuous variables, if they were normally distributed, were presented as means ± standard deviations and compared by ANOVA test. Bonferroni correction was performed in further comparisons between two groups. P value was significant when it was < 0.05. Continuous variables, if they were not normally distributed, were presented as median (quartile) and compared by nonparametric test. P value was significant when it was < 0.017 in further comparisons between two groups. Discrete variables were compared by Chi square test.

Spearman correlation analyses were made between the score of mental fatigue and the level of 5-HT in CSF, between the score of mental fatigue and the levels of iron and iron metabolism-related proteins in CSF and serum, among the levels of iron and tranferrin in CSF and age, disease duration, the scores of UPDRS III, tremor, rigidity, bradykinesia, postural and gait

abnormalities, HAMD and HAMA, and between the levels of 5-HT and iron and related proteins in CSF in PD group.

Multiple linear regression models were established, in which the level of 5-HT in CSF in PD group were set as dependent variables, whereas the score of mental fatigue, disease duration, H-Y stage, the scores UPDRS III, tremor, rigidity, bradykinesia, postural and gait abnormalities, HAMD and HAMA were set as independent variables. P value was significant when it was < 0.05.

Results

Frequency and assessment of fatigue in PD patients

Among the 518 PD patients, 250 cases (52.12%) were male and 268 (47.88%) were female. The average score of mental fatigue in fatigue and non-fatigue groups is 7.00 (6.00~8.00) and 4.00 (2.00~6.00) points, respectively. The disease duration varied from 3 month to 33 years, with a median of 2.5 years [interquartile range (IQR): 4.0 years]. The demographic characteristics are listed in **Table 1, Supplemental table 1 and Supplemental table 2**.

In the 518 PD patients with fatigue, 80 cases (15.44%) have fatigue before the onset of motor symptoms. The fatigue group shows more advanced H-Y stage, higher total UPDRS Ⅲ scores and higher scores of tremor, rigidity, bradykinesia, postural and gait abnormalities according to UPDRS III when compared with the non-fatigue group. The fatigue group also scores higher on HAMA and HAMD than the non-fatigue group, suggesting that individuals in the fatigue group have severer anxiety and depression than those in the non-fatigue group. The fatigue group and non-fatigue group do not differ in terms of demographic information, such as age, sex, disease duration and levodopa equivalent daily dose (**Table 1, Supplemental table 1 and Supplemental table 2**).

Relationship among the score of mental fatigue, the levels of 5-HT, iron and related proteins in CSF

Relationship between the score of mental fatigue and the level of 5-HT in CSF.

The level of 5-HT in CSF is compared among control, fatigue and non-fatigue groups (**Table 2**). The level of 5-HT in CSF in fatigue group is prominently lower than that in control and non-fatigue groups. Further analysis indicates that the score of mental fatigue increases with the decreased level of 5-HT ($r=-0.233$, $P<0.05$) in CSF.

Relationship between the score of mental fatigue and the levels of iron and related proteins in CSF.

The levels of iron, transferrin, ferritin and lactoferrin in CSF are compared among control, fatigue and non-fatigue groups (**Table 2**). The levels of iron and transferrin in CSF in fatigue group are prominently higher than that in control and non-fatigue groups. The level of transferrin in CSF in non-fatigue is strikingly higher than that in control group. Correlation analyses demonstrate mental fatigue score increases with the elevated levels of iron ($r = 0.372$, $P < 0.05$) and transferrin ($r = 0.323$, $P < 0.05$) in CSF in PD group.

Relationship between the level of 5-HT and iron and related proteins in CSF.

Further analyses indicate that 5-HT level decreases with the increased level of transferrin ($r=-0.492$, $P=0.008$) in CSF in PD group (**Table 3**). In the multiple linear regression models, we still find 5-HT level in CSF is significantly and negatively correlated with transferrin level ($r=-0.714$, $P=0.033$) after adjusting for confounders.

Relationship among the levels of 5-HT, iron and transferrin in CSF， age, age of onset, disease duration, the scores of UPDRS III, tremor, rigidity, bradykinesia, postural and gait abnormalities, depression and anxiety in PD group.

Analyses of the correlations of the level of 5-HT in CSF with age, age of onset, disease duration, the scores of UPDRS III, tremor, rigidity, bradykinesia and postural and gait abnormalities, depression and anxiety imply that 5-HT level is negatively correlated with the scores of rigidity ($r=-0.23$, $P =0.024$) and HAMD ($r=-0.79$, $P =0.046$).

Analyses of the correlations of iron level in CSF with age, age of onset, disease duration, the scores of UPDRS III, tremor, rigidity, bradykinesia and postural and gait abnormalities, depression and anxiety reveal that iron level in CSF is positively correlated with the scores of rigidity ($r = 0.96$, $P = 0.002$) and bradykinesia ($r = 0.19$, $P = 0.003$).

Analyses of the correlations of transferrin level in CSF with age, age of onset, disease duration, the scores of UPDRS III, tremor, rigidity, bradykinesia and postural and gait abnormalities, depression and anxiety indicate no significant correlations ($r = 0.31$, $P = 0.067$) .

Influencing factors of mental fatigue in PD group.

Multiple linear regression model (Type I) is established, in which mental fatigue in PD group is set as dependent variable, whereas the scores of HAMD, HAMA , UPDRS III, tremor, rigidity, bradykinesia and postural and gait abnormalities, the level of 5-HT in CSF, age, sex, disease duration and H-Y stage are set as independent variables. The results indicate that 5-HT level in CSF is the only influencing factors for mental fatigue score in PD group (regression coefficient = -0.175, P = 0.033), whereas the scores of HAMD, HAMA, UPDRS III, tremor, rigidity, bradykinesia and postural and gait abnormalities, age, sex, disease duration and H-Y stage do not enter the regression equation (**Supplemental table 3**).

Relationship between the score of mental fatigue and the levels of 5-HT, iron and related proteins in serum

Relationship between the score of mental fatigue and the level of 5-HT in serum

The level of 5-HT in serum is compared among control, fatigue and non-fatigue groups (**Table 4**). The decreased level of 5-HT in serum is observed in fatigue and non-fatigue groups comparing with control group. Further analysis shows the score of mental fatigue decreases with the reduced 5-HT level (r = -0.370, P = 0.022) in serum in PD group.

Relationship between the score of mental fatigue and the levels of iron and related proteins in serum.

The levels of iron, transferrin, ferritin and lactoferrin in serum are compared among control, fatigue and non-fatigue groups (**Table 4**). The data reveal that transferrin level in serum in fatigue group is prominently decreased comparing with non-fatigue and control groups. Further analyses imply no relationship between the score of mental fatigue and the levels of iron and related proteins in serum.

Relationship between the level of 5-HT and the levels of iron and related proteins in serum.

Correlation analyses are made between the level of 5-HT and the levels of iron and related proteins in serum. The data do not indicate any correlation among them (r=0.51, P>0.05).

Discussion

In this study, 58.88% of total PD patients have fatigue, indicating that fatigue is a very common non-motor symptom in PD patients, which is a little higher than that in previous report. The different prevalence between our and other investigations may be accounted for the differences in H-Y stage and disease duration of the patients and scales used for evaluating fatigue[1]. Eighty out of 518 PD patients (15.44%) were with fatigue prior to the appearance of motor symptoms, supporting that fatigue might be one of prodromal symptoms of PD. One study showed that fatigue frequently occurred in the 2 to 10 years premotor period [18]. Patients with fatigue might have a high risk of 1.56 to develop PD[19]. Fatigue could help to identify individuals at the earliest stages of PD. PD patients with fatigue in the present study showed a more advanced H-Y stage, severer motor symptoms and non-motor symptoms indicated by higher scores of total UPDRS III, HAMA and HAMD (**Table 1, Supplemental table 1 and Supplemental table 2**). Importantly, further analyses of each motor symptom in the PD patients revealed that the score of each motor symptom, including tremor, rigidity, bradykinesia and postural and gait abnormalities, in the fatigue group was significantly higher than that in the non-fatigue group (**Table 1, Supplemental table 1 and Supplemental table 2**), illustrating that fatigue worsened with disease progression [20]. Previous studies have reported that rigidity, bradykinesia [21] and postural and gait abnormalities [22,23] were related to fatigue of PD. The present study was the first to reveal that tremor is related to fatigue of PD. It might be that both tremor and fatigue have the same central origin, and their generation was linked to a failure in the basal ganglia-thalamo-cortical loop [24,25].

To our knowledge, this was the largest study assessing 5-HT level in CSF in PD patients, and exploring the relationship between 5-HT level in CSF and mental fatigue. Recently, growing evidence suggested that PD was not solely affecting the dopaminergic system, but also serotonergic system with the data from biochemical, animal, postmortem, and functional imaging studies [26]. Even in early stage of PD patients, it was also observed reduced serotonin transporter availability [27]. In this study, we found that 5-HT level in CSF in fatigue group was prominently lower than that in control and non-fatigue groups（**Table 2**）. Profoundly, we found that the score of mental fatigue increased with the declined 5-HT level in CSF, and the decreased 5-HT level is the only influencing factor for mental fatigue in PD patients (**Supplemental table 3**). In brain, the

serotonergic system originates from the brainstem raphe nuclei, suggesting that decreased 5-HT level in brain may predict mental fatigue in PD. As we all know, when Lewy bodies appeared in Braak stage 2 in the lower raphe nuclei and locus coeruleus, PD patients manifested with fatigue[28]. Post-mortem studies have observed a loss of serotonergic cell bodies with Lewy bodies aggregated in the raphe nuclei[29] and subsequently a global deficiency of serotonergic markers in cortical and subcortical structures that received raphe projections[30]. A recent study showed that PD patients with fatigue have a significant reduction of 5-HT transporter binding in basal ganglia and thalamus by utilizing 11C-DASB PET compared to the PD patients without fatigue[7]. However, another study has showed no association between raphe nuclei 5-HT transporter (SERT) availability and fatigue by using 123I-FP-CIT single photon emission computed tomography in early drug-naive PD patients[27], which was inconsistent with our study. It might be that PD patients recruited in the two studies were at different disease stages. The average H-Y stage of PD patients was 1.5±0.5 in the former study[27], which was lower than that in PD patients in our study (2.0±0.8 stage). Above two studies explored the relationship between 5-HT in focal brain region and fatigue in PD patients by using imaging method, which indirectly reflects 5-HT change in brain. PET imaging is very expensive, which is difficult for most of PD patients to bear the financial burden. Furthermore, the loss of serotonergic cell bodies with Lewy bodies aggregated in the raphe nuclei[29] and subsequently a global deficiency of serotonergic markers in cortical and subcortical structures that received raphe projections[30]. Thus, CSF is an optimal and objective source for allowing us to test and monitor the change of 5-HT level in the serotonergic system-containing brain regions in PD patients with fatigue. There is no study investigating the relationship between 5-HT in CSF and mental fatigue in PD. We for the first time to demonstrate that decreased 5-HT in CSF is related to mental fatigue in PD, implying that decreased 5-HT level in CSF might be a predictor for mental fatigue of PD.

There were rare studies investigating 5-HT level in serum in PD patients. 5-HT level in serum balanced by 5-HT secretion, catabolism and platelet uptake mechanisms [31]. In this study, we found the decreased level of 5-HT in serum in both fatigue and non-fatigue groups comparing with the control group (**Table 4**). Further analysis showed that the score of mental fatigue increased with the decline of 5-HT level in serum in PD group. The decreased 5-HT level in serum were consistent with its level in CSF in these PD patients with mental fatigue. This suggested that

serum could be a very reasonable alternative to CSF when measuring serotonin levels and be probably preferable in predicting 5-HT level in CSF in PD patients with fatigue since it was much less invasive.

We furtherly explored the mechanism for the decline of 5-HT level in CSF in PD patients with mental fatigue. Previous study have reported that 5-HT could protect against oxidase stress in PD patients [32]. Yet, several studies have proved that nigral iron was a trigger of oxidative stress in PD [33]. Recently, one study showed that 5-(Nmethyl-N-propargyaminomethyl)-8-hydroxyquinoline (M30), an iron chelator, could increase 5-HT level in the brain of PD rat [34]. By far, no study focused on the relationship between iron and its related proteins and mental fatigue of PD. In this study, the levels of iron and transferrin in CSF in the fatigue group were prominently higher than those in control and non-fatigue groups, and the level of transferrin in CSF in non-fatigue is strikingly higher than that in control group (**Table 2**). And the score of mental fatigue increased with the elevated levels of iron and transferrin in CSF in PD group, implying an excessive iron deposition in brain and an abnormal iron metabolism in brain in PD patients. Several studies have proved iron deposition in SN in PD patients[35]. Our study firstly find that increased iron level in CSF is related to fatigue. Iron in brain interstitium may bind to large molecules, such as transferrin, and then is transported into neurons. Excessive intake of exogenous iron may induce redundant iron deposition in the brain[36]. It may be explained that excessive iron deposits in brain region related to mental fatigue, such as raphe nuclei, resulting in symptom of fatigue. These results indicated a potential role of abnormal iron metabolism on mental fatigue in PD patients, and iron and transferrin might be the potential indicators for mental fatigue in PD patients.

In this study, transferrin levels in serum in the fatigue group and non-fatigue group were reduced comparing with that in the control group (**Table 4**). Further analyses showed no relationship between the score of mental fatigue and the levels of iron and related proteins in serum. Iron in serum could transfer into brain through blood-brain barrier (BBB), and thus it might also participate in the pathogenesis of PD[37]. Transferrin was the main receptor-mediated transporter of iron from periphery to brain across BBB and a transporter of iron throughout the brain[38]. Our previous work have found the decreased transferrin level in serum in PD patients with sleep disorders[39], as well as PD patients with rapid eye movement-sleep behavior disorder (RBD)[40]. Hence, we speculate that BBB of PD patients may be more seriously damaged than that

of control group, allowing the entry of transferrin from periphery to brain enormously, resulting in abnormal iron storage, transportation and accumulation in raphe nuclei and basal ganglia related to fatigue.

All PD patients recruited in this study came from the specialized neurodegenerative outpatient clinic in the Department of Neurology and Department of Geriatrics, Beijing Tiantan Hospital，which is the China National Clinical Research Center for Neurological Diseases. In Department of Neurology and Department of Geriatrics, more than 80% patients are from the whole country. Although the patients in this study came from one center, 518 PD patients are from 28 out of 32 provinces and municipalities of China, roughly representing PD patients in China.

In summary, the frequency of fatigue in PD patients is 58.88%. Fatigue group has more advanced disease stage, severer motor symptoms, including tremor, rigidity, bradykinesia, postural and gait abnormalities, and severer non-motor symptoms, such as anxiety and depression. Decreased 5-HT in CSF is closely associated with mental fatigue in PD patients. The elevated iron and transferrin level in CSF is significantly related to mental fatigue in PD patients which might result from the translocation of transferrin from peripheral system to brain. Overloaded iron may contribute to 5-HT dysfunction in brain related to mental fatigue in PD patients. Thus, 5-HT reuptake inhibitors and iron chelator may serve as novel targets of drug development for mental fatigue of PD.

The limitations of this study is that it is a cross-sectional study, therefore, causal relationships between the levels of 5-HT and transferrin in the CSF of PD patients and fatigue could not be determined. The data of PD patients are only from one center, it needs a large, nationwide and multicentric study to further investigate the mechanism of PD fatigue in the future.

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Table 1 Demographics information, motor and non-motor symptoms in non-fatigue and fatigue groups

	Non-fatigue group (213 cases)	Fatigue group (305 cases)	P value
Age	60.85±10.44	61.73±10.07	0.89
Male/Total [cases/total (%)]	108/213（50.70%）	162/305（53.11%）	0.79
Disease duration [years, median (quartile)]	2.00（1.00~4.00）	3.00（1.00~6.00）	0.23
Hoehn-Yahr stage [stage, mean ± SD]	1.80±0.72	2.19±0.83	**0.01***
Levodopa equivalent dose(mg, mean ±SD)	319.13±107.98	322.79±113.54	0.72
UPDRS III [points, median (quartile)]	18.00（11.50~25.50）	27.50（19.00~36.00）	**0.00****
Tremor	3.00（2.00~6.00）	5.00（2.00~8.00）	**0.02***
Rigidity	3.00（1.00~6.00）	5.00（2.00~8.00）	**0.00****
Bradykinesia	2.50（1.75~4.00）	4.00（2.00~6.00）	**0.00****
Postural and gait abnormalities	7.00（4.00~12.00）	11.00（6.00~16.00）	**0.00****
Mental fatigue [points, median (quartile)]	4.00（2.00~6.00）	7.00（6.00~8.00）	**0.00****
Total fatigue [points, median (quartile)]	6.00（4.00~9.00）	11.00（9.00~13.00）	**0.00****
HAMA[scores, median (quartile)]	5.00（2.00~9.00）	11.00（6.00~18.00）	**0.00****
HAMD[scores, median (quartile)]	5.00（3.00~11.00）	15.00（8.00~20.00）	**0.00****
MMSE[scores, mean ± SD]	26.93±3.61	26.37±3.33	0.78
PSQI[scores, mean ± SD]	7.12±3.00	8.64±4.74	0.21

HAMD=Hamilton Depression Scale (24 items); HAMA=Hamilton Anxiety Scale (14 items); UPDRS =Unified Parkinson's Disease Rating Scale;
MMSE=mini-mental state examination; PSQI= Pittsburgh Sleep Quality Index. *: P<0.05, ** P<0.01.

Table 2 The levels of 5-HT, iron and metabolism-related proteins in CSF among control, non-fatigue and fatigue groups

	Control group （29 cases）	Non-fatigue group (59 cases)	Fatigue group （63 cases）	P1	P2	P3
Neurotransmitters						

5-HT [ng/mL, median (quartile)]	10.617 （5.732~115.828）	8.934 （4.421~107.512）	5.546（4.312~338.01）	0.094	**0.000****	**0.000****
Iron and metabolism-related proteins						
Iron [nmol/mL, median (quartile)]	0.380（0.263~0.612）	0.411（0.261~0.8254）	0.632（0.321~0.845）	0.354	**0.000****	**0.004****
Transferrin [ug/ml, median (quartile)]	0.079（0.062~0.083）	0.104（0.084~0.123）	0.192（0.073~0.214）	**0.001****	**0.000****	**0.003****
Lactoferrin [ug/ml, mean ±SD]	148.471±65.153	138.822±61.371	134.295±53.764	0.231	0.114	0.614
Ferritin [ng/ml, median (quartile)]	5.291（2.592~20.723）	5.771（3.043~14.221）	5.854（3.632~17.231）	0.783	0.63	0.853

5-HT=serotonin;P1: non-fatigue group vs. control group; P2: fatigue group vs. control group, P3: non-fatigue group vs. fatigue group.

**P＜0.01.

Table 3 Correlation between the levels of 5-HT and transferrin in CSF in PD patients

Neurotransmitters (ng/mL)	Iron and related proteins	R	P value
5-HT	transferrin	-0.492	**0.008****

5-HT=serotonin;**P＜0.01.

Table 4 The levels of 5-HT, iron and metabolism-related proteins in serum among control, non-fatigue and fatigue groups

	Control group （29 cases）	Non-fatigue group （125 cases）	Fatigue group （145 cases）	P1	P2	P3
N**eurotransmitters**						
5-HT [ng/mL, median (quartile)]	415.812 （319.142~522.327）	217.323 （126.017~289.543）	230.619 （135.815~305.719）	**0.001****	**0.000****	0.635
Iron and metabolism-related proteins						
Iron [nmol/ml, mean ± SD]	3.322（2.624~4.861）	3.011（2.113~4.324）	3.121（2.871~4.434）	0.259	0.382	0.624
Transferrin [ug/ml ,median (quartile)]	0.145（0.177~0.564）	0.089（0.069~0.094）	0.076（0.066~0.084）	**0.000****	**0.000****	0.023
Lactoferrin [ug/ml, median (quartile)]	51.591 （45.214~86.022）	51.711（47.282~88.894）	50.882（44.981~87.805）	0.768	0.456	0.647
Ferritin [ng/ml, median (quartile)]	16.775 （6.241~48.983）	15.772（7.043~41.223）	16.401（7.455~43.742）	0.701	0.816	0.984

5-HT=serotonin;P1: non-fatigue group vs. control group; P2: fatigue group vs. control group, P3: non-fatigue group vs. fatigue group.

**P＜0.01.

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Competing financial interests

The authors declare no competing financial interests.