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Research article

Plasma transferrin level correlates with the tremor-dominant phenotype of Parkinson's disease

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ABSTRACT

Accumulating evidence suggests that iron metabolism may be involved in the pathophysiology of Parkinson's disease (PD), and particularly in motor phenotype. This investigation aimed to examine plasma iron metabolism related indicators in patients with tremor-dominant phenotype of PD and determine less invasive, potential markers from plasma, which could partially reflect pathophysiological mechanisms of the brain. Seventy-six PD patients were recruited and thirty-three of them were classified into the tremor-dominant PD (TD-PD) group and forty-three into the non-tremor dominant PD (NT-PD) group, as determined by clinical characteristics. Plasma iron, ceruloplasmin, transferrin and ferritin levels were measured using Beckman Coulter AU biochemical assays, immune transmission turbidimetry method, scatter turbidimetry method and chemiluminescence method, respectively. Spearman's correlation analysis and multiple linear regression analysis were used for further study. Compared to healthy controls, TD-PD patients exhibited lower plasma iron level ($p = 0.006$) and higher transferrin level ($p < 0.001$). Plasma transferrin level was much higher in the TD-PD as compared to NT-PD ($p = 0.003$). Furthermore, plasma transferrin level was positively correlated with the severity of tremor in TD-PD ($r = 0.358$, $p = 0.041$). Multiple linear regression further demonstrated significant associations of plasma transferrin level with severity of tremor in TD-PD (regression coefficient = 0.253, $P = 0.016$), independently from other confounding factors. The elevated plasma transferrin level, combining with decreased plasma iron level might be given considerable weight in the recognition of parkinsonian tremor.

1. Introduction

Parkinson's disease (PD) is considered as a neurodegenerative disorder, characterized by resting tremor, rigidity, bradykinesia, and gait disorder. According to clinical characteristics PD can possibly be classified into two main clinical phenotypes: tremor-dominant PD group (TD-PD) and non-tremor PD (NT-PD) group [1]. As one of the most important motor symptoms of PD, tremor usually occurs at rest at a frequency of 4–6 Hz [2]. Moreover, tremor mainly involves the distal limbs and is often visible as a pill-rolling movement. These features make it much more obvious than other motor symptoms, which could be recognized by patients or their families in the early phase.

Although tremor was observed by James Parkinson 200 years ago, the mechanisms underlying it has not been fully elucidated. Several reasons indicated that parkinsonian tremor might have different underlying pathophysiology processes from those of bradykinesia and rigidity [3]. TD-PD patients tend to have a slower disease progression, a benign clinical course and a better long-term prognosis as compared with NT- PD patients [4]. In addition, unlike NT-PD patients, severity of the dopaminergic deficit in the striatum seems to be less important in the TD-PD patients. Tremor is probably generated by neuronal mechanisms struggling to compensate for akinesia and rigidity [3]. Note that the pathogenesis of parkinsonian tremor involves the activity of some central neuronal systems in the cerebello-thalamo-cortical (CTC) circuit [5].

Emerging research indicated excessive iron depositions in substantia nigra (SN) and other nucleus in PD patients [6]. Neurodegeneration related to iron may be due to the defects in its metabolism and/or homeostasis, as well as subsequent accumulation in the specific brain regions [7]. To illustrate, as an iron metabolism-related protein, transferrin was significantly increased in periphery of PD patients compared with healthy controls [8], indicating that abnormal iron metabolism took part in the pathogenesis of PD. However, the results were controversial [9,10] Recently, it was reported that regional iron content was associated with motor dysfunction [11]. However, until now, no investigation detects the correlation between parkinsonian

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tremor and iron metabolism in plasma.

In this study, aimed to examine plasma iron metabolism related indicators in patients with tremor-dominant phenotype of PD and determine less invasive, potential markers from plasma, which could partially reflect pathophysiological mechanisms of the brain, we measured the plasma levels of four kinds of iron metabolism related indicators, including iron, ceruloplasmin, transferrin and ferritin in TD-PD, NT-PD and healthy controls among Chinese population, analyzing their relationship with parkinsonian tremor.

2. Methods

2.1. Participants

From February 2017 to January 2018, we enrolled 76 PD patients, diagnosed by neurologists who specialize in movement disorders from the First Affiliated Hospital of Nanjing Medical University, according to the UK Parkinson's brain bank criteria [12]. Exclusion criteria included PD patients with other neurological symptoms and signs, secondary Parkinsonism or Parkinson plus syndrome, severe hearing or visual loss. The severity of motor symptoms was assessed by the Hoehn and Yahr (H-Y) stage and Unified Parkinson Disease Rating Scale III (UPDRS III). The levodopa-equivalent daily dose (LEDD) was calculated for each patient. In fact, among these patients, 49 were drug-naïve or newly diagnosed patients, and 27 patients were on optimized anti-parkinsonian medication. Meanwhile, we recruited 50 age- and gender-matched healthy controls, who had no neurological and psychological disorders or a family history of PD. All participants were included based on the following criteria: (1) no histories of blood donation; (2) no alcohol or drug abuse; (3) no anemia or other systemic diseases; (4) no dysarthria or mental illness affecting emotional expression; (5) female participants should have been through menopause. The mini–mental state examination (MMSE) was used to detect cognitive impairments in all subjects (MMSE > 24). This study was approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University and each subject has signed informed consent form.

2.2. Classification of PD motor phenotypes

Briefly, the tremor score was derived from the sum of UPDRS III item 20 (tremor at rest) and item 21 (postural tremor) divided by 7, and the akinetic–rigid score was derived from the sum of items 18 (speech), 19 (facial expressions), 22 (rigidity), 27 (arising from chair), 28 (posture), 29 (gait), 30 (postural stability), and 31 (body bradykinesia and hypokinesia) on the UPDRS III divided by 12 [13]. Combining clinical characteristics and UPDRS III score, tremor dominant PD was defined as rest tremor as sole initial symptom or sustained dominance of tremor over bradykinesia and rigidity according to the neurologist. The criterion for inclusion in the non-tremor dominant group was predominantly bradykinetic and/or rigidity motor features with no or only mild rest tremor [14]. Of these 76 patients, 33 were put in the TD-PD group, and 43 put in the NT-PD group. Of the NT-PD, 17 had no visible tremor when examined.

2.3. Plasma sample collection and biological assays

Anti-parkinsonian drugs were withheld for three days if their condition allowed in PD patients. We collected a 3 ml venous blood sample from all participants on each empty stomach into these tubes containing Ethylene Diamine Tetraacetic Acid(EDTA), and then immediately centrifuged at 3000 rpm for 10 min at 4 $^{\circ}C$ to obtain plasma, which were then stored at -80 $^{\circ}C$ until analysis. Biochemical analysis was performed in the clinical laboratory of the First Affiliated Hospital of Nanjing Medical University. Plasma iron, ceruloplasmin, transferrin and ferritin levels were measured using Beckman Coulter AU biochemical assays, immune transmission turbidimetry method, scatter turbidimetry method and chemiluminescence method respectively.

2.4. Statistical analysis

All statistical analyses were operated by the SPSS 23.0 software (Chicago, IL, USA). In our research, the normality of the distribution of all variables was examined by Shapiro–Wilk statistic. Group comparisons were made using chi-square test for categorical variables, and one-way analysis of variance (ANOVA), Mann-Whitney U test or Kruskal-Wallis H test for continuous variables as appropriate, followed by least significant difference (LSD) method or Dunn-Bonferroni test for post hoc multiple comparisons when necessary. Spearman's rank correlation analysis was made among transferrin level in plasma and age, gender, disease duration, UPDRS III score, H&Y stage, LEDD akinetic–rigid score as well as tremor score in TD-PD. In order to further examine the impact of plasma transferrin level on tremor in TD-PD, one multiple linear regression model was established, in which level of transferrin in plasma in TD-PD group was set as dependent variable, whereas age, gender, disease duration, UPDRS III score, H&Y stage, LEDD as well as tremor score were set as independent variables. All findings were expressed as mean ± standard deviation (SD), and p values < 0.05 were generally deemed significant.

3. Results

3.1. Comparison of demographic and clinical characteristics between control, TD-PD and NT-PD groups

A total of 76 patients with PD (TD-PD: 33, NT-PD: 43) and 50 healthy controls participated in this research. There were no significant differences in age and gender between control, TD-PD and NT-PD groups. The results showed that NT-PD group had more advanced disease stage ($p = 0.008$), higher score of UPDRS III ($p = 0.003$), higher akinetic–rigid score ($p < 0.001$) and lower tremor score ($p < 0.001$) than TD-PD group. Moreover, no statistically significant differences were found for duration of the disease, MMSE score and LEDD between two PD groups. (Table 1).

Table 1
Demographic and clinical characteristics of control, TD-PD and NT-PD groups.

	Control (n = 50)	TD-PD (n = 33)	NT-PD(n = 43)
Age (years)	59.68 ± 6.39	59.82 ± 12.46	63.23 ± 10.36
Males: females	25:25	21:12	20:23
Disease duration (years)	NA	2.73 ± 2.45	3.28 ± 3.77
MMSE score	NA	28.36 ± 2.43	27.74 ± 2.48
Untreated patients, n (%)	NA	26(78.79)	23(53.49)
LEDD (mg/day)	NA	184.55 ± 173.48	251.02 ± 404.59
H&Y stage	NA	1.55 ± 0.68	2.07 ± 0.91*
UPDRS III score	NA	17.06 ± 6.28	24.07 ± 11.33*
Tremor score	NA	0.97 ± 0.39**	0.27 ± 0.36
Akinetic–rigid score	NA	0.33 ± 0.25**	1.06 ± 0.58

Abbreviation: Data are presented as mean ± SD. NA, not available; PD, Parkinson's disease; MMSE, mini–mental state examination; LEDD, levodopa-equivalent daily dose; H&Y stage, Hoehn and Yahr stage; UPDRS, Unified Parkinson's disease rating scale; TD, tremor-dominant; NT, non-tremor-dominant. Kruskal-Wallis H test and Dunn-Bonferroni post hoc method was adopted for age (years). Mann–Whitney U test was adopted for duration of the disease (years), MMSE score, LEDD, H&Y stage, UPDRS III score, tremor score and akinetic–rigid score. Chi-square test was adopted for male/female ratio. H&Y stage, UPDRS III score was higher in the NT-PD group as compared to TD-PD group (*$p < 0.01$). Tremor score was much higher and akinetic–rigid score was much lower in the TD group as compared to NT-PD group (**$p < 0.001$).