Change in Plasma Levels of Amino Acid Neurotransmitters and its Correlation with Clinical Heterogeneity in Early Parkinson's Disease Patients

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Keywords

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SUMMARY

Background: The correlation between plasma amino acid (AA) neurotransmitters and clinical heterogeneity in early patients with Parkinson's disease (PD) is still poorly understood. **Aims:** To examine the plasma levels of AA neurotransmitters in early patients with PD and to evaluate their correlation with PD subtypes. **Methods:** Based on the predominant symptoms, fifty-one patients with PD were enrolled and divided into four subgroups: (1) akinetic-rigid type (ART), (2) tremor-dominant type (TDT), (3) postural instability/gait difficulty type (PIGD), and (4) mixed type (MT). Plasma levels of AA were measured by HPLC-RF, and their potential diagnostic practicality and their association with PD subtypes were evaluated by the receiver operating characteristic (ROC) and correlation analysis, respectively. **Results:** Patients with PD exhibited markedly lower levels of Asp, Glu, Tau, L-ser, and lower values of Glu/GABA ratio than healthy controls. The ROC analysis revealed their high sensitivity (77.1–87.5%) and specificity (58.8–88.2%). Furthermore, the glutamic acid (Glu), γ -aminobutyric acid (GABA) level in the PIGD subtype was increased as compared with other subtypes and was negatively correlated with the ART/PIGD ratio. **Conclusion:** The decrease in plasma Asp, Glu, Tau, L-ser levels, and the value of Glu/GABA ratio may be helpful for early PD diagnosis. The elevated GABA level may be the biochemical basis for the specific symptoms of PIGD PD.

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Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder in the elderly, characterized by bradykinesia, rigidity, tremor, and postural instability. PD exhibits a significant clinical heterogeneity, but its pathogenesis and heterogeneity remain unclear. According to the predominant symptoms, patients with PD are classified into four types: tremor-dominant type (TDT), akinetic-rigid type (ART), postural instability/gait difficulty dominant (PIGD), and mixed type (MT) [1,2]. The different PD motor subtypes have different clinical courses [1,3].

There is evidence that the tremor-dominant patients with PD have a slower disease progression [4] and a benign clinical course, as well as a better long-term prognosis as compared to the nontremor-dominant PD. In contrast, the PIGD-dominant PD often progresses more rapidly than other motor subtypes and is in need of positive symptomatic treatment [5]. Furthermore, the cognitive

dysfunction is associated with the subtype of motor deficit. The cognitive function of the tremor-dominant PD is normal or nearly normal, whereas the nontremor-dominant PD with less response to levodopa treatment [6], especially PIGD-dominant PD, is often accompanied by more serious cognitive defects, affective disorders, sleep disorders, and other nonmotor symptoms [7,8].

It is well known that dopamine plays a critical role in the pathogenesis of PD. However, depletion of dopamine in the nigrostriatal system cannot effectively explain the diverse clinical heterogeneities of PD. Emerging evidence suggests that several amino acid neurotransmitters in brain, such as glutamic acid (Glu), γ -aminobutyric acid (GABA), taurine (Tau), and L-serine (L-ser), are involved in the pathogenesis of PD by disturbing the equilibrium of the basal ganglia circuit, affecting the survival of dopaminergic neurons and modulating N-methyl-D-aspartate (NMDA) receptor functions [9–13]. Moreover, several studies [14–17] suggest that Glu and GABA are related to the clinical heterogeneity of PD. Yu

et al. [14] found that restoring the balance between Glu and GABA in the basal ganglia can alleviate the tremor in a model of Parkinsonian tremor. Tremor in tremor-dominant PD after deep brain stimulation (DBS) is often decreased even without electrical stimulation, which may result from the rise of adenosine and Glu in the ventrolateral thalamus (VL) [15]. There is also evidence [16,17] that GABA is closely related with PIGD PD via controlling the function of pedunculopontine nucleus (PPN). However, it is still unclear whether other amino acid neurotransmitters, such as aspartic acid (Asp), glutamine (Gln), glycine (Gly), taurine (Tau), and L-ser, also involve in clinical heterogeneity of PD. Additionally, previous studies showed that plasma levels of amino acid neurotransmitters are partially related with the levels in central nervous system (CNS) [18,19]. Moreover, a few of studies [20,21] detected the levels of plasma amino acid neurotransmitters to indirectly explore the corresponding changes in PD in CNS. Hence, in this study, we measured the plasma levels of several amino acid neurotransmitters (three typical excitatory amino acids: Glu, Asp, Gln, three typical inhibitory amino acids: GABA, Tau, Gly, as well as L-ser) in early patients with PD and healthy controls and analyzed their correlation with different PD motor subtypes. We tried to explore the biochemical basis underlying the pathogenesis and clinical heterogeneity of PD and to seek potential indicators for early diagnosis of PD.

Patients and Methods

Patients

Patients with PD, who were newly diagnosed (untreated) or off medication (after 1-week withdrawal of antiparkinsonian medication), were included in this study. They were diagnosed by two neurologists (Kezhong Zhang and Lian Zhang) with a special skill in movement disorders according to the UK Parkinson's brain bank criteria [22]; the Unified Parkinson's Disease Rating Scale (UPDRS) [23] scores were evaluated individually in all patients to help us define the clinical subtype of PD and assess the severity of each patient. The mini-mental state examination (MMSE) [24] showed no detectable cognitive impairments in any cases. Age- and gender-matched healthy individuals were used as controls, devoid of movement disorders, parkinsonism, neurodegenerative

diseases (including dementias), or a family history of PD. Moreover, the enrolled subjects should avoid high- or low-amino acid diet for at least 1 week before the sample collection. Informed consent form was obtained from each subject.

Classification of PD Motor Subtypes

According to the predominant motor symptoms, there were two popular methods to identify motor subtypes of patients with PD [1,2]. However, each of the two methods alone failed to fully describe the clinical heterogeneity of PD; thus, we combined them according to the previous studies [1,2]. All patients were subtyped into one of the four clinical PD types using the criteria (Table 1) based on the UPDRS Part III (motor symptoms), a combination and modification of Korchounov's [2] and Jankovic's [1] methods. The four subtypes were (1) ART, (2) TDT, (3) PIGD, and (4) MT. As shown in Figure 1, the results of our grouping method were in good accordance with the predominant motor symptoms of enrolled patients with PD.

The Collection and Storage of Plasma Samples

The venous blood samples were collected in EDTA-containing tubes from patients and controls on an empty stomach, and then immediately centrifuged in a temperature lower than 4°C to obtain plasma. These plasma samples were stored under −80°C (less than 4 months of storage time) until the measurement.

Measurement of Plasma Amino Acid Neurotransmitters

The measurement of Asp, Glu, Gln, Gly, Tau, GABA, and L-ser was performed by HPLC-RF. Agilent ZORBAX XDB-C18 column (4.6 × 150 mm, 5 μm) was used at 40°C. The mobile phase A was methanol; the mobile phase B was composed of 70 mmol/L Na₂HPO₄, 0.4 mmol/L EDTA-2Na, 0.1% TEA. The flow rate was 1.0 mL/min, and precolumn fluorescence derivatization was chosen. The fluorometric detector was performed at 260 nm (excitation) and 450 nm (emission). The derivatives consisted of 10 mg OPA, 10 mg IBC, 1 mL methanol, 9 mL freshly prepared 0.2 mol/L H₃BO₃ (pH10.0).

Table 1 Clinical features and classification criteria of the four motor subtypes

Motor subtype	Clinical features	Classification criteria
TDT PD	Unilateral onset and having distinct tremor with mild bradykinesia and no significant rigidity and PIGD	TDT/ART ≥ 1.5 and TDT/PIGD ≥ 1.5
ART PD	Unilateral onset and having significant bradykinesia and rigidity without obvious tremor and PIGD	TDT/ART ≤ 0.5 and ART/PIGD ≥ 1.5
PIGD PD	Obvious PIGD (start hesitation, short steps, freezing gait, etc.) without significant tremor, bradykinesia, and rigidity	ART/PIGD ≤ 1.0 and TDT/PIGD ≤ 1.0
MT PD	Not having any prominent symptoms and with a clinical feature of combination of two or more major symptoms of PD	Not fitting into the other three subtypes

PD, Parkinson's disease; ART, akinetic-rigid type; TDT, tremor-dominant type; PIGD, postural instability and gait difficulty dominant; MT, mixed type; TDT/ART, the ratio of mean tremor score to mean akinetic-rigid score; TDT/PIGD, the ratio of mean tremor score to mean PIGD score; ART/PIGD, the ratio of mean akinetic-rigid score to mean PIGD score. Mean tremor score of each patient was the sum of UPDRS III items 20 and 21 divided by 7; mean akinetic-rigid score was the sum of UPDRS III items 22–26 and 31 divided by 14; mean PIGD score was the sum of UPDRS III items 27–30 divided by 4.

Figure 1 Distribution of mean tremor score, mean akinetic-rigid scores, and mean postural instability/gait difficulty (PIGD) score in PD motor subtypes. The mean akinetic-rigid score, the mean tremor score, and the mean PIGD score obtained for each patient are plotted along X-axis, Y-axis, and Z-axis, respectively. Tremor-dominant type PD (tremor-dominant type, open circle), $n = 20$; akinetic-rigid type PD (akinetic-rigid type, open square), $n = 11$; postural instability and gait difficulty dominant type PD (PIGD, closed triangle), $n = 8$; mixed-type PD (mixed type, cross), $n = 12$.

Statistical Analysis

All statistical analyses were performed in SPSS V.13.0 (SPSS, Chicago, IL, USA). The normality of the distribution of all variables was examined by Shapiro–Wilk statistic. Group comparisons were made using chi-square test or Fisher's exact test for categorical variables, and two-tailed t -test, the Mann–Whitney U test, ANOVA, or the Kruskal–Wallis H test, as appropriate, for continuous variables. Least significant difference (LSD) method or Nemenyi test was adopted for *post hoc* multiple comparisons, and adequate data transformation methods were chosen for original data deviated from normality or homogeneity of variance. The receiver operating characteristic (ROC) analysis was performed, and the area under the ROC curve (AUC) was calculated to evaluate the diagnostic value of different plasma amino acid neurotransmitters for distinguishing between early PD and healthy controls. For the ROC analysis, the optimum cutoff levels were chosen as a highest Youden index. The AUC ranges from 0.50 to 1, a larger value indicating increasing ability to discriminate disease status [25] and a value above 0.8 being considered good [26]. The correlation significance was detected by spearman rank correlation coefficient. Data were presented as mean $\pm$ standard error. Unless otherwise stated, P values < 0.05 were considered statistically significant.

Results

Demographic and Clinical Characteristics of Patients and Controls

A total of 51 patients with PD (ART: 11, TDT: 20, PIGD: 8, MT: 12) were enrolled in this study. Among these patients, 42 were drug-

naive and newly diagnosed patients; the others had terminated antiparkinsonian treatment for 1 week before this study. The average disease duration (1.9 ± 1.7 years) and Hoehn & Yahr stage (1.8 ± 0.7) of enrolled patients showed that they were at early stages. In addition, two patients had a family history of PD. There were 48 age- and gender-matched healthy controls. No statistical differences between all groups were found for age, disease duration, onset age, gender distribution, total UPDRS scores, MMSE scores, or the portion of untreated patients. Furthermore, the UPDRS III scores were lower in TDT and PIGD groups compared with ART group and MT group. In agreement with previous study [5], PIGD group in our study had the highest Hoehn and Yahr stage among all groups (Table 2).

Distribution of Mean Akinetic-Rigid Scores, Mean Tremor Scores, and Mean PIGD Scores

A 3D scattergram was used to describe the distribution of mean akinetic-rigid scores (X-axis), mean tremor scores (Y-axis), and mean PIGD scores (Z-axis; Figure 1). As shown in Figure 1, different subtypes of the patients with PD almost distributed in different regions. Hence, ART, TDT, and PIGD patients with PD were clearly distinguished, indicating that the grouping method in our study was effective. The distribution of the MT PD was surrounded by that of the other three subgroups, which suggested mixed symptoms of tremor, akinetic-rigid, and postural instability/gait difficulty. Moreover, the distribution of TDT group was tight, whereas the distributions of PIGD, ART, and MT group were loose.

Levels of Plasma Amino Acid Neurotransmitters and Glu/GABA Ratio in Patients with PD with Different Motor Subtypes and Healthy Controls

To determine the potential role of several amino acid neurotransmitters in pathogenesis and subtype classification of PD, we measured their plasma levels in patients with PD with different motor subtypes and controls. As shown in Table 3, patients with PD had lower plasma levels of Asp, Glu, Tau, and L-ser than controls. But there were no significant differences in the plasma concentrations of Gln, Gly, or GABA between PD group and controls. Interestingly, patients with PD exhibited a lower value of Glu/GABA ratio compared with their controls (Table 3). Together, there are lower levels of Asp, Glu, Tau, L-ser, and a lower value of Glu/GABA ratio in PD group (Table 3, Figure 2).

We next compared the levels of these plasma neurotransmitters among the four motor subtypes. Interestingly, the level of plasma GABA was higher in PIGD group compared with ART, TDT, and MT group (Table 4, Figure 3). However, there was no significant difference in the levels of the other detected amino acid neurotransmitters, as well as the Glu/GABA ratio among motor subtypes (Table 4). These results suggest that the genesis of postural instability/gait difficulty is closely associated with increased levels of GABA.

The ROC Analysis of Plasma Asp, Glu, Tau, L-ser, and Glu/GABA Ratio

Compared with the control group, the levels of plasma Asp, Glu, Tau, L-ser, and the value of Glu/GABA ratio of PD group

Table 2 Demographic and clinical characteristics of patients with PD and controls

	Control (n = 48)	Total PD (n = 51)	ART PD (n = 11)	TDT PD (n = 20)	PIGD PD (n = 8)	MT PD (n = 12)
Age (years), mean $\pm$ SD	63.7 $\pm$ 12.0	61.9 $\pm$ 13.2	56.8 $\pm$ 8.2	60.2 $\pm$ 15.4	70.4 $\pm$ 11.8	63.8 $\pm$ 12.1
Males: females	31:17	27:24	9:2	7:13	6:2	5:7
Duration of the disease (years), mean $\pm$ SD	NA	1.9 $\pm$ 1.7	1.6 $\pm$ 0.9	2.3 $\pm$ 2.3	2.1 $\pm$ 1.4	1.4 $\pm$ 1.0
Age at onset, mean $\pm$ SD	NA	60.0 $\pm$ 13.6	55.2 $\pm$ 7.9	57.9 $\pm$ 16.3	68.3 $\pm$ 11.6	62.4 $\pm$ 12.2
Hoehn and Yahr stage, mean $\pm$ SD	NA	1.8 $\pm$ 0.7	1.9 $\pm$ 0.5	1.5 $\pm$ 0.6	2.8 $\pm$ 0.4*	1.8 $\pm$ 0.6
UPDRS III score, mean $\pm$ SD	NA	23.3 $\pm$ 11.4	28.6 $\pm$ 12.0	18.0 $\pm$ 7.8 [†]	18.3 $\pm$ 7.2 [‡]	30.8 $\pm$ 12.7
UPDRS total score, mean $\pm$ SD	NA	37.8 $\pm$ 15.8	43.4 $\pm$ 14.5	30.8 $\pm$ 14.9	35.3 $\pm$ 10.7	46.1 $\pm$ 17.1
MMSE score, mean $\pm$ SD	27.8 $\pm$ 2.5	27.7 $\pm$ 3.0	28.1 $\pm$ 2.7	27.6 $\pm$ 4.0	27.3 $\pm$ 1.4	27.8 $\pm$ 2.3
Untreated patients, n (%)	NA	42 (82.4)	8 (72.7)	18 (90.0)	6 (75.0)	10 (83.3)
Antiparkinsonian treatment, n (%)						
Levodopa/benserazide	NA	7 (13.7)	2 (18.2)	2 (10.0)	1 (12.5)	2 (16.7)
Levodopa/carbidopa	NA	1 (1.96)	NA	NA	1 (12.5)	NA
Dopamine agonist	NA	3 (5.88)	1 (9.09)	NA	1 (12.5)	1 (8.33)
Amantadine hydrochloride	NA	2 (3.92)	NA	1 (5.00)	1 (12.5)	NA
Benzhexol	NA	2 (3.92)	2 (18.2)	NA	NA	NA
Patients with a family history of PD, n (%)	NA	2 (3.92)	NA	2 (10.0)	NA	NA

NA, not available; PD, Parkinson's disease; UPDRS, Unified Parkinson's disease rating scale; MMSE, mini-mental state examination; ART, akinetic-rigid type; TDT, tremor-dominant type; PI GD, postural instability and gait difficulty dominant; MT, mixed type. ANOVA and LSD for age (years), age at onset (years), UPDRS III score, and UPDRS total score. Kruskal–Wallis H test and Nemenyi test for MMSE score, duration of the disease (years), and Hoehn and Yahr stage. Fisher's exact test for male/female ratio and the portion of untreated patients. *Hoehn and Yahr stage of PI GD group was higher than that of ART ($P < 0.05$) group, TDT ($P < 0.001$) group, MT ($P < 0.01$) group, and total patients ($P < 0.01$). [†]UPDRS III scores were lower in TDT group as compared to ART ($P < 0.01$) group or MT ($P < 0.01$) group. [‡]UPDRS III scores were lower in PI GD group as compared to ART ($P < 0.05$) group or MT ($P < 0.05$) group.

Table 3 The plasma levels of several amino acid neurotransmitters and Glu/GABA ratio in patients with PD and controls

Neurotransmitters, Mean $\pm$ SD	Control (n = 48)	PD (n = 51)
Asp ($\mu\text{mol/L}$)	43.1 $\pm$ 15.2	24.4 $\pm$ 8.4*
Glu ($\mu\text{mol/L}$)	148 $\pm$ 49.5	86.6 $\pm$ 25.6*
Gln ($\mu\text{mol/L}$)	684 $\pm$ 151	663 $\pm$ 133
Gly ($\mu\text{mol/L}$)	290 $\pm$ 79.8	280 $\pm$ 103
Tau ($\mu\text{mol/L}$)	133 $\pm$ 46.1	77.2 $\pm$ 36.9*
GABA ($\mu\text{mol/L}$)	278 $\pm$ 91.1	248 $\pm$ 67.2
L-ser ($\mu\text{mol/L}$)	173 $\pm$ 44.3	132 $\pm$ 32.9*
Glu/GABA [†]	0.632 $\pm$ 0.543	0.360 $\pm$ 0.108*

PD, Parkinson's disease; Asp, aspartic acid; Glu, glutamic acid; Gln, glutamine; Gly, glycine; Tau, taurine; GABA, γ -aminobutyric acid; L-ser, L-serine. * $P < 0.001$, compared with controls. [†]The ratio of the plasma concentration of Glu and GABA.

were significantly lower (Table 3). Therefore, ROC analysis was performed to assess the potential diagnostic practicality of these reduced neurotransmitters in discriminating early patients with PD from healthy controls. As shown in Figure 4 and Table 5, these amino acid neurotransmitters appeared to be good candidates for diagnosing PD at early stages. Therefore, these results suggest that the changed amino acid neurotransmitters may be helpful for the early diagnosis of PD in the clinical practice.

The Correlation of Levels of Plasma Amino Acid Neurotransmitters and Glu/GABA Ratio with Patients with PD

Next, we asked whether the levels of these plasma neurotransmitters are correlated with PD. The correlation analysis suggested that plasma concentrations of Gly and GABA were negatively correlated with the ratio of mean akinetic-rigid score to mean PI GD score (ART/PI GD; correlation coefficient = -0.384 , $P < 0.001$ for both Gly and GABA). Plasma concentration of GABA was also positively correlated with age (correlation coefficient = 0.378 , $P < 0.001$) and onset age (correlation coefficient = 0.364 , $P < 0.001$), respectively. Moreover, the plasma concentration of L-ser was slightly correlated with MMSE score (coefficient = 0.297 , $P < 0.05$). Taken together, plasma concentrations of GABA, Gly, and L-ser are associated with several characteristics of early patients with PD (Table 6).

Discussion

In the past decades, great progress in the pathophysiology and clinical features of PD has been made. PD displays not only significant clinical heterogeneity but also complicated nonmotor symptoms [1–5]. Braak *et al.* [27] showed that the progressive pathologic changes in PD within the cerebrum are global, rather than only confined in the substantia nigra pars compacta. In addition to dopamine, which plays a critical role in PD, several amino

Figure 2 Plasma Asp, Glu, Tau, L-ser levels (A), and Glu/GABA (B) ratio in patients with PD and controls. ** $P < 0.001$, as compared to the controls. PD, Parkinson's disease; Asp, aspartic acid; Glu, glutamic acid; Tau, taurine; GABA, γ -aminobutyric acid; L-ser, L-serine; Glu/GABA, the ratio of the plasma concentration of Glu and GABA.

Table 4 The plasma levels of several amino acid neurotransmitters and Glu/GABA ratio in four motor subtype groups

Neurotransmitters, Mean $\pm$ SD	ART PD (n = 11)	TDT PD (n = 20)	PIGD PD (n = 8)	MT PD (n = 12)
Asp ($\mu\text{mol/L}$)	22.8 $\pm$ 8.1	23.2 $\pm$ 7.6	24.4 $\pm$ 9.2	27.6 $\pm$ 9.7
Glu ($\mu\text{mol/L}$)	88.6 $\pm$ 29.0	78.7 $\pm$ 17.5	97.1 $\pm$ 24.1	91.0 $\pm$ 33.3
Gln ($\mu\text{mol/L}$)	691 $\pm$ 120	647 $\pm$ 112	753 $\pm$ 132	604 $\pm$ 152
Gly ($\mu\text{mol/L}$)	251 $\pm$ 71.5	288 $\pm$ 129	289 $\pm$ 65.4	287 $\pm$ 105
Tau ($\mu\text{mol/L}$)	78.8 $\pm$ 28.2	68.8 $\pm$ 27.7	78.6 $\pm$ 35.6	88.6 $\pm$ 55.3
GABA ($\mu\text{mol/L}$)	229 $\pm$ 63.5*	239 $\pm$ 52.2*	310 $\pm$ 84.1	239 $\pm$ 65.6*
L-ser ($\mu\text{mol/L}$)	132 $\pm$ 21.6	136 $\pm$ 43.1	129 $\pm$ 25.3	126 $\pm$ 28.4
Glu/GABA	0.400 $\pm$ 0.150	0.340 $\pm$ 0.090	0.320 $\pm$ 0.040	0.380 $\pm$ 0.110

PD, Parkinson's disease; ART, akinetic-rigid type; TDT, tremor-dominant type; PIGD, postural instability and gait difficulty dominant; MT, mixed type; Asp, aspartic acid; Glu, glutamic acid; Gln, glutamine; Gly, glycine; Tau, taurine; GABA, γ -aminobutyric acid; L-ser, L-serine; Glu/GABA, the ratio of the plasma concentration of Glu and GABA. * $P < 0.05$, compared with PIGD group.

Figure 3 Plasma glutamic acid (Glu), γ -aminobutyric acid (GABA) levels in four motor subtypes. * $P < 0.05$, as compared to the PIGD group. GABA, γ -aminobutyric acid; PD, Parkinson's disease; ART, akinetic-rigid type; TDT, tremor-dominant type; PIGD, postural instability and gait difficulty dominant; MT, mixed type.

acid neurotransmitters, such as Glu, GABA, Tau, and L-ser, are also involved in the pathogenesis of PD [9–13]. Several decades ago, some studies [20,21] investigated indirectly the changes in amino acid neurotransmitters in PD brain by measuring the levels of plasma amino acid neurotransmitters, which may be affected by diet [28,29], disease status [20,30], and drug treatment [20,31]. Additionally, the permeability of the blood brain barrier

Figure 4 Receiver operating characteristic curves of plasma Asp, Glu, Tau, L-ser levels, and Glu/GABA ratio. Area under the curve (AUC) for plasma Asp, Glu, Tau, L-ser levels, and Glu/GABA ratio are 0.871, 0.892, 0.845, 0.786, and 0.840, respectively. Asp, aspartic acid; Glu, glutamic acid; Tau, taurine; GABA, γ -aminobutyric acid; L-ser, L-serine; Glu/GABA, the ratio of the plasma concentration of Glu and GABA.

Table 5 The results of the ROC analysis of plasma levels of Asp, Glu, Tau, L-ser, and the value of Glu/GABA ratio

	The optimum cut-off value	Sensitivity (%)	Specificity (%)	AUC
Asp	30.6 $\mu\text{mol/L}$	83.3	78.4	0.871
Glu	116 $\mu\text{mol/L}$	77.1	88.2	0.892
Tau	101 $\mu\text{mol/L}$	83.3	76.5	0.845
L-ser	131 $\mu\text{mol/L}$	87.5	58.8	0.786
Glu/GABA	0.382	85.4	70.6	0.840

AUC, area under the ROC curve; Asp, aspartic acid; Glu, glutamic acid; Tau, taurine; GABA, γ -aminobutyric acid; L-ser, L-serine; Glu/GABA, the ratio of the plasma concentration of Glu and GABA.

is increased in PD [32]. We therefore assumed that the plasma neurotransmitter concentrations might reflect partially and indirectly pathological changes in PD in CNS. However, only a few studies [14–17] have been carried out to investigate the correlation of Glu and GABA with PD clinical heterogeneity, and the biochemical basis of clinical heterogeneity, a remarkable characteristic of PD, has not been fully elucidated. Hence, in our study, we detected the levels of Asp, Glu, Gln, Gly, Tau, GABA, L-ser, and the value of Glu/GABA ratio in plasma to further assess their correlation with different motor subtypes of PD. Our findings provide insight into the pathogenesis and clinical heterogeneity of PD and may be helpful for the diagnosis of the early PD.

A number of studies tried to explore the biochemical basis of PD using various methods. A ^1H magnetic resonance spectroscopy (MRS) study revealed a significant negative correlation between UPDRS scores and the Glu level in the bilateral lentiform nucleus of patients with PD [33]. Griffith *et al.* [34] reported that the Glu level in the cerebral cortex was reduced in patients with PD. In line with these previous studies, we observed the decreased plasma Glu in patients with PD. We speculated that the decrease

might result from overinhibition of the direct pathway and over-excitation of indirect pathways in the basal ganglia circuit [35]. Nevertheless, some studies showed that the Glu level was elevated in PD animal models [36,37]. The loss of dopaminergic neurons was slowly progressive in patients with PD [27], but rapid in PD models; thus, the inconsistency may be attributed to different rates of the loss of dopaminergic neurons [38]. In addition, plasma level of Asp, which functions as excitatory amino acid similar to Glu, was also significantly decreased in patients with PD, suggesting that the alteration in Asp and Glu may share some common processes during PD pathogenesis.

Plasma Tau level also declined in patients with PD in our study. Tau was thought to protect dopaminergic cells through inhibiting the direct and indirect excitotoxicity in dopaminergic cells, alleviating oxidative stress, guarding the mitochondrial respiratory chain, and so on [10]. Decreased content of plasma Tau in patients with PD may be due to chronic consumption of the peripheral Tau pool to protect residual dopaminergic cells.

D-serine (D-ser), a coagonist of the NMDA receptor, is synthesized from L-ser in the brain tissue [39], but its role in the pathogenesis of PD remains unclear [12,40]. Previous work [40] demonstrated that the levels of D- and L-Ser in MPTP-induced PD mouse midbrain both decreased dramatically. Furthermore, the rate of D-serine synthesis in the brain tissue, especially in the fore-brain, was determined by L-ser availability [13]. Therefore, decreased levels of plasma L-ser in early patients with PD in our study may contribute to the pathogenesis of PD by affecting the synthesis of D-serine in the brain and controlling NMDA receptor functions. Additionally, our findings showed that Glu/GABA ratio was lower in patients with PD compared with healthy controls, although there was no significant change in the plasma GABA level in early patients with PD, which was in agreement with one previous report [41]. These findings suggest that imbalance between Glu and GABA plays a critical role in PD pathophysiology [35].

Table 6 The correlation of levels of plasma amino acid neurotransmitters and Glu/GABA ratio with some demographic and clinical characteristics of enrolled patients with PD

	Asp r	Glu r	Gln r	Gly r	Tau r	GABA r	L-ser r	Glu/GABA r
Age (years)	0.088	0.067	0.125	0.226	0.048	0.378**	0.031	−0.212
Disease duration (years)	0.073	0.175	0.122	−0.097	−0.001	0.113	0.132	0.111
Age at onset (years)	0.063	0.049	0.133	0.230	0.035	0.364**	0.031	−0.227
Hoehn and Yahr stage	0.049	0.243	0.155	0.005	0.083	0.190	−0.053	0.046
UPDRS III score	0.228	0.203	0.048	0.217	0.194	−0.012	0.141	0.176
UPDRS total score	0.256	0.217	0.028	0.246	0.206	0.056	0.160	0.120
Mean tremor Score	0.093	−0.063	−0.217	0.182	−0.076	−0.040	0.069	0.074
Mean akinetic-rigid Score	0.219	0.250	0.045	0.099	0.210	−0.047	0.132	0.230
Mean PIGD Score	0.117	0.163	0.130	0.275	0.072	0.194	0.023	−0.051
TDT/ART	0.033	−0.209	−0.233	0.015	−0.037	−0.063	−0.017	−0.071
TDT/PIGD	−0.005	−0.214	−0.242	−0.089	−0.075	−0.153	−0.025	0.010
ART/PIGD	−0.018	−0.076	−0.176	−0.384**	0.033	−0.384**	−0.002	0.245
MMSE score	0.199	0.223	0.129	−0.047	0.225	0.044	0.297*	0.262

r, Spearman's rho; Asp, aspartic acid; Glu, glutamic acid; Gln, glutamine; Gly, glycine; Tau, taurine; GABA, γ -aminobutyric acid; L-ser, L-serine; Glu/GABA, the ratio of the plasma concentration of Glu and GABA; UPDRS, Unified Parkinson's disease rating scale; MMSE, mini-mental state examination; PIGD, postural instability and gait difficulty dominant; TDT/ART, the ratio of mean tremor score to mean akinetic-rigid score; TDT/PIGD, the ratio of mean tremor score to mean PIGD score; ART/PIGD, the ratio of mean akinetic-rigid score to mean PIGD score. *Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.001 level (2-tailed).

However, some previous reports [21,42] showed different results. For example, Iwasaki *et al.* [21] found that the plasma levels of Asp, Glu, and Gly were significantly higher than healthy controls. The inconsistency may result from differences in the sample size, disease status, and measurement. Compared with the previous studies, we enrolled a relatively more patients, focused on the early PD and used a more stable and sensitive measurement (HPLC-RF).

The results from the ROC analysis showed that plasma levels of Glu, Asp, L-ser, Tau, and Glu/GABA ratio may be helpful for the early diagnosis of PD. An AUC value above 0.8 is considered good to discriminate disease status [26]. Among them, AUC for plasma Asp, Glu, Tau levels, and Glu/GABA ratio were larger than 0.8, implicating their potential practicality in the diagnosis of the early PD. As compared to dopamine transporter binding (DAT) single photon emission computed tomography (SPECT), this early diagnostic method may be economical, simple, sensitive, and specific. Of note, in our study, the ROC analysis was performed only between PD and healthy controls. Thus, the results from our ROC analysis were limited, only providing some clues for clinicians to diagnose early PD in combination with the clinical features. Notably, the specificity of these amino acid neurotransmitters still requires to be confirmed by comparisons between PD and other neurologic diseases (for instance, Alzheimer's disease, Huntington's disease, and stroke) with a larger sample size.

The comparisons between the four motor subtype groups showed that the plasma level of GABA in PIGD group was higher than the other motor groups and had an increase tendency ($P = 0.362$) as compared to the healthy control although there was no statistical difference. Several lines of evidence [16,43] have demonstrated that the special axial symptoms in PIGD patients with PD are closely related with PPN, which contains abundant cholinergic, glutamatergic, GABAergic neurons and has functional and anatomic association with basal ganglia [43]. In addition, DBS of PPN and microinjections of the GABA antagonist bicuculline into the PPN both improved gait dysfunction and postural instability in PD [16]. The specific effects of PPN stimulation on postural instability/gait difficulty may result from inhibiting the release of GABA in the VL [17]. Thus, we guessed that augmented levels of plasma GABA in PIGD patients with PD in our study may participate in the genesis of postural instability/gait difficulty via overexciting GABAergic pathway from PPN to VL, reducing the output of basal ganglia, and as a result producing the special axial symptoms

in PIGD PD [16,17,43]. Consistently, plasma levels of Gly and GABA were negatively correlated with ART/PIGD, further supporting that the genesis of postural instability/gait difficulty was closely associated with increased levels of inhibitory amino acids.

In conclusion, our results showed that the levels of plasma Asp, Glu, Tau, L-ser levels, and the value of Glu/GABA ratio were lower in patients with PD. However, the roles of the altered levels of amino acid neurotransmitters in PD remained to be further investigated. Among the four motor subtypes, the PIGD group had a higher level of plasma GABA, implicating its potential role in the genesis of postural instability/gait difficulty. However, the relatively small sample size of this work, especially in each motor subtype, may be insufficient to reveal the common changes in amino acid neurotransmitters in PD. Hence, more stringent studies with a larger sample size are needed to explore the dynamic changes in plasma amino acid neurotransmitters levels during PD progression. In addition, levels of amino acid neurotransmitters in the peripheral blood may be affected by the diet. Therefore, the samples should be obtained on an empty stomach and the enrolled subjects should avoid high- or low-amino acid diet for at least 1 week before the sample collection to minimize the influence of diet on plasma levels of amino acid neurotransmitters. Promisingly, the detection of some amino acid neurotransmitters levels in plasma may provide new insight into the pathogenesis and clinical heterogeneity of PD and be helpful for early PD diagnosis.

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Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

This study was approved by the ethics committee of Nanjing Medical University and therefore performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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