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Association of Serum Indirect Bilirubin Concentrations with Motor Subtypes of Parkinson's Disease

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Keywords
Parkinson's disease · Bilirubin · Motor subtypes

Abstract
Introduction: We aimed to investigate the change of serum indirect bilirubin (IBIL) concentrations in patients with Parkinson's disease (PD) and whether IBIL concentrations were associated with the motor subtypes of PD. ***Methods:*** A case-control study was performed to evaluate differences in bilirubin concentrations between 78 PD subjects and 78 controls. Venous blood samples were collected, and total bilirubin (TBIL), direct bilirubin (DBIL), and IBIL concentrations were analyzed between PD subjects and controls. PD patients were classified into three motor subtypes: tremor-dominant (TD), intermediate (I), and postural instability and gait disorder (PIGD). It was evaluated whether there were differences in IBIL concentrations between the different motor subtypes and between motor subtypes and controls. ***Results:*** PD patients had lower IBIL concentrations compared to controls (6.51 ± 4.03 vs. 10.82 ± 4.61, $p < 0.001$). There was no significant difference in IBIL concentrations between PD males and PD females (6.66 ± 3.64 vs. 6.22 ± 4.79, $p = 0.655$). IBIL concentrations had negative relationships with levodopa-equivalent daily dose (LEDD) ($R = -0.452$, $p < 0.001$) and positive relationships with tremor score ($R = 0.360$, $p = 0.001$). IBIL concentrations were significantly lower for PIGD than for TD subtype (4.88 ± 4.03 vs. 9.00 ± 4.15, $p < 0.001$). The lower IBIL concentrations in PD compared to controls were mainly driven by the PIGD patients. ***Conclusions:*** PD subjects showed lower levels of IBIL compared to controls. Higher IBIL levels were associated with TD motor subtype in PD, which could be related to the antioxidative properties of IBIL.

Introduction

Parkinson's disease (PD) has high clinical heterogeneity. Based on the motor symptoms, PD patients can be classified into tremor-dominant (TD), intermediate (I), and postural instability and gait disorder (PIGD) subtypes [1]. It is found that different motor subtypes have different prognoses. The prognosis of TD subtype was reported to be relatively good, with a higher quality of life than non-tremor patients [2].

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A major challenge is to unravel why different Parkinson's patients present with different subtypes of motor symptoms. It is likely that multiple factors play roles in the pathogenesis of PD phenotype, including mitochondrial function defects, abnormal protein regulation, and consecutive oxidative stress [1, 3]. Bilirubin is an important natural antioxidant in the human body and is a major contributor to the total antioxidant capacity of plasma [4]. Several previous studies have found that bilirubin is possibly a marker altering the risk of PD [5–7]. To our knowledge, no study has examined the association between bilirubin and motor subtypes of PD. Here, we report a case-control analysis of 78 Chinese PD patients to investigate: (i) the change of bilirubin concentrations in patients with PD in China; and (ii) whether the bilirubin correlates with the motor subtypes of PD. The results of this study might help to further understand the role of bilirubin in the pathogenesis of PD and reveal whether bilirubin can be used as an indicator for the classification of motor subtypes of PD.

Methods

This study was conducted at the Department of Neurology of the Beijing Tsinghua Changgung Hospital, China.

Patients

We analyzed the collected data from 84 patients (56 men and 28 women) with PD, who were examined at our outpatient clinic or ward from January 2018 to May 2019, taking or not taking anti-PD medication. Inclusion criteria were individuals who fulfilled the Movement Disorder Society clinical diagnostic criteria for PD [8]. Exclusion criteria were: (i) a diagnosis of secondary (such as vascular or drug-induced) or familial parkinsonism or a diagnosis of atypical parkinsonism, according to current diagnostic criteria; (ii) past history of kidney disease, liver and gallbladder disease, or with abnormal alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), or creatinine. A total of 1,061 controls were recruited from the Medical Examination Center at the same period of time. Controls with kidney disease, liver and gallbladder disease, or with abnormal ALT, AST, GGT, or creatinine were excluded. Among the 84 PD subjects and 1,061 controls enrolled in the study, 78 PD patients were selected and matched with 78 healthy controls in age and gender by using propensity score matching with a case-control matching ratio of 1:1.

Data Acquisition

We only accepted data from visits in "off" conditions, which present if a patient was in or close to their worst hypokinetic state and if the presence of extrapyramidal symptoms was in agreement with this statement. For all these patients, we collected information about their gender, age, course of disease, motor status, symptoms, and treatments. We used the Unified Parkinson's Disease Rating Scale (UPDRS) to classify motor subtypes. PD severity was determined by Hoehn and Yahr staging (H&Y). Levodopa-equivalent daily dose (LEDD) was calculated for each drug class [9].

Classification of Motor Subtypes

Patients were classified based on established methods into TD, I, or PIGD using items from the UPDRS [10]. Patients' tremor score was determined by adding items 16 and 20–21 (measures of rest, action, or postural tremor), and dividing by 8, and balance and gait score by adding items 13–15 (walking) and 29–30 (measures of gait and postural stability), and dividing by 5. Patients were classified as TD if the ratio of the tremor score divided by the balance and gait score was >1.50, PIGD if the ratio was <1, and as I if the ratio was between 1 and 1.50.

Laboratory Assessment

Venous blood was collected at rest in the morning after overnight fasting. Serum total bilirubin (TBIL), indirect bilirubin (IBIL), direct bilirubin (DBIL), ALT, AST, GGT, and creatinine levels were measured by an automatic biochemical analyzer (Siemens Analyzer, Germany). Laboratory assessments were performed at the same time as motor examination in the off conditions.

Statistical Analysis

The measurement data were presented as mean ± standard deviation (SD) and the numeration data as frequency and percentage. Demographic differences between groups were evaluated by χ^2, *t* test, or ANOVA as appropriate. The IBIL, DIIL, and TBIL concentrations were found to accord with normal distribution after being tested for normality. Differences in TBIL, DBIL, and IBIL concentrations between the PD group and controls were evaluated by *t* test. The correlations between IBIL and continuous variables (disease duration, LEDD, tremor score, balance and gait score) were evaluated with Pearson's correlation coefficient, while the correlation between IBIL and H&Y was evaluated with Spearman's correlation coefficient. A difference in IBIL values between patients requiring and not requiring levodopa was evaluated by means of a *t* test (two-tailed model). An ANOVA was used to evaluate whether there was a difference in IBIL concentrations between different motor subtypes. Post hoc analyses (LSD) were performed to compare significant associations in a pairwise manner. We used a multivariate logistic regression analysis to assess associations of IBIL and the motor subtypes while adjusting for potentially confounding variables. While performing the multivariate logistic regression analysis, the independent variables were grouped. Age was divided into three groups (group 1: ≥50 and <60 years; group 2: ≥60 and <70 years; group 3: ≥70 years). The course of disease was divided into three groups (group 1: <3 years; group 2: ≥3 years and <6 years; group 3: ≥6 years). H&Y was divided into three groups (group 1: 1–2.5; group 2: 3; group 3: 4–5). IBIL (μmol/L) was divided into four groups (group 1: <1.7; group 2: ≥1.7 and <6.8; group 3: ≥6.8 and <13.7; group 4: ≥13.7) with the 25, 100, and 200% of the official reference limit (<6.8) as the dividing point. LEDD (mg/day) was divided into four groups (0–300, 300–600, 600–900, 900–1,200) with the 25, 50, and 75% of the maximum LEDD of the patients in this study (1,200 mg/day) as the dividing point. The significance threshold was set to $p < 0.05$. Statistical analyses were performed with IBM SPSS Statistics 22.0.

Table 1. Descriptive values and statistics of the PD group and controls

	PD group (n = 78)	Controls (n = 78)	p value
Age, years	69.12±7.78	68.91±8.43	0.877
Male	52 (66.67)	52 (66.67)	1.000
H&Y			
Stage 1–2.5	46	–	–
Stage3	23	–	–
Stage 4–5	9	–	–
Disease duration, years	4.28±4.03	–	–
UPDRS III score	27.9±15.5	–	–
Drug-naive patients	18 (23.1)	–	–
Total LEDD, mg/day	347.9±332.4	–	–
TBIL, μmol/L			
Male	13.26±3.73	16.46±6.85	0.004
Female	12.30±4.17	13.44±4.60	0.351
Total	12.94±3.87	15.45±6.33	0.003
DBIL, μmol/L			
Male	6.61±3.54	5.16±2.10	0.012
Female	6.08±3.05	3.59±1.12	<0.001
Total	6.43±3.37	4.63±1.97	<0.001
IBIL, μmol/L			
Male	6.66±3.64	11.30±4.99	<0.001
Female	6.22±4.79	9.85±3.61	0.003
Total	6.51±4.03	10.82±4.61	<0.001

Data are presented as n (%) or mean ± SD as appropriate.

Results

Descriptive values and statistics are shown in Table 1. Compared to controls, significantly lower TBIL and IBIL concentrations and significantly higher DBIL concentration were observed in PD subjects (TBIL, $p = 0.003$; IBIL, $p < 0.001$; DBIL, $p < 0.001$) (Table 1). PD females had lower IBIL concentrations compared to control females ($p = 0.003$), and the same was observed for PD and control males ($p < 0.001$) (Table 1). But there was no significant difference in IBIL concentrations between PD males and PD females (6.66 ± 3.64 vs. 6.22 ± 4.79, $p = 0.655$).

After that, PD subjects only were evaluated. We analyzed whether disease duration, LEDD, H&Y, tremor score, and balance and gait score related to IBIL levels. IBIL concentrations had negative relationships with LEDD ($R = -0.452$, $p < 0.001$) and positive relationships with tremor score ($R = 0.360$, $p = 0.001$) (Fig. 1, 2) , but were not correlated with disease duration ($R = -0.156$, $p = 0.172$), H&Y ($R = -0.190$, $p = 0.095$), or balance and gait score ($R = -0.176$, $p = 0.123$). The IBIL concentrations were significantly lower for patients requiring levodopa than for those not requiring levodopa (5.37 ± 3.54 vs. 10.31 ± 3.21, $p < 0.001$).

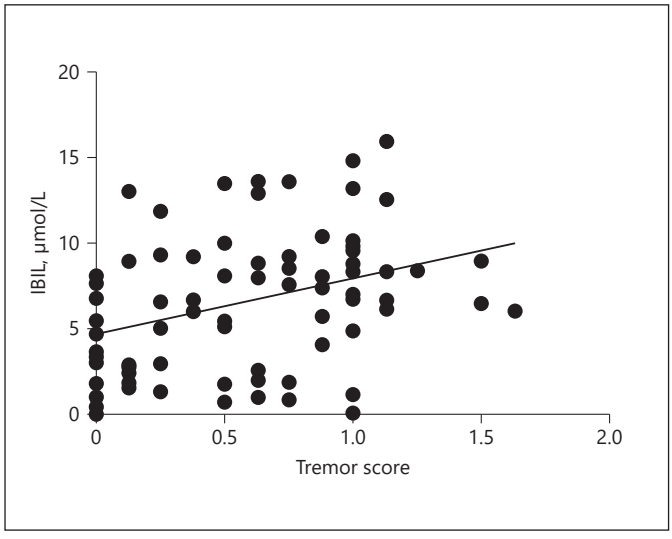

Fig. 1. Correlation between IBIL and tremor score. Serum IBIL concentrations were positively correlated with tremor score ($R = 0.360$, $p = 0.001$).

With regard to PD motor subtypes, it was analyzed whether bilirubin concentrations were associated with different motor subtypes. Descriptive values and differ-

Table 2. Descriptive values and differences in sex, age, disease duration, H&Y, and total LEDD and bilirubin levels between patients with TD, PIGD, and I subtypes

	PIGD (n = 46)	I (n = 13)	TD (n = 19)	p value
Male	31 (67.4)	7 (53.8)	14 (73.7)	0.498
Age, years	70.09±6.92	70.92±9.21	64.11±8.78	0.014
Disease duration, years	4.27±3.80	5.45±5.38	3.53±3.53	0.422
H&Y	2.73±0.87	2.58±0.67	2.03±1.05	0.019
Total LEDD, mg/day	427.99±349.16	274.04±254.87	204.61±287.57	0.031
TBIL, μmol/L	11.85±3.36	13.00±2.95	15.55±4.46	0.002
IBIL, μmol/L	4.88±3.54	8.65±2.52	9.00±4.15	<0.001
DBIL, μmol/L	6.97±3.53	4.35±1.75	6.54±3.41	0.044

Data are presented as n (%) or mean ± SD as appropriate.

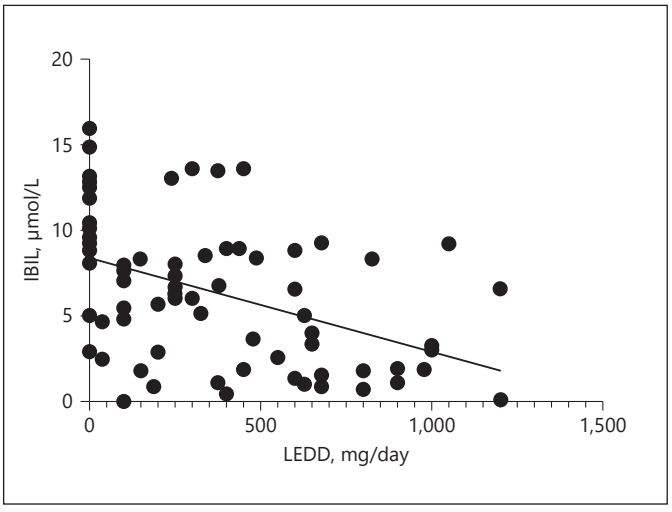

Fig. 2. Correlation between IBIL and LEDD. Serum IBIL concentrations were negatively correlated with LEDD (R = –0.452, p < 0.001).

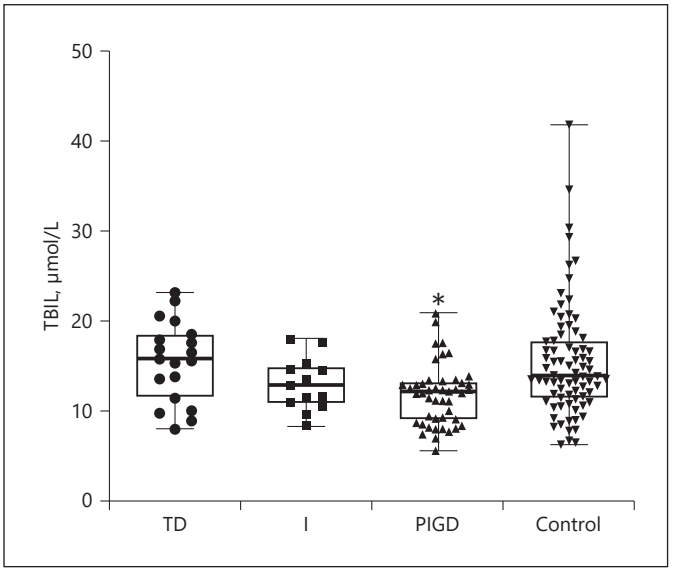

Fig. 3. Differences in TBIL concentrations between TD, PIGD, and I subtypes and controls. Box and whisker plot showing the differences in TBIL levels between TD (n = 19), PIGD (n = 46), and I (n = 13) subtypes and controls (n = 78). ANOVA indicated that TBIL concentrations were different across TD, PIGD, and I subtypes (TBIL, p = 0.002). * p < 0.05 compared with the control group (t test).

ences in sex, age, disease duration, H&Y, and LEDD between patients with TD, PIGD, and I subtypes are shown in Table 2. ANOVA indicated that TBIL, DBIL, and IBIL concentrations were different across TD, PIGD, and I subtypes (TBIL, p = 0.002; DBIL, p = 0.044; IBIL, p < 0.001) as shown in Table 2 and Figures 3–5. Post hoc analyses revealed that IBIL and TBIL concentrations were significantly lower for PIGD than for TD (IBIL, p < 0.001; TBIL, p < 0.001), while the DBIL concentrations were not significantly different between PIGD and TD (p = 0.632). Multivariate logistic regression analysis indicated that when potential confounders including sex, age, disease duration, LEDD, and H&Y stage were taken into account, there was still a linear relationship between IBIL and motor subtypes (p = 0.004). The bilirubin levels of TD, I, and PIGD were compared to those of controls, respectively. The results showed that IBIL concentrations were different between PIGD and controls, but not significantly different between TD and controls as well as between I and controls, which indicated that the lower IBIL concentrations in PD were mainly driven by the PIGD patients (Table 3; Fig. 3–5).

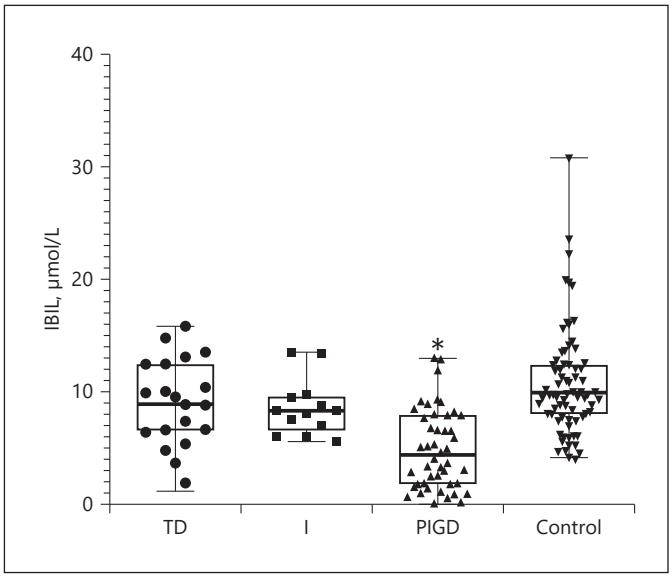

Fig. 4. Differences in IBIL concentrations between TD, PIGD, and I subtypes and controls. Box and whisker plot showing the differences in IBIL levels between TD (n = 19), PIGD (n = 46), and I (n = 13) subtypes and controls (n = 78). ANOVA indicated that IBIL concentrations were different across TD, PIGD, and I subtypes (p < 0.001). * p < 0.05 compared with the control group (t test).

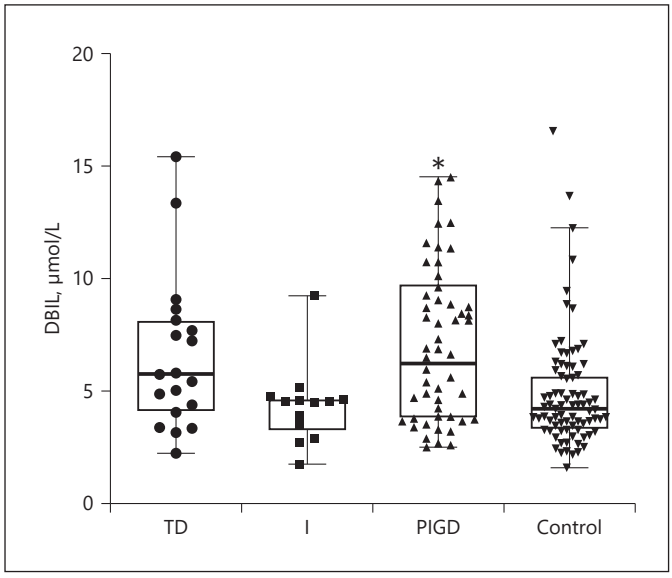

Fig. 5. Differences in DBIL concentrations between TD, PIGD, and I subtypes and controls. Box and whisker plot showing the differences in DBIL levels between TD (n = 19), PIGD (n = 46), and I (n = 13) subtypes and controls (n = 78). ANOVA indicated that DBIL concentrations were different across TD, PIGD, and I subtypes (p = 0.044). * p < 0.05 compared with the control group (t test).

Discussion

The main findings of our current study were: (1) PD patients had lower IBIL concentrations compared to controls; (2) IBIL concentrations had negative relationships with LEDD in PD patients; and (3) IBIL concentrations were significantly lower for PIGD than for TD.

Lower IBIL Concentrations in PD Compared to Controls

Bilirubin is a metabolic end product of heme catabolism. TBIL consists of water-soluble DBIL and liposoluble IBIL. The highly lipophilic IBIL might interact with cell membranes to protect against lipid peroxidation [11]. It was reported that IBIL exerted anti-ROS properties and therefore had protective effects, whereas DBIL was rarely mentioned [5]. Here we used the IBIL concentration as a biomarker of PD and PD motor subtypes rather than the DBIL or TBIL concentration.

Our study found that PD patients had lower IBIL concentrations compared to controls. This trend were also observed when males and females were assessed separately. Our results are in line with previous findings by Qin et al. [5], who found decreased levels of serum IBIL in 425 Chinese PD patients and 460 controls. They explained that IBIL was the only endogenous lipophilic antioxidant in the human body that could protect neurons from oxidative stress, and lower serum IBIL concentrations influenced PD development by reducing endogenous antilipid peroxidation resistance [5]. However, studies of Scigliano et al. [6] and Moccia et al. [7] conducted in Caucasians concluded otherwise that serum bilirubin concentrations increased in patients with PD. They speculated that heme oxygenase (HO) upregulation within the substantia nigra might be an adaptive response to increased oxidative stress occurring in PD and was likely to be responsible for increased bilirubin which was formed by HO [7]. Compar-

Table 3. Comparison of bilirubin levels between motor subtypes and controls (p values of t test)

	p value		
	TD-control	I-control	PIGD-control
TBIL	0.951	0.176	**0.001**
DBIL	**0.002**	0.627	**<0.001**
IBIL	0.120	0.103	**<0.001**

Values in bold indicate statistical significance.

ing these reports, we find that the TBIL concentrations of a Chinese healthy population reported in our paper and that of Qin et al. [5] are much higher than those of a Caucasian healthy population in the papers of Scigliano et al. [6] and Moccia et al. [7] (Chinese: 12.30 μmol/L [5] and 15.55 μmol/L (our report) vs. Caucasian: 10.62 μmol/L [6] and 8.72 μmol/L [7]). We speculate that these differences are more likely due to intrinsic differences between the two populations such as diet and ethnic origin among others. Different bilirubin levels lead to different antioxidant capacity. Higher bilirubin levels in the Chinese play a stronger role in antilipid peroxidation in preventing PD development, while lower bilirubin levels in Caucasians are not enough to prevent the occurrence of PD but just increase compensatively as a metabolite of HO after the occurrence of PD. In addition, our study found that the lower IBIL levels in PD were mainly driven by the PIGD patients. Therefore, apart from the ethnic factor, the difference in the proportion of PIGD patients included in the studies may also explain the discrepant findings with previous studies.

A Negative Relationship between IBIL Concentrations and LEDD in PD Patients

LEDD is an indirect estimate of dopaminergic deficit in PD. Our study found lower IBIL concentrations in patients requiring levodopa than in those not requiring levodopa and a significant inverse association between IBIL concentrations and LEDD, indicating that decreased levels of IBIL may relate to a more malignant PD type.

Lower IBIL Concentrations in PIGD than TD Patients

Our study found that IBIL concentrations were significantly lower for PIGD than for TD and were positively correlated with TD score, which is the first to evaluate IBIL concentrations in patients with different PD motor subtypes.

Our findings raise the question regarding which biochemical and pathological mechanisms may explain the relationship between bilirubin and motor subtypes of PD. There are different physiological mechanisms underlying tremor and bradykinesia, which have been suggested by several imaging studies and postmortem analysis revealing a tight association of nigrostriatal dopaminergic deficit with bradykinesia but not with tremor [12–14]. It is speculated that higher levels of bilirubin reduce the oxidative stress damage to dopaminergic neurons so as to help PD patients against the PIGD subtypes with worse prognosis. Our results suggest that the motor phenotype may be associated with IBIL levels.

Limitations

Finally, some limitations need to be addressed. First, the sample size of the PD group is small. Second, reduced serum bilirubin concentrations are not specific to PD, as they have been linked to a variety of diseases, including metabolic syndrome, diabetes, cardiovascular disease, and cancer [15–17] and can be confounded by other factors including BMI, cholesterol, current smoker, alcohol drinker, regular exercise, diabetes, hypertension, and so on, which were not discussed in our study [18]. Third, it was speculated in our study that lower IBIL levels reflected reduced antioxidant capacity in PD, especially in PIGD patients. Further research studies evaluating whether PIGD patients have higher levels of ROS compared to TD patients and controls are needed to provide more evidence of this speculation. Furthermore, comparisons of bilirubin levels between Chinese and Caucasians are only based on differences in the mean bilirubin levels reported in the paper. Whether the differences are statistically significant and whether gene polymorphisms modifying bilirubin metabolism lead to differences in bilirubin levels under different genetic backgrounds will be confirmed by larger samples, cross-regional, genomic research studies.

Conclusions

Our study found decreased IBIL levels in PD patients. Although there is still some uncertainty as there are several other potential confounders of bilirubin measures, it is possible that persons with decreased IBIL concentrations lack an endogenous defense system to prevent oxidative stress from damaging and destroying dopaminergic cells in the substantia nigra. Moreover, it was found that IBIL level was related to the motor subtypes, providing additional evidence of the reliability of bilirubin as a potential protective substance against the PIGD subtypes with worse prognosis.

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Statement of Ethics

The study protocol was approved by the ethics committee of Beijing Tsinghua Changugung Hospital (IRB No. 18186-0-01), and all patients signed informed consent to participate in the study.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Conceptualization: Jian Wu. Data curation: Jun Li and Lei Zhao. Formal analysis: Jun Li and Jian Wu. Investigation: Jun Li, Lei Zhao, Zhong Wang, and Xiuying Zhao. Methodology: Jian Wu and Jun Li. Resources: Jun Li, Lei Zhao, Zhong Wang, Xiuying Zhao, and Jian Wu. Writing – review and editing: Jun Li and Jian Wu.

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