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ORIGINAL ARTICLE

MiR-19b-3p Serves as a Potential Diagnostic Biomarker for Parkinson's Disease

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SUMMARY

Background: **Hundreds of miRNAs have been reported to be dysregulated in Parkinson's disease (PD), providing valuable assistance in improving its diagnosis. The purpose of this study was to analyze the expression and diagnostic value of miR-19b-3p in PD patients, as well as its relationship with inflammatory factors.**
Methods: **We recruited 50 PD patients and 50 healthy age- and gender-matched controls and collected demographic data and biochemical parameters from both groups. RT-qPCR was used to detect miR-19b-3p levels in serum. ELISA was used to detect serum levels of TNF-α and IL-1β. Receiver operating characteristic (ROC) curves were constructed to evaluate the diagnostic value of miR-19b-3p in PD patients.**
Results: **Serum miR-19b-3p expression was downregulated in PD patients compared with healthy controls. A negative correlation was found between miR-19b-3p levels and the MDS-UPDRS score in PD patients ($r = -0.686$, $p < 0.01$). In ROC curve analysis, the area under the curve of miR-19b-3p for prediction of PD was 0.779. In PD patients, serum miR-19b-3p levels were negatively correlated with serum IL-1β ($r = -0.556$, $p < 0.01$) and TNF-α levels ($r = -0.592$, $p < 0.01$).**
Conclusions: **Serum miR-19b-3p might serve as a diagnostic and predictive biomarker for PD. The association detected between miR-19b-3p and two common markers of inflammation (IL-1β and TNF-α) may suggest a role for miR-19b-3p in PD-associated neuroinflammation.**
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KEYWORDS

MiR-19b-3p, Parkinson's disease, inflammatory factors, biomarker

INTRODUCTION

Parkinson's disease (PD) is a heterogeneous neurological disorder with both motor and non-motor characteristics, affecting approximately seven million people in 2020 [1]. Alarmingly, the number of PD patients is expected to double by 2030, mainly due to an aging population [2,3]. PD first presents with non-motor symptoms and gradually develops into motor symptoms, seriously affecting the patient's quality of life [4]. However, the treatment of PD is still symptomatic, and currently no compound with potential neuroprotective properties *in*

vitro or *in vivo* has shown any impact on the progression of PD in clinical trials [5]. Therefore, early and accurate diagnosis of PD is particularly important. Currently, most studies based on PD biomarkers use patient cerebrospinal fluid (CSF) samples to assess the correlation with PD progression and severity [6]. However, the global application of these CSF-based biomarkers is still limited, mainly due to the invasive nature of CSF collection. Thus, identifying biomarkers that can be measured in the blood will allow for an easier, less painful, and less dangerous diagnosis. In this regard, several non-coding RNAs (including microRNAs, lncRNAs, and circRNAs) have been shown to be differentially regulated in PD and can be measured in the patient's blood to develop new diagnostic strategies [7,8]. MiR-19b-3p belongs to the miR-17/92 cluster, which is generally upregulated in human B-cell lymphoma. This miRNA cluster has been found to have important biological significance in cancer and other diseases [9]. In addition, miR-19b-3p has been reported to be involved in regulating different cellular inflammatory responses in several human diseases, such as Crohn's disease and rheumatoid arthritis [10]. Sequencing results from CSF and CSF exosomes in PD patients showed a decrease in miR-19b-3p expression, revealing a possible connection between PD and miR-19b-3p [11,12]. In this study, we further validated the expression of miR-19b-3p in the peripheral blood of PD patients and determined its diagnostic value. Hoping to provide further evidence for the role and diagnostic value of miR-19b-3p in the pathogenesis of PD, we also explored the potential association between miR-19b-3p expression and levels of inflammatory factors in the serum of PD patients.

MATERIALS AND METHODS

Participants

A total of 50 PD patients were enrolled from the Affiliated People's Hospital of Jiangsu University between 2021 and 2023, and 50 age- and gender-matched healthy volunteers were included as a control group. The diagnosis of PD was performed according to the 2015 MDS PD diagnostic criteria. All patients were evaluated for the severity of their condition using the MDS score. The exclusion criteria for patients included: atypical or secondary parkinsonism disorders, immunodeficiency disorder, mental illness, malignant tumors, and inability to cooperate.

This study was approved by the institutional Ethics Board Committee of the Affiliated People's Hospital of Jiangsu University and the People's Hospital of Jurong City and was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All study participants provided written informed consent.

Serum collection

To prevent the impact of levodopa on blood samples, all subjects discontinued levodopa 48 hours before sampling. Blood samples were collected from participants and immediately centrifuged to separate the serum. The serum samples were stored at -80°C for further use. In addition, demographic and general hematological indicators of the patients were recorded for subsequent analysis.

RNA extraction and RT-qPCR

Total miRNA was extracted by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. Reverse transcription was performed using a reverse transcription kit (Takara, Tokyo, Japan). Expression levels of miR-19b-3p were measured by quantitative RT-PCR on an ABI 7500 real-time PCR instrument, using a fluorescent-based quantitative PCR kit (Takara, Tokyo, Japan). U6 was used as an internal standard. Relative gene expression of miR-19b-3p was analyzed by using the $2^{-\Delta\Delta ct}$ method. The primer sequence for miR-19b-3p (Sangon Biotech, Shanghai, China) was: Forward (5′ to 3′): TGTGCAAA TCCATGCAAAACTGA.

ELISA determination of inflammatory factors

To evaluate the inflammation level of PD patients, serum levels of IL-1-β and TNF-α were measured according to the instructions of ELISA kit (AiFang bilolgical, Hunan, China).

Statistical analysis

All data were statistically analyzed and plotted using SPSS 25.0 software (IBMCorp., Armonk, NY, USA) and GraphPad Prism 5.01 software (GraphPad Software Inc.). Based on whether the data conforms to a normal distribution, mean ± SD and the median and interquartile range (IQR) were used to record the baseline characteristics and miR-19b-3p levels. Chi-squared tests, Mann-Whitney tests, or independent sample *t*-tests were used to compare baseline characteristics and miR-19b-3p levels between PD patients and the control group. Spearman's rank correlation testing was applied to determine the correlation between miR-19b-3p, MDS-UPDRS, and inflammatory factors. Receiver operating characteristic (ROC) curve and area under the ROC curve (AUC) analysis was used to evaluate the predictive ability of miR-19b-3p for PD.

RESULTS

Demographic characteristics of study subjects

The demographic characteristics, clinical information, and routine laboratory test results of the participants, including age, gender, BMI, as well as blood glucose, lipid, and uric acid (UA) levels, are shown in Table 1. There was no significant difference in age and gender between the control group and PD patients. In contrast, significant differences were noted among these groups in the levels of low-density lipoprotein (LDL), total cholesterol (TC), UA, homocysteine (HCY), and miR-

Table 1. Comparison of basic information between the two groups.

	PD (n = 50)	Control (n = 50)	t/χ^2	p
Age (years)	67.36 ± 7.03	66.74 ± 8.96	0.385	0.701
Men (%)	28 (56)	23 (46)	1	0.317
BMI (kg/m^2)	23.01 ± 2.31	22.63 ± 2.68	0.756	0.452
FPG (mmol/L)	6.36 ± 2.61	6.65 ± 1.79	-0.649	0.518
TG (mmol/L)	1.59 ± 1.05	1.47 ± 0.74	0.652	0.516
TC (mmol/L)	3.65 ± 1.02	4.08 ± 1.07	-2.073	0.041
LDL (mmol/L)	2.35 ± 0.79	2.80 ± 0.87	-2.685	0.009
HDL (mmol/L)	1.38 ± 0.55	1.23 ± 0.36	1.592	0.115
UA (μmol/L)	289.24 ± 100.71	337.54 ± 109.97	-2.290	0.024
HCY (μmol/L)	9.57 ± 3.15	8.29 ± 2.91	2.103	0.038

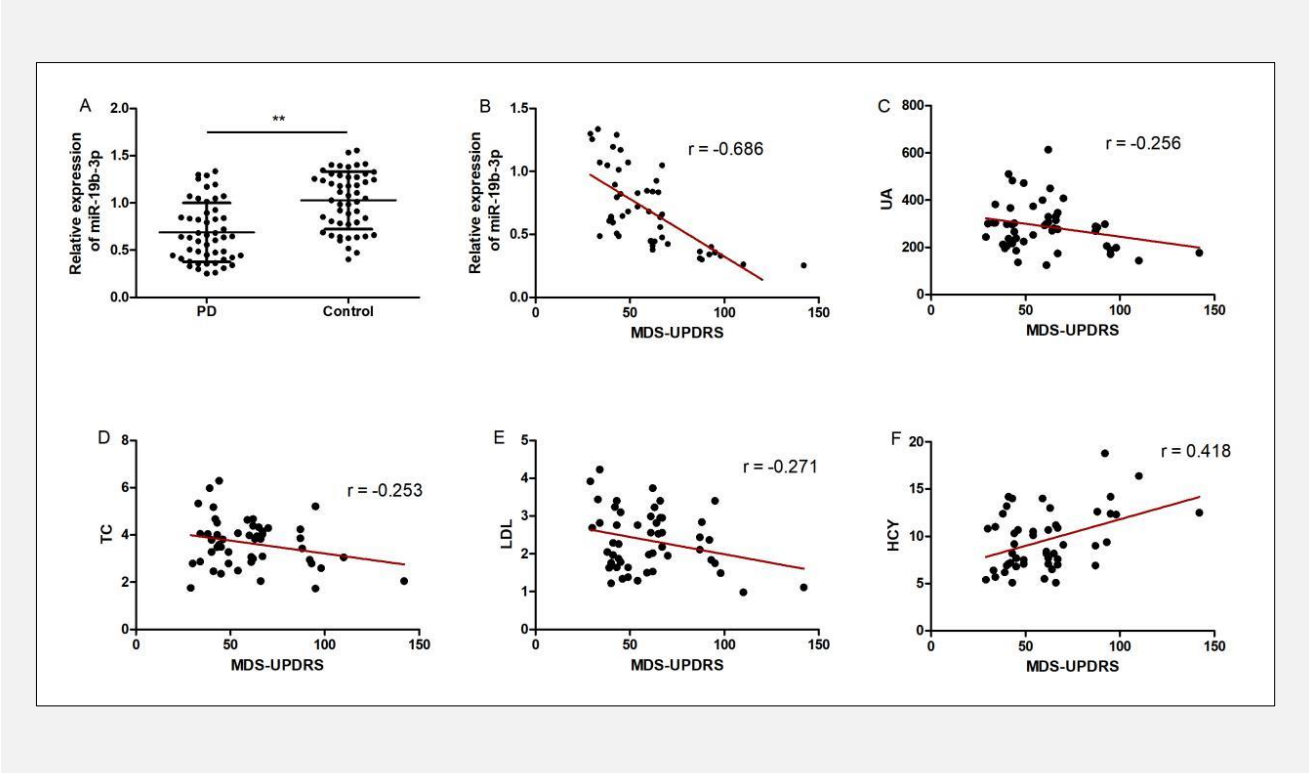

Figure 1. Expression of miR-19b-3p in serum samples of PD patients (A). Correlation between miR-19b-3p, UA, TC, LDL, and HCY and MDS-UPDRS scores (B - F).

19b-3p (all $p < 0.05$). The disease duration and H&Y staging of PD patients were (4.09 ± 2.35) years and 2.59 ± 1.02, respectively.

Expression of serum miR-19b-3p in patients with PD

Serum levels of miR-19b-3p in PD patients and healthy controls were estimated by RT-qPCR (Figure 1). The results showed that the levels of miR-19b-3p were significantly downregulated in PD patients, compared with the control group (0.69 ± 0.31 vs. 1.03 ± 0.30, $p < 0.001$).

Correlation analysis between two sets of differential indicators and PD severity

A correlation analysis was conducted between two sets of baseline data with significant differences recorded

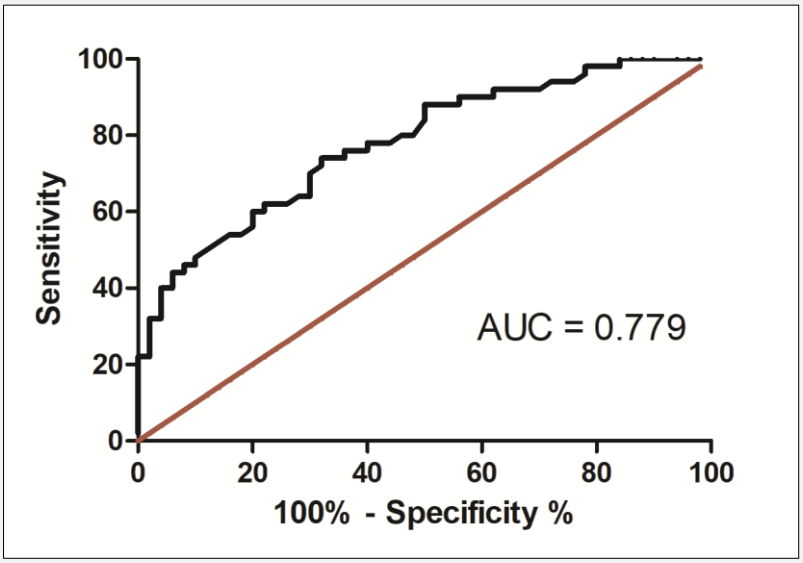

Figure 2. Diagnostic value of serum miR-19b-3p levels in patients with PD.

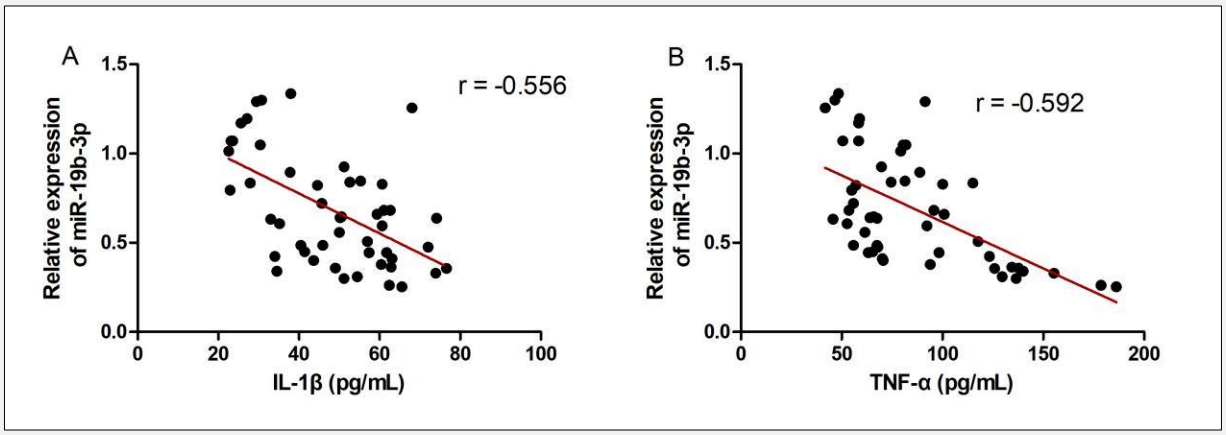

Figure 3. Relationship between serum miR-19b-3p levels and inflammatory factors.

for several indicators (LDL, TC, UA, HCY, and miR-19b-3p) and the MDS-UPDRS scores of PD patients (Figure 1). The results showed that the correlation between HCY, miR-19b-3p, and MDS-UPDRS scores was significant. Specifically, the correlation between miR-19b-3p and MDS-UPDRS was the highest (r = -0.686), while the correlation between HCY and MDS-UPDRS was average (r = 0.418).

ROC analysis of miR-19b-3p-based diagnosis of PD

To evaluate the potential diagnostic value of miR-19b-3p in PD, we plotted a ROC curve (Figure 2), which

showed an area under the curve of 0.779, sensitivity of 74%, and specificity of 68%.

Correlation analysis between miR-19b-3p and inflammatory factors

We used Spearman's rank correlation analysis to investigate the relationship between levels of miR-19b-3p and inflammatory factors (IL-1β and TNF-α) in the serum of PD patients (Figure 3). The results showed a negative correlation between miR-19b-3p and IL-1β ($r = -0.556$, $p < 0.01$) and between miR-19b-3p and TNF-α levels ($r = -0.592$, $p < 0.01$).

DISCUSSION

This study, which included 50 PD patients and 50 normal controls, aimed at investigating the potential association between serum miR-19b-3p expression and PD diagnosis and severity. Whereas no significant intergroup differences were noted regarding age, gender, BMI, FBG, TG, and HDL, serum UA and miR-19b-3p levels were lower, while serum HCY, TC, and LDL levels were higher, in PD patients compared to normal controls. In the correlation analysis between serum UA, HCY, TC, LDL, miR-19b-3p, and MDS-UPDRS in PD patients, miR-19b-3p had the highest correlation, while HCY had a moderate correlation, with MDS-UPDRS. In addition, ROC analysis showed that miR-19b-3p has certain clinical value in diagnosing PD.

UA is the end product of purine metabolism and exhibits neuroprotective effects through its antioxidant properties [13]. Consistent with our findings, precious research has shown that the levels of UA in peripheral blood, CSF, and brain tissue of PD patients are significantly lower than those of same-age control groups [14, 15]. However, due to the influence of various factors, UA cannot currently be used as a biomarker for PD. HCY is an excitatory amino acid that can easily cause neurotoxicity when present in excess. Indeed, elevated HCY levels are considered to contribute to the pathogenesis of various neurodegenerative diseases, including PD, by inducing oxidative stress, endoplasmic reticulum stress, and neuroinflammation [16]. A meta-analysis showed that HCY is a risk factor for cognitive impairment in patients with PD and PD comorbidities [17]. Our study also found that HCY levels in PD patients were significantly higher than those in the control group. While there are few studies reporting HCY as a diagnostic biomarker for PD, one study identified HCY as a potential predictive factor for advanced PD dementia and vascular PD dementia [18].

With the development of second-generation sequencing, hundreds of miRNAs that may be involved in the pathogenesis of PD have been discovered in recent years. The abnormal expression of some specific miRNAs has been thoroughly studied, which may provide new ideas for the diagnosis and treatment of PD [19]. Goh et al. [20] identified 15 dysregulated miRNAs in the CSF and blood of PD patients from 34 literature reports. Among those, five miRNAs genes, i.e. miR-30, miR-29, let-7, miR-485, and miR-26, were most closely related to the onset of PD.

Compared with healthy control samples, in the postmortem brain of PD patients, the expression of miR-30c-2 in the substantia nigra is upregulated and correlates positively with miR-30c-2 expression in white blood cells. This suggests the potential of detecting miR-30c-2 levels in peripheral blood white blood cells as a diagnostic biomarker for PD [21]. MiR-124 was reported to confer neuroprotective effects. In a PD mouse model, Esteves et al. [22] used extracellular vesicles derived from umbilical cord blood mononuclear cells as biological carriers to deliver miR-124-3p. Their results showed that this approach protected the integrity of dopaminergic neurons in the substantia nigra and striatal fibers, revealing the promising prospects for miR-124-3p in PD treatment.

MiR-19b-3p was first reported in tumors, with a correlation observed between high miR-19b-3p expression, tumor cell proliferation, and apoptosis inhibition [23, 24]. Ibáñez et al. [25] conducted a transcriptomics meta-analysis of three common central nervous system diseases, namely Alzheimer's disease, PD, and schizophrenia and three equally common cancers (lung cancer, prostate cancer, and colorectal cancer). Their study showed that two overlapping genes were upregulated in central nervous system diseases and downregulated in cancer, providing support for the inverse comorbidity hypothesis.

According to such hypothesis, it is speculated that miR-19b-3p may be downregulated in PD. Such downregulation has been confirmed in AD [26] and verified in at least five sequencing reports related to PD [11,12,27-29]. Consistent with those reports, our study found that compared with the control group, the expression level of miR-19b-3p in the peripheral blood of PD patients was significantly downregulated. However, discrepant results emerged from a study conducted in Turkish PD patients, which found that the expression of miR-19b-3p in their serum was not different from that of healthy controls [30]. While we believe that this may be related to differences in genetic backgrounds and experimental methods, it must be noted that taking levodopa can also affect the expression levels of miRNAs in peripheral blood.

We also evaluated the diagnostic potential of miR-19b-3p. ROC analysis results showed that the AUC of serum miR-19b-3p in distinguishing PD patients from healthy individuals was -0.686, indicating high accuracy. Therefore, we believe that miR-19b-3p may serve as a candidate biomarker for the diagnosis of PD.

Various miRNAs have been linked to the pathogenesis of PD, by influencing neuroinflammation, apoptosis, synaptic function, mitochondrial function, and immune regulation [20]. In LPS-treated cells (simulating sepsis), the expression of miR-19b-3p was decreased, while its overexpression reduced the levels of inflammatory fac-

tors (TNF-α, IL-6) [31]. Therefore, we hypothesized that miR-19b-3p may affect the pathological process of PD by regulating neuroinflammation. We preliminarily evaluated the expression level of miR-19b-3p in the serum of PD patients and its correlation with TNF-α and IL-1β. The results showed that the expression level of miR-19b-3p was negatively correlated with TNF-α and IL-1β levels. However, we are not yet clear about the signaling pathways through which miR-19b-3p regulates inflammatory responses.

Some limitations exist in this study. First, this is a cross-sectional study and cannot evaluate the changes in serum miR-19b-3p levels during the progression of PD. Second, the sample size is small, and since the selection of research subjects has regional limitations, it does not represent the overall population. Third, although we have established an association for miR-19b-3p and PD, the specific mechanism of action is still unclear.

In summary, our study found that the expression level of miR-19b-3p in the serum of PD patients is reduced, correlates with the severity of the disease, and may serve as a potential biomarker for diagnosis. Although additional evidence is clearly needed, we propose that miR-19b-3p might participate in the occurrence and development of PD by regulating the inflammatory response.

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Declaration of Interest:

No potential conflicts of interest were disclosed.

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