Original Article

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Plasma extracellular vesicle pathognomonic proteins as the biomarkers of the progression of Parkinson's disease

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SUMMARY: Parkinson's disease (PD) is a progressive neurodegenerative disorder for which reliable blood biomarkers to predict disease progression remain elusive. Plasma extracellular vesicles (EVs) have gained attention as a promising biomarker platform due to their stability and ability to cross the blood-brain barrier. This study explored the potential of EV-cargo proteins, specifically α-synuclein, tau, and β-amyloid, as biomarkers of PD progression. A cohort of 55 people with PD (PwP) and 58 healthy controls (HCs) underwent annual assessments of plasma EV proteins, cognition, and motor symptoms. EVs were isolated and validated using standardized methods, with pathognomonic proteins quantified *via* immunomagnetic reduction assays. Associations between biomarker changes and clinical symptom progression were analyzed. Over an average of 3.96 visits for PwP and 2.25 visits for HCs, PwP exhibited a distinct pattern of plasma EV protein changes linked to motor symptom progression, particularly in the Unified PD Rating Scale (UPDRS) part II score. Notably, changes in plasma EV α-synuclein levels were significantly correlated with changes in motor and cognitive symptoms, suggesting its central role in disease progression. These findings highlight the potential of plasma EV biomarkers, especially α-synuclein, as indicators of ongoing pathogenesis and as candidates for evaluating α-synuclein-targeted therapies in PD.

Keywords: sodium glucose transporter 2 inhibitors, type-2 diabetes mellitus, cype-2 diabetes mellitus, canagliflozin, sodium glucose transporter 2 inhibitors

1. Introduction

Parkinson's disease (PD) is a challenging neurodegenerative disease regarding the diagnosis and prognosis prediction (*1*). The progression characteristics of PD results in severe disability in the people at advanced stage of disease. However, the speed of deterioration varies. The urgent need for reliable biomarkers for PD is underscored by the potential of early predicting the disease prognosis and the development of disease modification therapies (*2,3*). However, the quest for definitive blood biomarkers has been fraught with inconsistencies (*4*) and the results were mixed (*5,6*). The instability of free-form proteins and nucleic acids in the blood are vulnerable to spontaneous degradation in the blood or storage, which can significantly alter their concentration and detectability (*7-9*). The blood-brain barrier (BBB) also poses a significant obstacle, and the selective permeability of BBB limited the accurate reflection of brain pathology in the bloodstream for neurological disease (*10*). Conversely, cerebrospinal fluid (CSF) is an optimal source of providing more accurate biomarkers, but the invasive procedure to obtain CSF limited its widespread application (*11,12*).

Recent advances in the development of blood extracellular vesicle (EV) biomarkers offer a promising avenue for improving the diagnosis and monitoring the progression of PD (*11-14*). EVs are small, membrane-bound, cell-derived carrying with proteins, lipids, and nucleic acids (*15*). One of the key advantages of EV-contained protein biomarkers is their enhanced stability compared to free-form proteins and nucleic acids *(16-18*). Moreover, EVs possess the remarkable ability to cross the BBB (*19*). This capability enhances the potential of EVs to serve as reliable indicators of neurological conditions like PD.

α-Synuclein, tau, and β-amyloid (Aβ) play critical pathological roles in the development and progression of PD, making them key targets for biomarker research (*20-22*). α-Synuclein is the main component of Lewy bodies, a pathological hallmark of PD. α-Synuclein aggregates disrupt cellular function, leading to neuronal death and

the characteristic motor symptoms of PD (*23*). The progression of Lewy bodies from the brainstem to the cortex, as described by Braak staging (*24*), underscores the association between the progression of α-synuclein pathology and clinical deterioration. Tau is the main component of neurofibrillary tangles, which disrupt the normal functioning of neurons, contributing to neurodegeneration and cognitive decline, one of the main progression indicators in PD (*25*). Aβ forms extracellular plaques that contribute to neuroinflammation and oxidative stress, exacerbating neuronal damage. The presence of Aβ plaques in PwP suggests a complex interplay between different proteinopathies in the disease's pathology (*26,27*). The associations between these pathognomonic proteins and the progression of clinical manifestations of PD position them as promising candidates for prognostic biomarkers, targets for disease-modifying treatments, and parameters for neuroprotective clinical trials.

Previous studies have explored these proteins as blood biomarkers for PD, but results have been inconsistent (*28*). Recent research has shifted focus to blood EVs as carriers of these pathognomonic proteins. Encapsulated within EVs, α-synuclein, tau, and Aβ are protected from degradation, making them more stable and detectable in blood samples. Elevated levels of EV-associated α-synuclein have been observed in PwP, offering a more reliable biomarker due to the vesicles' ability to cross the blood-brain barrier and reflect central nervous system pathology (*29*). Similarly, EV-associated tau and Aβ levels have shown potential as biomarkers, correlating with disease severity and progression (*13,14*). These findings underscore the promise of blood EV pathognomonic proteins as accurate and non-invasive biomarkers for diagnosing and monitoring PD, representing a significant advancement in neurodegenerative disease research. This study hypothesizes that in a longitudinal PD cohort, blood EV pathognomonic proteins — namely α-synuclein, tau, and Aβ — could serve as biomarkers of PD progression, particularly in relation to motor and cognitive aspects.

2. Materials and Methods

2.1. Study population

The first PD cohort was established in November 2016, led by Dr. C.T. Hong (approval of Joint Institutional Review Board of Taipei Medical University with approval no. N201609017), and the second PD cohort, led by Dr. C.C. Chung (approval of Joint Institutional Review Board of Taipei Medical University with approval no. N201801043) at the same institute, was established in May 2018. Subsequently, the two cohorts collaborated. Study participants were followed annually with assessments including the Unified Parkinson's Disease Rating Scale (UPDRS) (motor function in PD), Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA) (cognitive function in PD), and blood sampling for plasma EV analysis.

However, the COVID-19 pandemic significantly impacted participants' willingness to undergo follow-up assessments, as these required extended hospital visits. Consequently, we terminated the two cohorts. In 2022, after the pandemic, a third PD cohort (approval of Joint Institutional Review Board of Taipei Medical University with approval no. N202205008) was launched. Participants from the first two cohorts were invited to resume follow-up assessments as part of the third cohort. The data from all three cohorts were then integrated. The study protocol was illustrated in Figure 1.

PD was diagnosed according to the UK Parkinson's Disease Society Brain Bank Diagnostic Criteria (*30*). Participants with PD were limited to those in the early to mid-stages, defined by Hoehn and Yahr stages I~III. The HCs were free from significant neurodegenerative diseases and disabilities, and were regularly monitored in outpatient clinics for chronic conditions such as

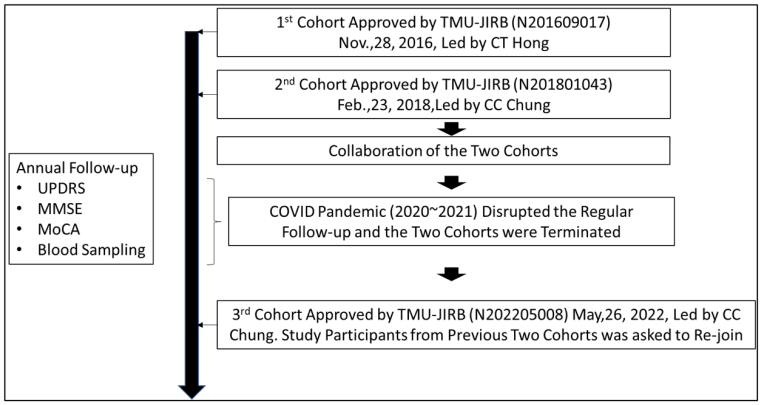

Figure 1. The diagram of the establishment of the study cohort and the assessment of the study participants during the visit. UPDRS, Unified Parkinson's Disease Rating Scale; MMSE, mini-mental status exams; MoCA, Montreal Cognitive Assessments, MoCA.

hypertension, diabetes, hyperlipidemia, headaches, or vertigo. In total, 140 PwP and 66 HCs completed their initial visit. The dropout rate and non-compliance with annual follow-ups were significant during the COVID-19 pandemic, leading to variations in the number of cases over the follow-up period.

Given that this cohort had previously examined the cross-sectional and 1-year follow-up results of plasma EV biomarkers in PD (*13,14*), this study now focuses on longer-term follow-up. As a result, the current analysis includes only PwP with three or more visits and HCs with two or three visits in the cohort.

2.2. Clinical assessment

Each participant underwent an interview to gather baseline demographic information. Trained nurses assessed the cognitive function of all participants using the Taiwanese versions of the MMSE and the MoCA. Additionally, all participants were evaluated using Parts I, II, and III of the UPDRS during an outpatient visit. The interval between the last dose of anti-PD medication and the UPDRS Part III assessment was not documented; therefore, it was assumed that PwP were in their "on" state. Tremor, akinetic rigidity (AR), and postural instability and gait disturbance (PIGD) subscores were derived from the subitems in UPDRS Part III and calculated according to modifications from the previous study (*31*).

2.3. Plasma EV isolation and validation

The details of plasma EV isolation and validation have been published previously (*14*). Venous blood samples were collected from all study participants during clinic visit, and plasma isolation was performed within 3 hours after venous blood sampling. Later on, storage plasma EV was isolated using the exoEasy Maxi kit according to the manufacturer's instructions. The isolated EVs were validated based on the presence of surface markers, such as CD63, CD9, and CD81; their morphology was determined using transmission electron microscopy; and particle size analysis was conducted through nanoparticle tracking.

2.4. Immunomagnetic reduction assay for quantifying α-synuclein, tau, and β-amyloid

The details of the immunomagnetic reduction assay for quantifying plasma EV α-synuclein, tau, and Aβ have been described previously[2]; these analyses were conducted by MagQu Co (New Taipei, Taiwan). According to their instructions, the assay limit of detection was 1.39, 26, and 77 fg/mL for α-synuclein, tau, and Aβ, respectively.

2.5. Statistical analysis

All statistical analyses were performed using IBM SPSS, version 26 (IBMin, Armonk, NY, USA). Generalized estimating equations evaluated associations between clinical symptom progression and plasma EV biomarkers. Spearman correlation assessed the relationship between biomarkers and age- and sex-adjusted clinical symptoms in PwP. The adjusted UPDRS, MMSE and MoCA scores have been standardized to Z scores to account for variations in age and gender across different visits. This standardization process involves calculating the mean and standard deviation of UPDRS, MMSE and MoCA scores within each age and gender group, and then converting individual scores to Z scores. A Z score indicates how many standard deviations an individual's score is from the mean of their age and gender group. Positive Z scores signify scores above the group mean, while negative Z scores indicate scores below the group mean. P value < 0.05 was considered statistically significant.

3. Results

3.1. Demographic data

In total, the clinical and plasma EV biomarkers data from 55 PwP who had three or more visits and 58 HCs with two or three visits were analyzed. There was no significant difference of age, gender distribution and baseline cognition (MMSE and MoCA) between two groups (Table 1). Regarding the progression of disease, for PwP, the UPDRS III remained stable between 1st to 3rd visits, and substantially deteriorated at 4th and 5th follow-up. In terms of cognition, there was no significant change of MMSE for PwP during the follow-up. At baseline, there was no significant difference of plasma EV α-synuclein, tau, and Aβ between PwP and HCs, despite a trend of lower of these plasma EV pathognomonic proteins in PwP. During follow-up, the change of plasma EV α-synuclein, tau, and Aβ was significantly different between PwP and HCs with the adjustment of age and sex. In general, an increase tendency was noted in PwP whereas the levels of plasma EV α-synuclein, tau, and Aβ were variable without substantial difference in HCs during the follow-up period of time (Table 1).

3.2. Association between the change of plasma EV α-synuclein, tau, and Aβ with changes in clinical motor and cognition symptoms during the whole couse of follow-up

Considering only PwP, Figure 2 demonstrated the dynamic change of the clinical parameters and plasma EV pathognomonic proteins during the whole course of follow-up. In general, the increase trend was observed in all plasma EV pathognomonic proteins, in parallel with the change of UPDRS part II and III. The changes of cognitive parameters (MMSE and MoCA) were not

Table 1. Baseline demographic data of study participants (with completed baseline and follow-up)

	HCs, $n = 58$					PwP, $n = 55$					p-value
Age (y/o)	66.05 ± 7.06					68.11 ± 6.67					0.11*
Female	22					25					0.45*
	Visit					Visit					
	1	2	3	4	5	1	2	3	4	5	
Clinical Parameters											
MMSE	27.09 ± 3.55	27.16 ± 3.72	-	-	-	26.55 ± 3.21	26.50 ± 4.16	26.23 ± 4.23	28.37 ± 1.42	27.82 ± 1.82	0.40*
MoCA	23.21 ± 4.64	24.21 ± 5.28	-	-	-	22.25 ± 4.76	22.92 ± 5.15	22.33 ± 5.48	25.53 ± 3.53	25.18 ± 3.86	0.95*
UPDRS-II	-	-	-	-	-	7.13 ± 4.62	10.04 ± 5.36	11.46 ± 6.61	8.26 ± 5.37	10.77 ± 5.18	
UPDRS-III	-	-	-	-	-	21.67 ± 8.86	20.08 ± 7.84	20.13 ± 9.08	27.58 ± 7.93	29.32 ± 8.42	
Plasma EV Proteins											
α-synuclein (fg/mL)	80.9 ± 31.6	68.2 ± 27.6	75.4 ± 30.2	-	-	56.0 ± 35.6	73.0 ± 27.6	69.8 ± 26.0	76.0 ± 12.8	82.2 ± 16.6	0.012[$]
Tau (pg/mL)	4.65 ± 1.99	5.16 ± 2.32	5.75 ± 2.21	-	-	3.55 ± 2.24	5.78 ± 2.41	5.17 ± 2.25	6.02 ± 3.55	5.23 ± 3.21	0.017[$]
Aβ (pg/mL)	1.37 ± 0.50	1.41 ± 0.36	1.52 ± 0.22	-	-	1.13 ± 0.46	1.49 ± 0.33	1.25 ± 0.37	1.33 ± 0.58	1.53 ± 0.51	0.001[$]

Aβ, β-amyloid; EV, extracellular vesicle; HC, healthy control; PwP, people with Parkinson's disease; MMSE, Mini-Mental State Examination; MoCA, Montreal cognitive assessment; UPDRS, unified Parkinson's disease rating scale. Data was presented as mean ± standard deviation. *, p value was obtained from the comparison at visit 1. [$], p-value was obtained from the generalized estimated equation to compare the trend of change with the adjustment of age and sex.

substantial. The regression model with the adjustment of age and sex further delineated the association between the plasma EV pathognomonic proteins with clinical parameters (Table 2). IT was found that plasma EV α-synuclein and Aβ levels from baseline to the end of the follow-up were significantly associated with changes in UPDRS II scores during the same period, reflecting alterations in daily functional activity over the follow-up period. This association suggests that increases in plasma EV α-synuclein and Aβ could serve as indicators of worsening daily functional activity in PwP. However, no association was found between changes in plasma EV α-synuclein, tau, or Aβ levels from baseline to the end of the follow-up with changes in UPDRS III, MMSE, or MoCA scores during the same period (Table 2).

3.3. Correlation of changes in plasma EV α-synuclein, tau, and Aβ with changes in clinical motor and cognition severity at each follow-up time-point

To thoroughly delineate the association between changes in plasma EV α-synuclein, tau, and Aβ and clinical progression, correlation analysis was performed at each follow-up time point. The severity of motor and cognitive symptoms was adjusted for age and sex, then normalized and presented as changes in Z scores. A significant association was found between changes in plasma EV α-synuclein and changes in age- and sex-adjusted UPDRS II and MMSE scores at each follow-up time point (Table 3 and Figure 3). These associations suggest that plasma EV α-synuclein could serve as a real-time indicator of changes in the severity of motor and cognitive function in PwP.

4. Discussion

The present study demonstrated that PwP exhibited a distinct pattern of changes in plasma EV proteins compared to HCs, which were significantly associated with alterations in PD-related daily functioning. Furthermore, changes in plasma EV α-synuclein levels showed a significant correlation with changes in UPDRS-II scores and cognitive function. These findings suggest that blood EV pathognomonic proteins may reflect the progression of brain pathology in PD, with changes in plasma EV α-synuclein levels serving as an indicator of clinical disease progression.

In the pathogenesis of PD, α-synuclein, tau, and Aβ play crucial roles. Misfolded α-synuclein aggregates disrupt neuronal function, leading to neurodegeneration (*23*). Hyperphosphorylated tau forms neurofibrillary tangles that impair axonal transport and synaptic function (*32*). Aβ is also implicated in PD by contributing to neuroinflammation and oxidative stress through amyloid plaque formation (*26,33*). Research has increasingly focused on the potential of these proteins as biomarkers for PD (*34*). Decreased levels of CSF α-synuclein

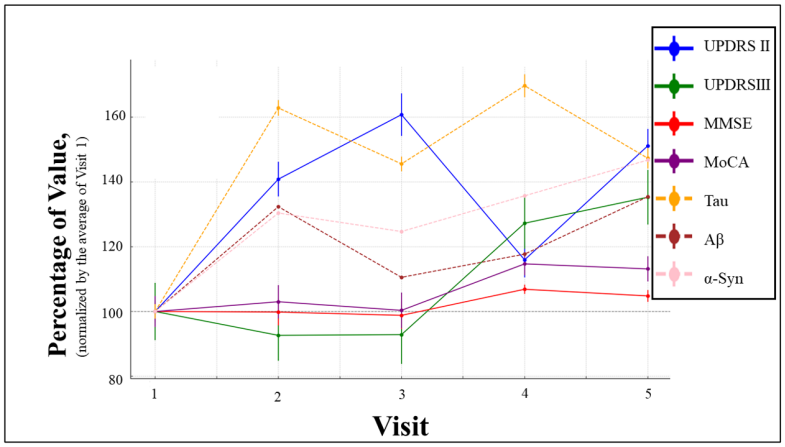

Figure 2. The dynamic change of motor symptoms (assessed by total score of Unified Parkinson's Disease Rating Scale, UPDRS part II and III), cognition (assessed by mini-mental status exams, MMSE and Montreal Cognitive Assessments, MoCA) and plasma extracellular vesicle pathognomonic proteins (Tau, β-amyloid [Aβ] and α-synuclein [α-Syn] in people with Parkinson's disease during 1st to 5th visits. Data of each parameter was normalized by the average of 1st visit and presented as percentage with standard deviation.

Table 2. Regression models for the association between the changes in plasma extracellular vesicle (EV) pathognomonic proteins with the changes in clinical assessments in people with Parkinson's disease during the whole course of follow-up after adjustment of age and sex. Data are presented as standardized beta value (*p* value)

	Changes in Plasma EV		
	Tau	Aβ	α-synuclein
Changes in			
UPDRS-II	0.23 (0.854)	2.05** (0.008)	0.04** (0.001)
UPDRS-III	-0.09 (0.686)	0.33 (0.727)	0.02 (0.287)
MMSE	0.01 (0.825)	-0.50 (0.218)	−0.02 (0.954)
MoCA	0.004 (0.954)	0.05 (0.916)	-0.01 (0.553)

MMSE, Mini-Mental State Examination; MoCA, Montreal cognitive assessment; UPDRS, Unified Parkinson's Disease Rating Scale. *, $p < 0.05$; **, $p < 0.01$.

Table 3. Association between the changes in plasma extracellular vesicle (EV) pathognomonic proteins with the changes in age, sex-normalized clinical parameters in people with Parkinson's disease at each point of visit. Data are presented as correlation coefficient (ρ) and *p* value

	Change of age & sex-normalized clinical parameters						
Change of Plasma EV Protein	UPDRSII	UPDRSIII	Tremor	AR	PIGD	MMSE	MoCA
α-synuclein							
ρ	0.211*	0.041	0.086	0.001	0.059	-0.275**	-0.079
p-value	0.026	0.673	0.372	0.994	0.537	0.004	0.411
Tau							
ρ	0.106	-0.053	-0.053	-0.018	-0.112	0.000	0.043
p-value	0.269	0.584	0.580	0.853	0.242	0.997	0.657
Aβ							
ρ	0.152	0.012	0.069	-0.003	-0.043	-0.067	0.025
p-value	0.112	0.899	0.473	0.978	0.657	0.484	0.795

Aβ, β-amyloid; AR, akinetic rigidity; MMSE, Mini-Mental State Examination; MoCA, Montreal cognitive assessment; PIGD, postural instability and gait disturbance; UPDRS, Unified Parkinson's Disease Rating Scale. *, $p < 0.05$; **, $p < 0.01$.

and Aβ have been noted in PD, while higher levels of phosphorylated tau in CSF have been associated with cognitive impairment in PwP (*35*). Our group has previously published findings highlighting the association between elevated levels of tau and Aβ in plasma EVs and cognitive decline in PwP (*14*). Additionally, we observed that PwP exhibit a distinct pattern of increased levels of α-synuclein, tau, and Aβ within blood EVs compared to controls in a short-term follow-up period of time (*13*). These findings support the potential of EV-associated proteins as biomarkers for PD progression, emphasizing the need for further research to validate their clinical utility and understand the underlying mechanisms. In the present study, we observed no significant baseline differences in the levels of α-synuclein, tau, and Aβ between PwP and HCs. However, the trend of these

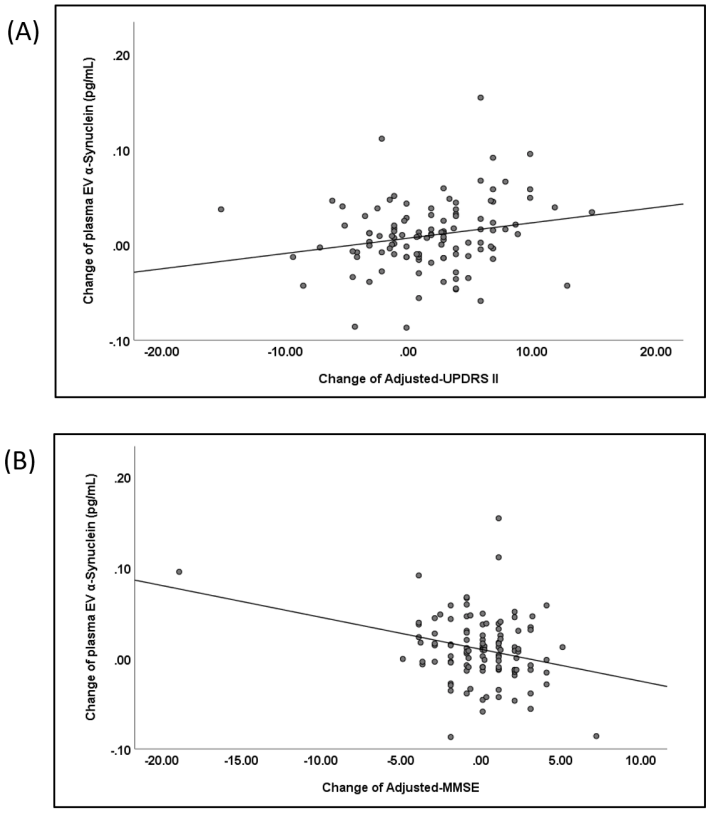

Figure 3. The association between the change of plasma extracellular vesicle (EV) α-synuclein with the change of adjusted Unified Parkinson's Disease Rating Scale, (UPDRS) part II (A), and the change of adjusted Mini-Mental Status Examination (MMSE) (B).

proteins over the follow-up period revealed significant differences. Specifically, while the levels of these proteins tended to remain stable in HCs, there was a noticeable increase in PwP over time. This trend suggests a progressive accumulation of these proteins in PwP, which aligns with the disease's neurodegenerative nature. These findings supported the hypothesis that blood EVα-synuclein, tau, and Aβ could serve as biomarkers of PD progression.

Previous studies had identified several blood biomarkers, including neurofilament light chain (*36*), uric acid (*37*) and inflammatory cytokines (*38*), as potential indicators of PD progression. Among these, the most extensively studied biomarker is α-synuclein. Elevated baseline levels of plasma ser129-phosphorylated α-synuclein have been associated with a higher risk of motor symptom progression (*39*). However, it has also been observed that plasma total α-synuclein levels increase over time, up to 20 years of follow-up, while phosphorylated α-synuclein levels remain constant during the same period (*40*). Overall, studies on blood α-synuclein as a biomarker for PD progression have yielded inconsistent results, likely due to variations in quantification methods and contamination from red blood cells. For blood exosomal α-synuclein, a small-scale study demonstrated that longitudinally increases in α-synuclein, rather than baseline levels, were associated with a higher risk of motor symptom progression in PD (*41*). However, this association was not confirmed in other study (*42*). Furthermore, the relationship between blood free α-synuclein and blood EV α-synuclein has yet to be investigated in PD. In the present study, the changes in the levels of plasma EV α-synuclein, tau, and Aβ were significantly correlated with the progression in the UPDRS part II scores, reflecting the daily functional abilities of PwP. This association underscores the potential of these proteins as biomarkers for monitoring disease progression and daily functional decline in PD. These findings are consistent with previous research indicating that increases in these protein levels, particularly within blood extracellular vesicles, are associated with PD motor progression (*41*). This study further supports the utility of monitoring these biomarkers longitudinally to better understand and predict the trajectory of PD. We also identified a significant association between changes in blood EV α-synuclein levels and age- and sex-adjusted changes in UPDRS-II and MMSE scores. Notably, it was the change in blood EV α-synuclein, rather than its absolute value, that predicted disease progression. This suggests that the dynamic change in α-synuclein levels correlates with the progression of PD pathology and its clinical manifestations. This relationship highlights the potential of blood EV α-synuclein as a sensitive biomarker for monitoring PD progression and evaluating therapeutic efficacy, particularly for α-synuclein-targeted treatments.

The correlation between the progression of α-synuclein pathology and clinical disease markers supports its use as a more nuanced biomarker compared to traditional motor symptom scores. This could significantly enhance the precision of therapeutic monitoring and the assessment of novel treatments aimed at reducing α-synuclein pathology. These insights provide a valuable framework for future research and clinical applications, emphasizing the importance of longitudinal biomarker monitoring in PD management.

The present study demonstrated that PwP exhibited a distinct pattern of changes in plasma EV proteins compared to HCs, which were significantly associated with alterations in PD-related daily functioning. Furthermore, changes in plasma EV α-synuclein levels showed a significant correlation with changes in UPDRS-II scores and cognitive function. These findings suggest that blood EV pathognomonic proteins may reflect the progression of brain pathology in PD, with changes in plasma EV α-synuclein levels serving as an indicator of clinical disease progression. This aligns with the understanding that α-synuclein pathology is central to PD, while tau and Aβ are more strongly associated with the cognitive aspects of PD (*22*). The cerebral multimorbidity hypothesis, which posits the coexistence of mixed pathologies in neurodegenerative diseases, supports the presence of tau and Aβ in PwP (*43*). However, our findings indicated that each neurodegenerative disease maintains distinct pathological patterns and preferences. This underscores the complexity of neurodegenerative diseases and the necessity for disease-specific biomarkers and treatment strategies. The lack of significant findings for tau and Aβ suggests that while these proteins contribute to the broader landscape of neurodegeneration, α-synuclein remains the primary marker for PD. This distinction is crucial for developing targeted therapies and improving patient outcomes in PD.

Despite its strengths, this study has several limitations that warrant discussion. First, the study design excluded participants who deteriorated too rapidly or severely to return for follow-up visits. This exclusion likely introduced a bias, resulting in a cohort that may represent slower disease progression. Furthermore, the COVID-19 pandemic significantly affected participant retention, particularly among healthy controls, leading to a higher dropout rate and reduced sample size. Second, the study utilized total plasma EVs as biomarkers rather than neuron-derived exosomes, which are more specific to neuronal changes associated with PD. Neuron-derived exosomes could provide a higher degree of specificity and sensitivity, and future studies should explore this approach to enhance biomarker detection. Third, assessments of motor symptoms using the UPDRS were conducted during the PwP's "on" medication state, potentially masking the full extent of motor dysfunction. This methodological limitation may have influenced the observed associations between biomarkers and clinical parameters. Fourth, while the statistical methods employed provided valuable insights, the study did not integrate artificial intelligence (AI)-based analyses. AI approaches could uncover complex, non-linear relationships between biomarkers and disease progression, improving predictive accuracy and enhancing biomarker utility. Incorporating AI in future studies could address this gap and provide more nuanced interpretations of the data. Finally, the relatively short follow-up period, despite being supplemented by data from integrated cohorts, limits the ability to fully capture the long-term dynamics of disease progression. Future studies with extended follow-up durations and more frequent assessments will be critical to validate and expand upon these findings.

5. Conclusion

In conclusion, while our study provides valuable insights into the potential of plasma EV α-synuclein as a biomarker for PD progression, which was associated with cognition and daily functional activity, these limitations highlight the need for cautious interpretation of the results and underscore the importance of designing more robust and comprehensive studies in the future.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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