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Exploratory mediation analysis of associations between serum biomarkers of neuronal injury, astrocyte reactivity, neuroinflammation, and neurotrophic support and fatigue in Parkinson's disease

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ABSTRACT

Introduction and aim. Fatigue is a debilitating non-motor symptom in Parkinson's disease (PD). This exploratory cross-sectional mediation analysis investigated whether specific serum biomarkers mediate the relationship between PD and fatigue severity.

Material and methods. Ninety PD patients and 45 matched controls were enrolled in the study. Fatigue was assessed using the Fibro-Fatigue scale (FF). Serum levels of ten biomarkers (neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP), brain-derived neurotrophic factor (BDNF), β-amyloid-42, α-synuclein, ubiquitin carboxy-terminal hydrolase L1 (UCHL1), high-mobility group box 1 (HMGB1), R-spondin 1 (RSPO1), Dickkopf-1 (DKK1), and sclerostin) were quantified by enzyme-linked immunosorbent assay. Mediation analysis (PROCESS Macro, Version 4.2, Model 4) tested whether these biomarkers mediated the relationship between PD status and FF score.

Results. The overall model predicting FF scores was highly significant ($F(26,108)=54.08$, $p<0.001$, $R^2=0.929$). Mediation analysis revealed significant indirect effects from PD status to fatigue via GFAP, HMGB1, BDNF, and NSE. Moderation analysis indicated that the biomarker fatigue relationship was significantly modified by PD status only for HMGB1 and BDNF. For GFAP and NSE, the interactions were non-significant. Bootstrapped analyses confirmed significant indirect effects of PD on fatigue through elevated levels of NSE (effect=1.743), HMGB1 (effect=1.207), GFAP (effect=1.101), and BDNF (effect=0.921).

Conclusion. PD-related fatigue is significantly mediated by neuroinflammation (HMGB1), astroglial activation (GFAP), neuronal injury (NSE), and a paradoxical BDNF response. Disease-specific moderation was confirmed only for HMGB1 and BDNF.
Keywords. fibro-fatigue, mediation study, neuronal damage biomarkers, Parkinson's disease, Wnt pathway biomarkers

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder pathologically defined by the degeneration of dopaminergic neurons in the substantia nigra and the accumulation of α-synuclein in Lewy bodies, leading to profound dopamine deficiency in the striatum.[1,2] The cardinal motor symptoms, bradykinesia, tremor, rigidity, and postural instability form the basis of the clinical diagnosis.[3,4] Non-motor symptoms are increasingly recognized for their devastating impact on quality of life.[5] Among these, chronic fatigue stands out as one of the most prevalent and disabling symptoms, affecting over 50% of patients and severely impairing daily functioning. However, its underlying pathophysiological mechanisms remain poorly understood.[6,7] A pathway critically implicated in PD pathogenesis is the canonical Wnt/β-catenin signaling cascade. This pathway is essential for the development, maintenance, and survival of midbrain dopaminergic neurons.[8,9] Its activation supports neurogenesis and synaptic plasticity and protects neurons by improving mitochondrial function and reducing oxidative stress.[10,11] Endogenous agonists and antagonists govern the precise modulation of this pathway. For instance, Dickkopf-1 (DKK1) is a potent antagonist whose upregulation is linked to neuronal damage, while R-spondin 1 (RSPO1) acts as an agonist that potentiates Wnt signaling and can attenuate DKK1-mediated inhibition.[12,13] Other relevant modulators include sclerostin, a Wnt antagonist,[14] and high-mobility group box 1 (HMGB1), a mediator of neuroinflammation with elevated levels in PD that can interact with α-synuclein to potentiate neurodegeneration.[15,16] Dysregulation of this central pathway is recognized as a contributor to neurodegeneration.[12,13,17] In parallel, a panel of circulating biomarkers provides insight into underlying neurodegenerative processes. Proteins such as α-synuclein and β-amyloid-42 are being explored for their diagnostic potential, as they reflect core pathological hallmarks.[18,19] Furthermore, biomarkers indicative of active neuronal damage and glial response have emerged. Neuron-specific enolase (NSE) and ubiquitin carboxy-terminal hydrolase L1 (UCHL1) are elevated following neuronal injury.[20] Recently, Al-Hakeim and colleagues[21] reported that serum neuronal damage markers (NSE, GFAP, S100B) correlate with affective and chronic fatigue symptoms in a cohort of 70 PD patients. However, that study did not examine a broader panel of inflammatory and astroglial markers, nor did it test for mediation or disease-specific conditional effects. Conversely, brain-derived neurotrophic factor (BDNF) supports the survival of dopaminergic neurons, although its utility as a clear biomarker is debated.[22,23] Glial fibrillary acidic protein (GFAP), released into the blood upon astrocytic activation, shows promise for monitoring disease

progression in PD.[24,25] Despite these advances, a critical gap persists. While the role of Wnt signaling in neuronal survival is well established, its potential effects on specific non-motor manifestations, particularly fatigue, remain largely unexplored. Furthermore, it is unclear whether the subjective experience of fatigue is reflected in quantifiable changes in circulating biomarkers of neuronal damage, neuroinflammation, and glial activation. Thus, the specific novelty of the present study is twofold. First, rather than reporting simple associations between biomarkers and fatigue, we formally test a statistical mediation model in which circulating biomarkers mediate the relationship between PD and fatigue. Second, we examine whether these mediation effects are disease-specific, i.e., whether the link between biomarker elevation and fatigue severity differs between PD patients and controls. To the best of our knowledge, this exploratory mediation approach has not previously been applied to fatigue in PD. It is important to emphasize that this analysis tests statistical indirect effects consistent with a causal model; however, causality cannot be established from this cross-sectional design, and all findings are hypothesis-generating. We hypothesized that fatigue severity in PD might be associated with altered levels of biomarkers indicative of neuronal damage (NSE, UCHL1), astrocytic activation (GFAP), neuroinflammation (HMGB1), and neurodegeneration (α-synuclein and β-amyloid-42). Given the established role of Wnt signaling in neuronal survival, we also explored whether Wnt pathway modulators (DKK1, RSPO1, sclerostin) were associated with fatigue, though these analyses were considered exploratory.

Aim

This exploratory cross-sectional study aimed to investigate whether circulating levels of selected biomarkers (NSE, GFAP, BDNF, HMGB1, α synuclein, β amyloid 42, UCHL1, DKK1, RSPO1, and sclerostin) mediate the relationship between PD status and fatigue severity. A secondary aim was to test whether the biomarker–fatigue association differs between PD patients and controls (disease specific moderation). Given the exploratory nature of the analysis, all findings are intended to generate hypotheses for future longitudinal mechanistic studies, not to establish biological causality or clinical recommendations.

Material and methods

Study design and participants

This case-control study enrolled ninety patients with a confirmed diagnosis of PD and forty-five age- and sex-matched healthy controls. Participants were recruited from Al-Sadr Medical City, Al-Najaf Teaching Hospital, and Al-Furat Al-Awsat Center for Neurosciences in Najaf, Iraq, between February and May 2025. The study protocol was approved by the Institutional Ethics Committee of the University of Kufa (MEC-110/2025) and was conducted in accordance with the World Medical Association's Declaration of Helsinki. All participants provided written informed consent before enrollment.

PD diagnosis was established using the UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria.[26] All patients presented with bradykinesia plus at least one other cardinal feature (resting tremor, rigidity, or postural instability) and exhibited no signs suggestive of atypical Parkinsonism. To rule out confounding conditions, individuals with concurrent liver or kidney disease were excluded. Furthermore, to control for systemic inflammation, only participants with serum C-reactive protein (CRP) levels below 6 mg/L were included.[27]

Clinical assessments

An experienced neurologist conducted a semi-structured interview to collect sociodemographic and clinical information from both control subjects and patients. Motor and non-motor symptoms associated with PD were assessed using the Movement Disorders Society Revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS), as previously outlined. [28] The MDS-UPDRS is divided into four parts: Part I (non-motor Experiences of Daily Living, nM-EDL), Part II (motor Experiences of Daily Living, M-EDL), Part III (motor examination), and Part IV (motor complications).[28] Neurologists conducted the MDS-UPDRS assessment in patients, and ratings from the four domains were used for statistical analysis. The Hoehn and Yahr staging method was also used to rate PD severity.[29] The severity of chronic fatigue syndrome (CFS) and fibromyalgia was assessed by a senior psychiatrist using the Fibro-Fatigue (FF) scale.[30] The FF scale assesses both mental and physical aspects of central fatigue, including exhaustion, lack of energy, and cognitive fatigue, without directly measuring pain or fibromyalgia tender points. However, fatigue in PD may overlap with depressive symptoms, sleep disturbances (e.g., insomnia, REM sleep behavior disorder), or medication effects (e.g., dopaminergic agents, sedatives). While we did not systematically exclude or separately measure these factors, our statistical model included key covariates (age, sex, BMI, exercise, and tobacco use disorder (TUD)) to reduce potential confounding. Nevertheless, the possibility that some variance in FF scores reflects depression, sleep dysfunction, or medication side effects cannot be excluded, and this is addressed as a limitation.

Assays

Fasting venous blood samples (5 mL) were collected from all participants at approximately 9:00 a.m. After clotting, samples were centrifuged at 1200×g for 5 minutes at room temperature. The resulting serum was aliquoted into three Eppendorf tubes, and any hemolyzed samples were discarded. Aliquots were stored at -80 °C until analysis. Serum concentrations of key biomarkers were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits from Wuhan USCN Business Co., Ltd. (China). The key biomarkers included α-synuclein (Catalog E3002h; range 0.1–15 ng/mL), β-amyloid-42 (Catalog E1706h; range 1–60 pg/mL), BDNF (Catalog E0115h; range 15–2000 pg/mL), DKK1 (Catalog E1672h; range 31.2–2000 pg/mL), GFAP (Catalog E0186h; range 0.1–20 ng/mL), HMGB1 (Catalog

E0722h; range 0.05–15 ng/mL), NSE (Catalog E0999h; range 0.5–50 ng/mL), RSPO1 (Catalog E3921h; range 0.1–10 ng/mL), Sclerostin (Catalog E2845h; range 15.6–1000 pg/mL), and UCHL1 (Catalog E3156h; range 0.1–12 ng/mL). The inter- and intra-assay coefficients of variation for all ELISAs were below 10%. Each sample was run in duplicate, and the mean optical density was used to calculate concentration. All assays were performed according to the manufacturer's protocols, and samples with high analyte concentrations were diluted 1:5 with the provided diluent.

Statistical analysis

An a priori power analysis using G*Power 3.1.9.7 indicated that a total sample size of 134 participants would provide 90% power to detect a medium effect size (f=0.28) at an α level of 0.05. Data normality was assessed using the Kolmogorov-Smirnov test. Normally distributed continuous data were presented as mean±standard deviation (SD) and compared using one-way analysis of variance (ANOVA). Non-normally distributed data are reported as medians (25^{th}–75^{th} percentiles) and were compared using the Mann-Whitney U test. Categorical variables were analyzed using chi-square (χ^2) tests.

Mediation and moderation were tested separately, not as a single moderated mediation model. First, moderation was tested using linear regression with an interaction term (PD status × biomarker) to determine whether the biomarker–fatigue relationship differed between PD patients and controls. Second, simple mediation was tested using PROCESS Macro (Version 4.2, Model 4; Hayes, 2022) with PD status as the independent variable, fatigue as the dependent variable, and all ten biomarkers entered as parallel mediators. Covariates (age, sex, BMI, exercise, TUD) were included in all models. The indirect effect of PD on fatigue via each biomarker was considered significant if the bias-corrected bootstrap 95% confidence interval (5,000 resamples) did not cross zero.

The significance of the indirect effects was determined using bias-corrected bootstrap confidence intervals (CIs) based on 5,000 bootstrap samples. An indirect effect was considered statistically significant if the 95% CI did not include zero. Variance inflation factors (VIFs) were calculated for all predictors in each mediation model. All VIFs were <2.5 (range 1.12–2.31), indicating no problematic multicollinearity. Bootstrapped standard errors (5,000 resamples) were used for all indirect effects. Confidence intervals were stable across repeated resampling. Residuals were approximately normal for all models, and Breusch-Pagan tests were non-significant ($p>0.05$ for all models)**.** All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 28.0, with a two-tailed significance level of $p<0.05$.

Results

Sociodemographic and clinical data

The sociodemographic and clinical characteristics of the study participants are summarized in Table 1. No significant differences were observed between the PD patient group and the healthy control group in age,

BMI, sex distribution, residency, physical activity levels, TUD, or marital status, indicating successful matching across these baseline variables**.** However, significant intergroup differences were identified in several key areas. Patients with PD had significantly lower levels of formal education ($p<0.05$). Employment status also differed markedly, with a significantly higher proportion of PD patients unemployed or retired, likely reflecting the disease's functional impact. Furthermore, a positive family history of PD was significantly more prevalent in the patient group compared to controls ($p<0.05$). Clinically, the PD cohort exhibited moderate disease severity, as indicated by a mean Hoehn and Yahr stage of 2.77±0.87. Motor symptoms were quantified using the Unified Parkinson's Disease Rating Scale (UPDRS). The mean score for Part III (Motor Examination) was 54.27±11.38, and for Part IV (Motor Complications) was 16.77±4.11, confirming substantial motor impairment. Regarding pharmacological management, 70.0% (63/90) of patients were on levodopa therapy. A subset of patients was also treated with other common antiparkinsonian agents, including procyclidine (Kemadrin; 27.8%, 25/90) and carbidopa-levodopa (Sinemet; 25.6%, 23/90).

Table 1. Demographic and clinical parameters in patients with PD and healthy controls*

Parameter	Healthy controls	PD Patients	F/χ^2	df	p
Age (years)	61.311±5.351	63.722±13.154	1.392	1/133	0.240
Sex (female/male)	19/26	40/50	0.061	1	0.806
BMI (kg/m^2)	27.019±2.27	26.797±4.131	0.113	1/133	0.737
TUD, no/yes	31/14	72/18	2.046	1	0.152
Exercise, no/yes	32/13	70/20	0.722	1	0.369
Employment, no/yes	12/33	16/74	1.438	1	0.023
Rural/Urban	11/34	17/73	0.563	1	0.453
Family history, no/yes	44/1	61/29	15.921	1	<0.001
Age of onset (years)	–	58.811±13.164	–	–	–
Total-FF	11.467±2.634	40.289±7.312	–	–	–
TOTAL-m-EDL	–	29.189±7.55	–	–	–
TOTAL-PARTIII	–	54.267±11.38	–	–	–
TOTAL-Part IV	–	16.767±4.105	–	–	–
Hoehn & Yahr stage	-	2.772±0.868	–	–	–
TOTAL-nM-EDL	–	29.111±7.102	–	–	–
Levodopa, no/yes	–	27/63	–	–	–
Kemadrin, no/yes	–	65/25	–	–	–

Sinemet, no/yes	–	67/23	–	–	–

* BMI – body mass index, TUD – tobacco use disorder

Results of the mediation study

A multiple mediation analysis was conducted using Hayes' PROCESS Macro (Version 4.2, Model 4) to examine whether a panel of ten biomarkers mediated the relationship between PD status and FF score, while controlling for sex, TUD, exercise, age, and BMI. The analysis was performed on a sample of 135 participants.

Effects of PD on biomarker levels

A series of linear regression models was first estimated to examine the effect of PD status on each of the ten biomarkers. As summarized in Table 2, PD was a significant positive predictor of eight biomarkers. Specifically, patients with PD exhibited significantly elevated levels of NSE (β=1.379, p<0.001), DKK1 (β=14.011, p<0.001), HMGB1 (β=1018.094, p=0.003), GFAP (β=0.798, p=0.002), BDNF (β=8.143, p=0.018), β-Amyloid-42 (β=9.942, p=0.048), α-Synuclein (β=5.620, p=0.033), and UCHL1 (β=0.545, p=0.012). No significant effects were found for RSPO1 or sclerostin.

Mediation model for FF scores

A comprehensive regression model predicting FF score from PD status, all ten biomarkers, their interactions with disease status, and the covariates was highly significant (F (26, 108)=54.08, p<0.001), accounting for 92.9% of the variance in FF (R^2=0.929) as seen in Table 2. The test of highest-order unconditional interactions revealed significant moderation effects for two biomarkers. This high R^2 value indicates well-fitting model, and the significant F-test confirms that the overall model has strong predictive power. Specifically, the interactions between PD status and both HMGB1 (R^2-change=0.005, β=0.001, p=0.010) and BDNF (R^2-change=0.003, β=0.114, p=0.033) were statistically significant. The nature of these interactions is detailed in the conditional effects section below and visualized in Figure 1.

Table 2. Associations between Parkinson's disease status (X) and circulating biomarker mediators (M) after controlling for age, BMI, exercise, sex, and TUD. This table shows the regression models with each biomarker as the outcome variable

Biomarker (M)	Effect of X on M (coefficient)	SE	t-value	p	R^2	Model p
NSE	1.379	0.209	6.603	<0.001	0.283	<0.001
DKK1	14.011	2.287	6.127	<0.001	0.255	<0.001
GFAP	0.798	0.251	3.173	0.002	0.108	0.022

HMGB1	1018.094	335.348	3.036	0.003	0.098	0.037
α-Synuclein	5.620	2.601	2.161	0.033	0.085	0.072
Sclerostin	-0.442	1.589	-0.278	0.781	0.080	0.095
BDNF	8.143	3.385	2.406	0.018	0.065	0.188
UCHL1	0.545	0.215	2.534	0.012	0.058	0.257
β-Amyloid-42	9.942	4.969	2.001	0.048	0.057	0.271
RSPO1	24.835	22.339	1.112	0.268	0.026	0.754

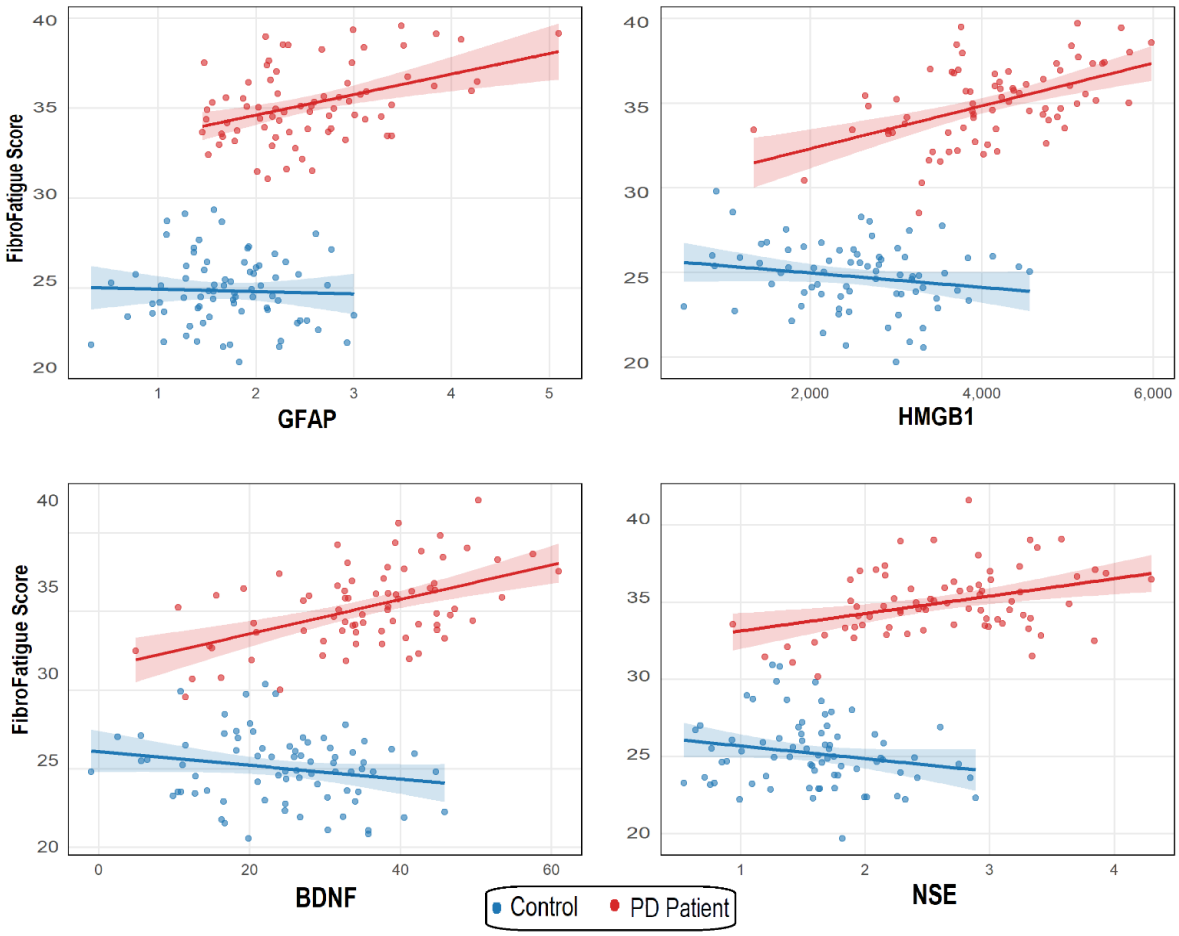

Fig. 1. Moderating effect of PD status on biomarker–fatigue relationships. Significant interactions ($p < 0.05$) were observed for HMGB1 and BDNF, with associations present only in PD patients. For GFAP and NSE, interactions were non-significant ($p>0.05$), indicating no disease-specific moderation

Conditional direct effects of biomarkers on FF scores

The interaction effects between PD status (X) and the biomarkers (M) on FF scores (Y) are presented in Table 3. A significant interaction (PD status×biomarker) was observed for HMGB1 ($p=0.010$) and BDNF ($p=0.033$), indicating that the associations between these two biomarkers and fatigue differ between PD

patients and controls. The interactions were not significant for GFAP (p=0.469) and NSE (p=0.264), suggesting that their associations with fatigue do not significantly differ by PD status. Other biomarkers (α-synuclein, etc.) also showed non-significant interactions.

Table 3. Interaction effects between Parkinson's disease status and biomarkers on fatigue scores: results of simple regression analyses. The moderation effects represent the X × M interaction on FF score, X=Parkinson's disease status, and M=proposed biomarker mediators (M); the conditional effect of M on Y in controls (X=0) is not significant*

Biomarker (M)	PD status × Biomarker interaction p-value	Effect of M on FF in PD patients (SE) [95% CI]	Effect of M on FF in controls (SE) [95% CI]	Significant interaction
HMGB1	0.01	0.001 (<0.001) [0.001-0.002]	-0.001 (0.001) [-0.003 to 0.001]	Yes
BDNF	0.033	0.113 (0.028) [0.057-0.169]	-0.001 (0.018) [-0.037 to 0.035]	Yes
GFAP	0.469	1.380 (0.332) [0.722-2.037]	0.915 (0.876) [-0.822 to 2.652]	No
NSE	0.264	1.263 (0.442) [0.387-2.140]	0.447 (0.654) [-0.849 to 1.743]	No
α-Synuclein	0.445	0.063 (0.033) [-0.002 to 0.128]	0.031 (0.040) [-0.049 to 0.111]	No

* Conditional effects are derived from regression models including all covariates (age, sex, BMI, exercise, TUD), a significant interaction indicates that the biomarker's effect on fatigue differs between PD patients and controls

Direct, indirect, and total effects

The direct, indirect, and total effects of the biomarkers on the FF score in PD are presented in Table 4. The bootstrapped analysis of indirect effects revealed four significant mediation pathways through which PD status correlates with FF scores. The pure natural direct effect, accounting for all mediators, remained significant (β=22.598, SE=1.114, p<0.001, 95% CI [20.391–24.806]). The results in Table 4 present the bootstrap confidence intervals for specific indirect effects, indicating significant mediation via four biomarkers.

A statistically significant indirect effect of PD on fatigue was observed through elevated levels of NSE (Effect=1.743, 95% BootCI [0.366-3.293]), HMGB1 (Effect=1.207, 95% BootCI [0.266-2.380]), GFAP (Effect=1.101, 95% BootCI [0.464-1.936]), and BDNF (Effect=0.921, 95% BootCI [0.140-1.869]). The

indirect effects through the remaining biomarkers were not statistically significant. The direct and indirect effects of diagnosis (presence of PD) on functional score through blood biomarkers are presented in Figure 2. The total effect of PD on FF was significant (b=28.693, BootSE=0.939, 95% BootCI [26.828, 30.524]).

Table 4. Bootstrapped estimates of indirect associations between Parkinson's disease status and fatigue scores via biomarkers*

Indirect pathway	**Effect (Boot)**	**BootSE**	**BootLLCI**	**BootULCI**	**p**
PD → NSE → FF	1.743	0.735	0.366	3.293	<0.05
PD → HMGB1 → FF	1.207	0.543	0.266	2.38	<0.05
PD → GFAP → FF	1.101	0.371	0.464	1.936	<0.05
PD → BDNF → FF	0.921	0.445	0.14	1.869	<0.05
PD → DKK1 → FF	0.738	0.502	-0.292	1.692	>0.05
PD → α-Synuclein→ FF	0.355	0.252	-0.156	0.844	>0.05
PD → β-Amyloid-42→ FF	0.223	0.273	-0.191	0.88	>0.05
PD → Sclerostin → FF	0.02	0.14	-0.296	0.315	>0.05
PD → UCHL1→ FF	-0.047	0.196	-0.407	0.384	>0.05
PD → RSPO1 → FF	-0.164	0.186	-0.598	0.125	>0.05
Total indirect effect	6.095	-	-	-	-
Pure direct effect	22.598	1.114	20.391	24.806	<0.001
Total effect	28.693	0.939	26.828	30.524	<0.001

* Bootstrapped 95% confidence intervals (5,000 resamples) that do not cross zero indicate significant indirect effects, models controlled for age, sex, BMI, exercise, and TUD, LLCI – lower level confidence interval, ULCI – upper level confidence interval

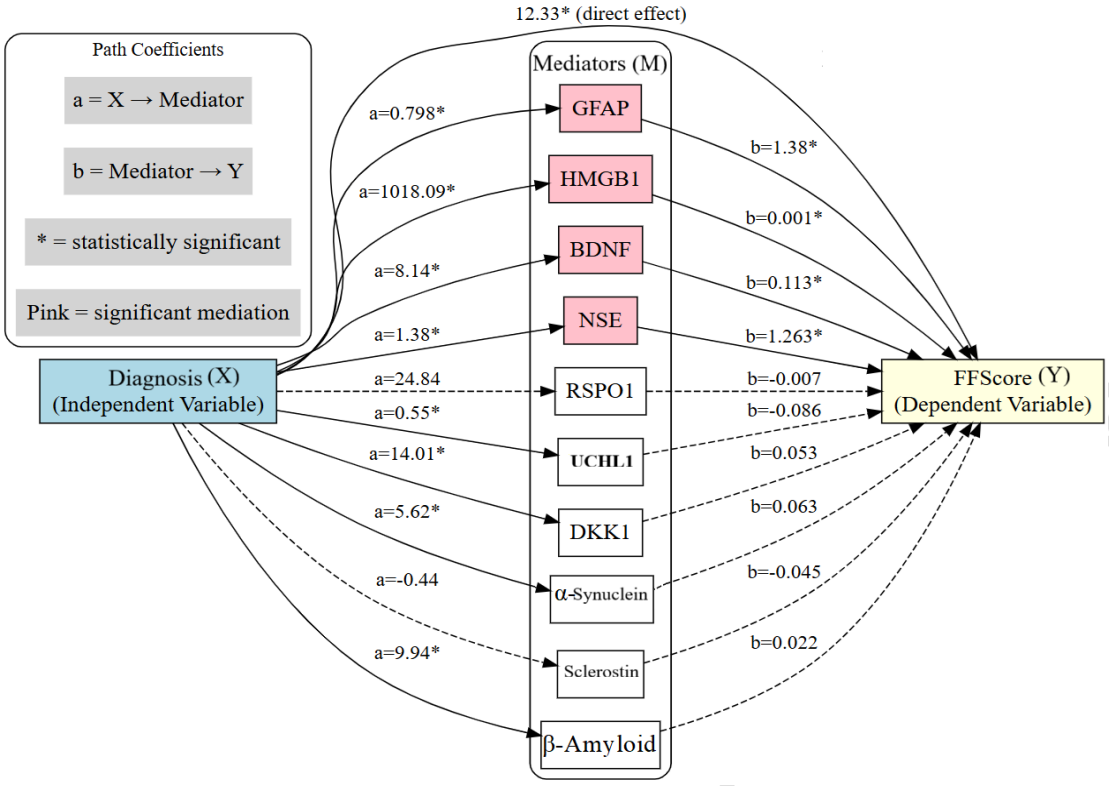

Fig. 2. Graphical diagram of the final model with key mediation and moderation pathways of fatigue in Parkinson's disease

Discussion

The primary finding of this exploratory cross-sectional mediation analysis is that GFAP, HMGB1, BDNF, and NSE each show significant indirect (mediated) effects from PD status to fatigue. Moderation analysis revealed that the biomarker-fatigue relationship was disease-specific (i.e., differed significantly between PD patients and controls) only for HMGB1 and BDNF, suggesting that the PD pathophysiological environment may be required for their association with fatigue. For GFAP and NSE, the non-significant interactions suggest that their mediating roles did not differ significantly between groups. These findings are associational only; causality cannot be inferred from this cross-sectional design.

The indirect effects observed through GFAP, HMGB1, BDNF, and NSE are statistically consistent with the hypothesis that dysregulation within these molecular pathways may be associated with fatigue severity in PD. However, cross-sectional mediation does not establish causation. Interestingly, Table 3 shows that only HMGB1 and BDNF had significant interactions with PD status, meaning their relationships with fatigue differ between PD patients and controls. GFAP, a structural protein released during astrocytic activation and reactive gliosis,[31,32] was significantly elevated in PD patients compared to controls and statistically mediated the PD-fatigue association. Previous studies reported that serum GFAP levels are elevated in PD and correlate with cognitive decline,[24,33] indicating astrocytic remodeling as a response to

neurodegeneration.[33,34] The present results extend these observations by suggesting that astroglial activation may also be statistically linked to non-motor symptoms, specifically fatigue.

HMGB1 plays a central role in orchestrating inflammatory cascades upon release, driving microglial activation and progressive neurodegeneration, thereby establishing a feed-forward cycle of damage.[35,36] The role of HMGB1, a significant damage-associated molecular pattern (DAMP) and key regulator of neuroinflammation, highlights the essential involvement of innate immune activation in this process.[35,37] Elevated HMGB1 levels were associated with higher fatigue scores only among PD patients (conditional β=0.001, p=0.001). This aligns with previous work linking HMGB1 to chronic neuroinflammation in PD,[15,35] and extends it by associating HMGB1 with a specific non-motor symptom. HMGB1-induced microglial activation and mitochondrial dysfunction could, in theory, be associated with a perceived energy deficit, a core feature of fatigue.[38] Nevertheless, serum HMGB1 may also originate from peripheral sources. NSE was elevated in PD and showed a significant indirect (mediated) effect on fatigue (Table 4). However, the PD status × NSE interaction was not significant (p=0.264, Table 3), indicating that the association between NSE and fatigue did not differ significantly between PD patients and controls. Elevated NSE is well documented as a marker of acute or ongoing neuronal injury in various neurological disorders.[20,32] Thus, NSE may reflect a general fatigue mechanism rather than a PD-specific pathway.

BDNF, typically considered neuroprotective and supportive of dopaminergic neuron survival,[39] was unexpectedly elevated in PD patients and positively associated with fatigue severity, with a conditional effect present only in the PD group (β=0.113, p<0.001). This finding appears paradoxical, given BDNF's established role in neuronal health [23,39]. Several speculative explanations exist, none of which can be confirmed by the present data. First, serum BDNF may reflect peripheral (not central) sources and could be influenced by medication effects, disease stage, or comorbid conditions, none of which were fully controlled in this exploratory analysis. Second, if the finding is replicable, speculative explanations could include a maladaptive compensatory response or context-dependent signaling in inflamed neural tissue. However, longitudinal studies measuring central and peripheral BDNF are needed before any mechanistic conclusions can be drawn.

The overall findings are consistent with a hypothesized model in which central fatigue in PD may be related to neuroinflammation and neuronal-astroglial dysfunction. However, causality is not established. Figure 3 illustrates the hypothesized integrative pathway of GFAP, HMGB1, BDNF, and NSE in PD fatigue. This cascade is proposed to disrupt critical brain regions involved in motivation, reward, and the perception of effort, including the basal ganglia and prefrontal cortex.

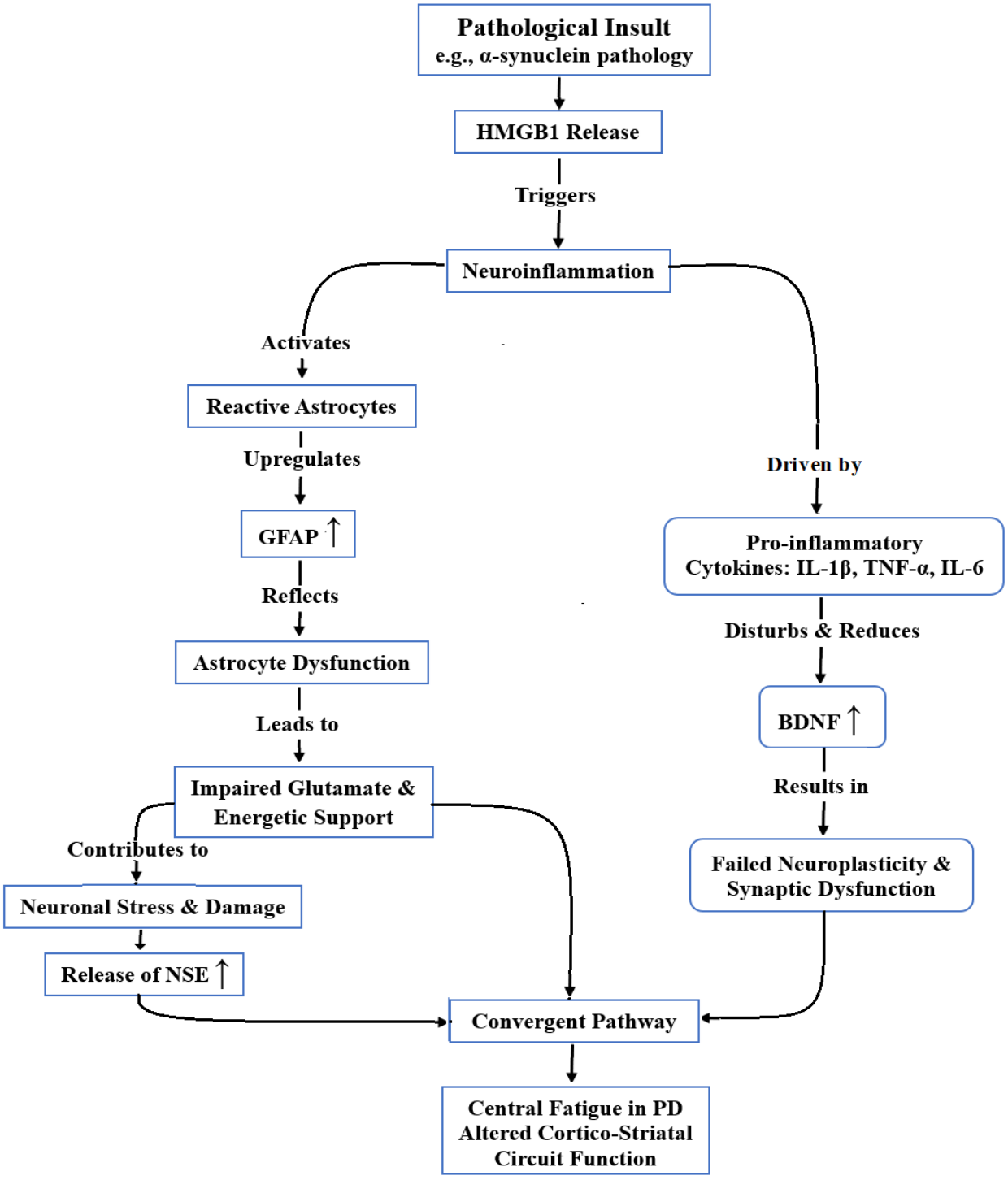

Fig. 3. Hypothetical integrative pathway linking GFAP, HMGB1, BDNF, and NSE to fatigue in Parkinson's disease

While α-synuclein, β-amyloid-42, UCHL1, RSPO1, DKK1, and sclerostin may be involved in PD pathogenesis or progression, they did not statistically mediate the relationship between PD and fatigue in our model. This dissociation suggests that different non-motor symptoms may be associated with distinct

biomarker profiles. For example, β-amyloid-42 has been linked to dopa-resistant gait disturbances and oxidative stress, rather than fatigue.[40,41] Similarly, Wnt pathway modulators (DKK1, RSPO1, sclerostin) may be more relevant to neuronal survival and motor progression than to fatigue perception. These null findings should be interpreted cautiously, given the sample size and the exploratory nature of the analysis.

Study limitations

Several limitations should be acknowledged. First, the cross-sectional design precludes conclusions about causality, directionality, reverse causation, or unmeasured confounding. Second, biomarkers were measured in blood, not cerebrospinal fluid or brain tissue, and we lack neuroimaging to confirm central origin. Third, the FF scale does not fully distinguish fatigue from depression, apathy, sleep disturbances, or medication effects, and residual confounding cannot be excluded. Fourth, the case-control design and single-country sample (Iraq) limit generalizability. Finally, although Hoehn and Yahr stage was recorded, it was not included in the mediation model; future studies should examine whether disease stage modifies these effects.

Conclusion

The results of the present study are consistent with a statistical mediation model in which PD is associated with fatigue indirectly through elevated levels of specific biomarkers of neuroinflammation (HMGB1), astroglial activation (GFAP), neuronal injury (NSE), and a paradoxical BDNF response. However, causality cannot be established from this cross-sectional design. The disease-specific moderation effects (biomarkers predicting fatigue only in PD for HMGB1 and BDNF) generate testable hypotheses for future longitudinal and mechanistic studies. These findings identify candidate biomarkers that warrant further investigation in independent cohorts.

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Declarations

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Author contributions

Conceptualization, T.A. and H.A.; Methodology, T.A.; Software, H.A.; Validation, H.A., T.A. and Z.Z.; Formal Analysis, H.A.; Investigation, T.A.; Resources, H.A.; Data Curation, H.A.; Writing-Original Draft Preparation, T.A.; Writing-Review & Editing, H.A.; Visualization, T.A.; Supervision, H.A.; Project Administration, H.A.; Funding Acquisition, T.A.

Conflicts of interest

The authors declare no conflicts of interest.

Data availability

The datasets used during the current study are available from the corresponding author upon reasonable request

Ethics approval

The study was approved by the Institutional Ethics Committee of the University of Kufa (MEC-110/2025).

Use of AI and AI-assisted technologies in the writing process

No artificial intelligence (AI) tools or AI-assisted technologies were used in the writing, editing, or preparation of this manuscript.

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