

A Profile of Nanoparticle-Based Plasma Neurodegenerative Biomarkers for Cognitive Function Among Patients Undergoing Hemodialysis

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Purpose: This study aimed to compare the plasma levels of nanoparticle-based neurodegenerative biomarkers between hemodialysis (HD) participants with grossly normal cognitive function and healthy controls.

Patients and Methods: A cohort of participants undergoing maintenance HD and healthy controls were enrolled for comparison between July and October 2021. The immunomagnetic reduction method was used to measure plasma neurodegenerative biomarkers A β ₁₋₄₀, A β ₁₋₄₂, tau protein, and neurofilament light chain (NfL). The clinical dementia rating (CDR) was used to evaluate cognitive function. A receiver operating characteristic curve was used to discriminate between HD participants and healthy controls.

Results: There were 52 and 18 participants in the HD and healthy control groups, respectively. The mean age of the HD participants was 62 years, and that of the healthy controls was 57 years. The mean HD vintage in the HD cohort was 11.8 years. HD participants demonstrated significantly higher plasma levels of A β ₁₋₄₂, tau protein, A β ₁₋₄₂ \times tau, and NfL and A β ₁₋₄₂/A β ₁₋₄₀ ratio and significantly lower plasma A β ₁₋₄₀ levels than healthy controls. The measured plasma biomarkers could not discriminate between CDR0 and CDR0.5 HD participants. The area under the curve of the study biomarkers to discriminate HD participants from healthy controls ranged from 0.987 (A β ₁₋₄₂ \times tau) to 0.889 (NfL).

Conclusion: The plasma levels of nanoparticle-based neurodegenerative biomarkers were higher in HD participants with grossly normal cognitive function than in healthy controls. These findings imply that neurodegenerative changes appear in HD participants. A profile of plasma neurodegenerative biomarkers could be considered a potential surrogate for evaluating long-term cognitive function in HD participants.

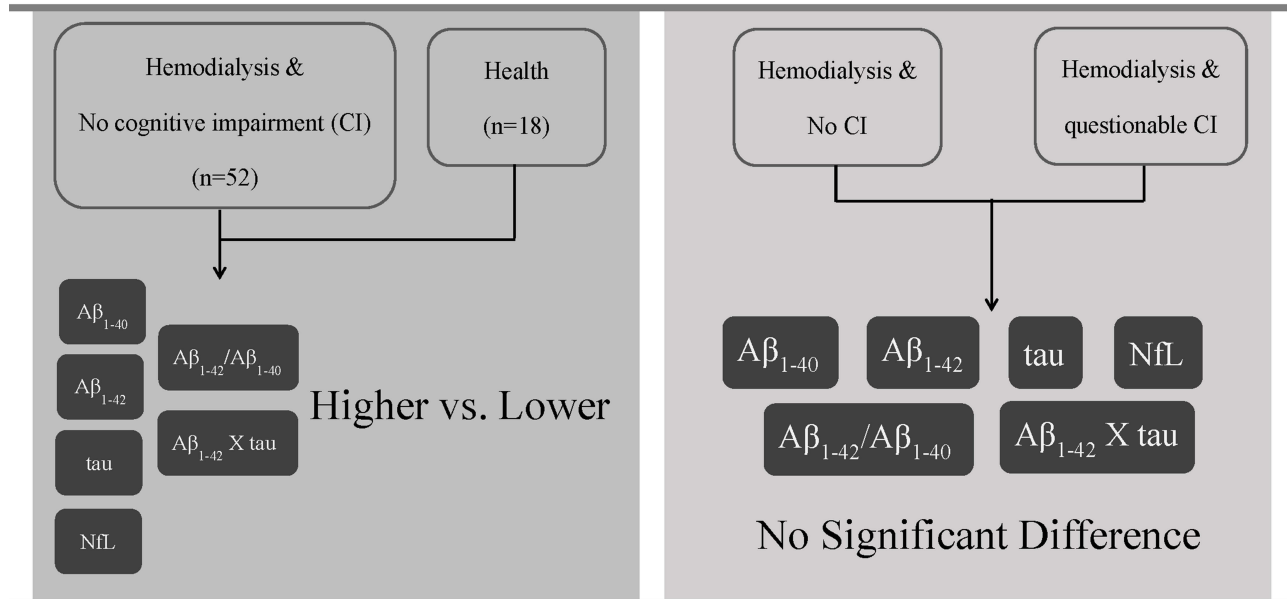
Keywords: A β proteins, cognitive function, hemodialysis, tau protein

Introduction

Patients with chronic kidney disease (CKD) and those undergoing dialysis are at risk of cognitive impairment (CI).^{1,2} The clinical characteristics of CI include language and memory deficits, cognitive slowing, and diminished executive function. The potential pathological factors include white matter disease,³ silent brain infarcts,⁴ demographic profiles,⁵ vascular lesions,⁶ uremic toxin retention,⁷ uremic hyperparathyroidism,⁸ and hemodialysis (HD)-related disequilibrium brain insult.⁹ CI in CKD and dialysis patients leads to disability in ordinary life, increasing the incidence of hospitalization and mortality.¹⁰ Based on our previous study² and the aforementioned studies, uremic milieu contributes to degenerative changes in the brain. Reports have demonstrated that aberrant functional connectivity in the default mode network exists in the medial frontal lobe, parietal lobe, and thalamus in dialysis patients.^{11,12} Additionally, the ventral striatum volume is correlated with memory, execution, and depression in dialysis patients.¹³ Conclusively, CKD and dialysis populations often present with neuropsychological impairments that are correlated with alterations in the neurohormone levels and default in the nervous network.

Graphical Abstract

A profile of nanoparticle-based plasma neurodegenerative biomarkers for cognitive function among patients undergoing hemodialysis



Conclusion: plasma levels of nanoparticle-based neurodegenerative biomarkers are higher in hemodialysis subjects with grossly normal cognitive function compared with healthy controls.

The clinical characteristics of CI in CKD resemble those observed in Alzheimer's disease (AD) and parkinsonism. The main pathological hallmarks of AD are the presence of a large number of senile plaques and neurofibrillary tangles in the cerebral cortex and hippocampus.^{14,15} The major component of senile plaques in AD is the $A\beta$ protein. In patients with CKD, the serum $A\beta$ protein levels are higher than usual, resulting from decreased clearance of $A\beta$ proteins in the blood.¹⁶ This observation suggests that AD and CKD share similar pathological processes. Another pathological change in AD is the presence of neurofibrillary tangles. Abnormal phosphorylated tau proteins are the main constituent proteins of neurofibrillary tangles.¹⁷ Previous study in aluminum overdose in dialysis patients has shown that association between changes of tau proteins and AD-like brain pathological changes.¹⁸ Given the aforementioned observations, CKD and AD share potential markers for CI, such as increased $A\beta$ protein and tau protein expression. Therefore, measuring these markers in the blood may be used as a proxy for disease progression.

Blood-based marker detection has been reported to differentiate between healthy individuals and those with mild CI due to AD or AD-like dementia.^{15,19,20} However, the levels of these markers in the blood are extremely low, commonly at the level of pg/mL. Recently, ultrasensitive assay techniques have been developed to substantially increase the sensitivity and reliability. These assays include modified enzyme-linked immunosorbent assay, surface-based fluorescence intensity distribution, multiplex electrochemiluminescence, multiplexed flow-matrix analysis, selected reaction monitoring mass spectrometry, single-molecule array, and immunomagnetic reduction (IMR).^{21,22} Several previous studies have shown the feasibility of the IMR methods for detecting

nanoparticles such as A β and tau proteins in AD and CI populations.^{21,23} Accordingly, disease progression can be followed up, and a novel therapeutic strategy could be expected.

We hypothesized that potential nanoparticle-based plasma neurodegenerative biomarkers could be detected using the IMR method in HD participants with grossly normal cognitive function. Consequently, a monitoring protocol can be developed to reduce the incidence of CI in HD participants.

Materials and Methods

Participants

The study was conducted from July to October 2021. Patients who received maintenance outpatient HD thrice weekly at Kaohsiung Chang Gung Memorial Hospital in Taiwan were enrolled. The inclusion and exclusion criteria were followed up in our previous study.² The inclusion criteria were age of ≥ 18 years and the ability to provide basic interviews. The exclusion criteria included history of cerebral stroke or brain injury by nonmedical causes; drug-usage history, which may influence cognitive function, including chemotherapy; other comorbidities, which may affect cerebral function, for examples, liver cirrhosis, cancer, and psychiatric diseases; malnutrition, defined by a serum albumin level of < 3.5 g/dL; chronic alcohol use; pregnancy; hospitalization within 3 months prior to enrollment; and absence of caregiver for providing medical history.² Dialyzers for HD had a surface area of 2.0 m² and used bicarbonate-based dialysate. A total of 100 eligible HD patients were screened. Finally, 52 HD participants were enrolled in the analysis (Figure 1). Healthy controls were recruited voluntarily in the outpatient clinic by posting protocol notifications. A total of 18 healthy controls were enrolled because of a shortage of research budget and time restriction in the poster declaration.

Data Collection and Analytic Parameters

Informative data included demographics, educational levels, comorbidities, HD vintage, and circulating parameters. All blood samples from HD participants were obtained mid-week (Wednesday and Thursday). The measured parameters included serum albumin, urea nitrogen, creatinine, calcium, phosphate, cholesterol, triglyceride, ferritin, intact parathyroid hormone, hemoglobin, HD adequacy index (Kt/V), urea (Daugirdas method),²⁴ A β_{1-40} , A β_{1-42} , tau protein, and neurofilament light chain (NfL). Blood samples for biochemical measurements were obtained using commercial kits and an autoanalyzer (Hitachi 7600–210, Hitachi Ltd., Tokyo, Japan). The albumin levels were measured by the bromocresol green method. Blood drawn from participants was collected in ethylenediaminetetraacetic acid (EDTA) collection tubes, centrifuged according to the instructions of the package inserts, and stored at -80 °C until use in plasma A β_{1-40} , A β_{1-42} , tau protein, and NfL biomarker assays. Finally, blood samples in a dry-ice package were delivered to MagQu Co., Ltd., in

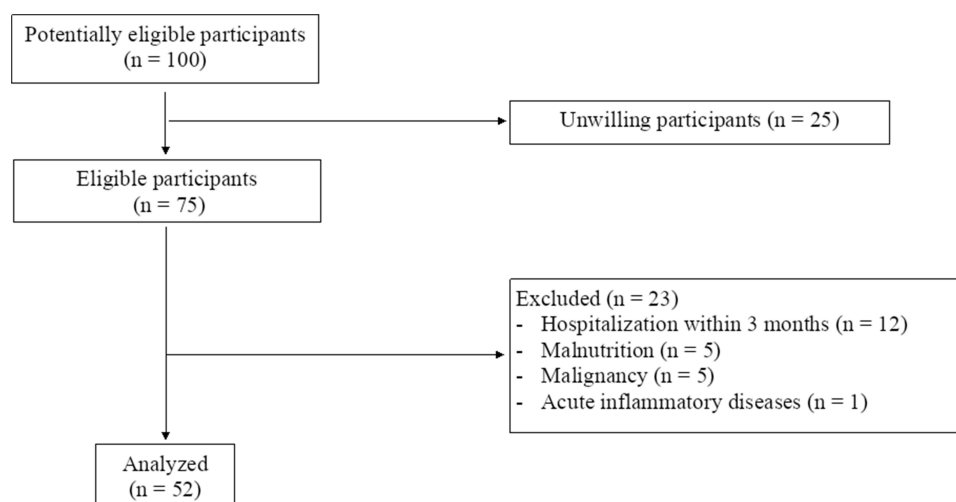


Figure 1 Participants' deposition.

New Taipei City, Taiwan, for biomarker measurements. The IMR method used to detect plasma $A\beta_{1-40}$, $A\beta_{1-42}$, tau protein, and NfL followed the protocol described in a previous study.²⁵

Mini-Mental State Examination

General cognitive function was assessed using the Mini-Mental State Examination (MMSE), which has been validated in a Chinese population.²⁶ We used clinical dementia rating (CDR) scores to stratify the functional scores of HD participants.^{2,27} A rating score of CDR0 indicated no dementia, and CDR0.5, CDR1, CDR2, and CDR3 indicated questionable, mild, moderate, and severe dementia, respectively. The necessary information was obtained through interviews with the patients and reliable informants (eg, family members) by a qualified reviewer based on the CDR assessment protocol.

Statistical Analysis

The baseline demographic characteristics and laboratory measurements of HD participants and healthy controls are presented as frequency (percentage), mean (standard deviation), and median (interquartile range). The difference in distribution between subgroups was estimated using the chi-square test, Fisher exact test, independent two-sample *t*-test, or Wilcoxon rank-sum test. The Pearson and Spearman correlation tests were conducted to evaluate the correlation between the CI plasma biomarkers and associated clinical factors. Receiver operating characteristic (ROC) curve analysis was performed to discriminate between HD participants and healthy controls according to CI plasma biomarkers. Statistical significance was set at $p < 0.05$. All analyses were performed using R software (R Development Core Team 2021, version 4.0.5).

Results

Baseline Characteristics

The mean age of the HD participants was 62 years, and that of the healthy controls was 57 years. There were equal numbers of men and women in the HD and healthy control groups. HD participants had comorbidities, diabetes ($n=15$), hypertension ($n=44$), and cardiovascular disease ($n=15$). Most of the participants reached the 12-year educational level: the HD participants ($n=40$) and healthy controls ($n=16$). HD participants demonstrated significantly higher plasma levels of $A\beta_{1-42}$, tau protein, $A\beta_{1-42} \times \text{tau}$, and NfL and a greater $A\beta_{1-42}/A\beta_{1-40}$ ratio than healthy controls. In contrast, the $A\beta_{1-40}$ levels were significantly lower in the HD group than in healthy controls (Table 1).

Demographics and Plasma Biomarkers in HD Patients

Table 2 presents the dichotomous results for various demographic and laboratory factors between CDR0 and CDR0.5 in HD participants ($n=9$). Patients in CDR0.5 showed significantly lower serum albumin and triglyceride levels than those in CDR0. The levels of $A\beta_{1-42}$, $A\beta_{1-40}$, tau protein, NfL, and $A\beta_{1-42} \times \text{tau}$ and $A\beta_{1-42}/A\beta_{1-40}$ ratio were not significantly different between the CDR0 and CDR0.5 groups.

Correlation Analysis Results for Plasma Biomarkers in HD Participants

In the correlation analysis, the plasma tau protein levels were significantly negatively associated with sex and Kt/V-urea. The plasma $A\beta_{1-40}$ levels were also negatively associated with hypertension. The plasma $A\beta_{1-42}$ levels were significantly positively associated with cholesterol levels (Table 3).

ROC Curve Analysis for Discriminating HD Participants from Controls

Table 4 shows the ROC curve analysis of plasma biomarkers for discriminating HD participants from healthy controls. The area under the curve (AUC) for all plasma biomarkers was >0.9 , except for NfL (0.889). The distribution of sensitivity in all plasma biomarkers ranged from 0.750 ($A\beta_{1-40}$) to 0.962 ($A\beta_{1-42} \times \text{tau}$). The specificity distribution ranged from 0.889 (tau and NfL) to 0.962 ($A\beta_{1-42} \times \text{tau}$). Figure 2 shows the ROC curve analysis using figure expression.

Table 1 Baseline Characteristics

Characteristics	Controls	Hemodialysis	p value
N (female:male)	18 (9:9)	52 (26:26)	0.221 ^a
Age (years)	57.8 ± 7.3	62.2 ± 10.2	0.044
Hb (g/L)	13.71 ± 1.79	10.58 ± 0.98	<0.001
BUN (mg/dL)	17.15 ± 6.24	65.46 ± 16.69	<0.001
Cr (mg/dL)	0.95 ± 0.32	10.90 ± 2.50	<0.001
Albumin (g/L)	4.31 ± 0.62	4.05 ± 0.28	0.068
HD vintage (years)	–	11.83 ± 7.64	–
DM (n)	–	15	–
Hypertension (n)	–	44	–
Cardiovascular disease (n)	–	15	–
Educational years			0.495 ^b
≤6 years (n)	0	0	
6–12 years (n)	2	12	
12 years (n)	16	40	
tau (pg/mL)	20.53 ± 2.23	27.78 ± 5.29	<0.001
Aβ ₁₋₄₂ (pg/mL)	16.13 ± 0.43	17.15 ± 0.43	<0.001
Aβ ₁₋₄₀ (pg/mL)	54.18 ± 5.12	42.90 ± 5.54	<0.001
Aβ ₁₋₄₂ ×tau	331.45 ± 40.47	476.81 ± 93.94	<0.001
Aβ ₁₋₄₂ /Aβ ₁₋₄₀	0.30 ± 0.03	0.41 ± 0.05	<0.001
NfL (pg/mL)	7.06 ± 2.03	10.77 ± 2.66	0.002

Notes: P-value is estimated using chi-squared test^a, Fisher's exact test^b or independent two-sample t-test.

Abbreviations: Hb, hemoglobin; BUN, blood urea nitrogen; Cr, creatinine; HD, hemodialysis.

Spearman Correlation Between MMSE Parameters in HD Participants

Table 5 shows correlation estimation between MMSE parameters (orientation to time, place, attention and calculation, recall, and language) and plasma biomarkers in HD participants (n=7). There was no significant correlation between them.

Discussion

The end-stage renal disease population has been recognized to have a higher incidence of CI than the general population.^{28,29} Accordingly, there is an urgent need to determine a practical and validated method for early detection of CI in end-stage renal disease populations. Furthermore, it is more complicated in the HD population owing to the complex uremic milieu and solute clearance during HD sessions. Based on previous reports, there are similar clinical characteristics of CI between CKD, AD, and parkinsonism.^{14,15,18,30} Therefore, detection of plasma Aβ proteins is considered a reasonable method for screening for CI in end-stage renal disease populations. Moreover, the plasma Aβ protein levels increase when kidney function is reduced.³¹ Consequently, it is reasonable to expect the plasma Aβ protein levels to increase in HD participants compared with those in healthy controls. However, it is unclear whether increased plasma Aβ protein levels are related to cognitive function in the HD population. Moreover, the relationships between other plasma nanoparticle-based neurodegenerative biomarkers, such as tau and NfL, and cognitive function in the HD population also need to be further investigated. In the present study, we compared nanoparticle-based plasma neurodegenerative biomarkers between HD participants with normal cognitive function and healthy controls. We found higher plasma levels of Aβ₁₋₄₂, tau protein, Aβ₁₋₄₂ × tau, and NfL and a greater Aβ₁₋₄₂/Aβ₁₋₄₀ ratio in HD participants than in healthy controls (Table 1). Moreover, there was a high AUC value for discriminating HD participants from healthy controls (Table 4). Further research is required to establish whether long-term blood monitoring of neurodegenerative components plays a role in reducing the incidence of CI in HD participants.

HD can affect the plasma levels of Aβ₁₋₄₀, Aβ₁₋₄₂, and tau protein. In a previous study, hemodialyzers effectively removed blood Aβ₁₋₄₀ and Aβ₁₋₄₂ during HD sessions; in contrast, hemodialyzers could not remove tau protein.³² However, tau protein was observed to have a small influx into blood during HD sessions.³² One speculation is that the

Table 2 Demographics and Plasma Biomarkers in Hemodialysis Patients (n=9)

Characteristics	CDR 0	CDR 0.5	p value
N (female:male)	5 (3:2)	4 (1:3)	0.524 ^a
Age (years)	58.8 ± 11.4	72.0 ± 9.5	0.190
Hb (g/L)	11.08 ± 0.54	10.9 ± 0.35	0.282
BUN (mg/dL)	73.6 ± 28.53	55.25 ± 14.22	0.128
Cr (mg/dL)	11.764 ± 2.94	9.71 ± 0.16	0.097
Albumin (g/L)	4.29 ± 0.12	3.84 ± 0.23	<0.010
Ca (mg/dL)	9.04 ± 1.30	10.03 ± 0.65	0.286
P (mg/dL)	5.72 ± 2.28	4.22 ± 0.63	0.190
Cholesterol (mg/dL)*	163.00 (140.00–169.00)	153.50 (148.00–155.75)	0.556
Triglyceride (mg/dL)*	191.00 (167.00–246.00)	98.00 (91.75–107.75)	0.049
Ferritin (ug/dL)*	180.50 (148.20–184.00)	215.25 (130.98–401.42)	0.999
iPTH(pg/dL)*	347.60 (220.50–1042.70)	513.60 (424.85–590.75)	0.905
Kt/V-urea	1.72 ± 0.34	1.63 ± 0.09	0.413
HD vintage (years)	13.2 ± 3.42	10.25 ± 4.57	0.164
DM (n)	2	2	1.000 ^a
Hypertension (n)	4	3	1.000 ^a
Cardiovascular disease (n)	1	3	0.206 ^a
Educational level			0.444 ^a
≤6 years (n)	0	0	
6–12 years (n)	0	1	
12 years (n)	5	3	
tau(pg/mL)	23.78 ± 1.92	27.80 ± 5.11	0.108
Aβ ₁₋₄₂ (pg/mL)	17.14 ± 0.66	17.14 ± 0.14	0.498
Aβ ₁₋₄₀ (pg/mL)	43.31 ± 3.51	39.14 ± 3.48	0.060
Aβ ₁₋₄₂ xtau	407.1 ± 28.8	476.3 ± 85.7	0.103
Aβ ₁₋₄₂ /Aβ ₁₋₄₀	0.398 ± 0.040	0.440 ± 0.037	0.073
NfL(pg/mL)	11.10 ± 3.31	10.34 ± 1.94	0.340

Notes: P-value is estimated using Fisher's exact test^a or Wilcoxon rank-sum test. *Expressed as median (interquartile range).

Abbreviation: iPTH, intact parathyroid hormone.

efflux ability of the blood–brain barrier may be disturbed by uremic toxins, especially during HD sessions,³³ thus resulting in a small increase in the blood tau protein levels. In addition, the plasma t-tau levels were reported not to be dependent on HD vintage in a cross-sectional study.³² On the contrary, the plasma Aβ₁₋₄₀ and Aβ₁₋₄₂ levels significantly decline in longer HD vintage, especially in cases exceeding 4 years.³² However, these results in clinical observational studies need to be further validated by well-designed studies in the future. Our HD participants had received HD therapy for more than 4 years. Based on previous clinical observations, we assumed that the plasma levels of Aβ₁₋₄₀, Aβ₁₋₄₂, and tau protein in our HD participants were relatively constant in cross-sectional measurements and could be applied to examine their associations with cognitive function in our HD cohort.

Higher plasma levels of Aβ₁₋₄₂ and a greater Aβ₁₋₄₂/Aβ₁₋₄₀ ratio have been reported in healthy controls that do not transition to cognitive decline.³⁴ It has also been observed that an increased Aβ₁₋₄₂/Aβ₁₋₄₀ ratio and Aβ removal via HD are related to slight cognitive function improvement or maintenance of cognitive function in HD participants.³⁵ In the present study, HD participants demonstrated significantly higher plasma levels of Aβ₁₋₄₂ and a greater Aβ₁₋₄₂/Aβ₁₋₄₀ ratio than healthy controls. The peripheral Aβ levels are affected by age and gene expression: increased Aβ efflux in the blood–brain barrier at the choroid plexus with transporter genes LRP-1 and P-gp and decreased Aβ influx with transporter gene LRP-2.³⁶ Thus, increasing age demonstrated more Aβ retention in the cerebrospinal fluid.³⁶ Moreover, aberrations in gene expression (single nucleotide polymorphisms, DNA methylation, miRNA, and lincRNA) could decrease the Aβ levels in the blood.³⁴ We did not examine gene expression in our participants. Therefore, we hypothesized that this limitation may contribute to these contradictory results. Further comparison between

Table 3 Correlation Analysis Results for Plasma Biomarkers in Hemodialysis Subjects (n=52)

	tau ^a (n=52)	Aβ ₁₋₄₂ ^a (n=52)	Aβ ₁₋₄₀ ^a (n=52)	NfL ^b (n=9)
tau	1.000	0.151	0.079	-0.083
Aβ ₁₋₄₂	0.151	1.000	-0.070	0.517
Aβ ₁₋₄₀	0.079	-0.070	1.000	-0.300
NfL ^b	-0.083	0.517	-0.300	1.000
Education level (<12 vs >12 years)	0.031	0.149	0.070	-0.411
Age, years	-0.122	-0.139	-0.176	-0.050
Sex (female vs male)	-0.296*	-0.133	-0.190	0.346
HD vintage, years	-0.237	-0.051	0.166	-0.126
DM	0.009	0.133	0.217	0.433
Hypertension	0.135	0.089	-0.326*	0.518
Hb	-0.039	-0.095	0.053	-0.076
Albumin	0.104	-0.145	0.256	0.467
Ferritin	-0.131	0.183	-0.139	0.383
Cholesterol	-0.135	0.308*	0.055	-0.133
Triglyceride	-0.172	0.078	-0.039	0.527
Ca	0.215	-0.220	-0.054	-0.050
P	-0.091	0.077	0.009	-0.017
iPTH	0.039	-0.019	-0.178	0.033
Kt/V-urea	-0.375**	-0.008	-0.023	-0.067

Notes: ^aCorrelation analysis were conducted using Pearson correlation test. ^bCorrelation analysis were conducted using Spearman correlation test. **p*<0.05, ***p*<0.001.

Table 4 ROC Analysis for Discriminating Hemodialysis Subjects from Controls

Biomarker	Cut-off Value	Sensitivity	Specificity	AUC
tau	22.65 pg/mL	0.846	0.889	0.935
Aβ ₁₋₄₂	16.71 pg/mL	0.865	0.944	0.960
Aβ ₁₋₄₀	46.95 pg/mL	0.750	0.944	0.923
Aβ ₁₋₄₂ ×tau	374.3	0.962	0.962	0.987
Aβ ₁₋₄₂ /Aβ ₁₋₄₀	0.345	0.827	0.944	0.954
NfL	8.86 pg/mL	0.889	0.889	0.889

Abbreviations: ROC, receiver operating characteristic curve; AUC, area under the curve.

CDR0 and CDR0.5 HD participants revealed no significant differences in the plasma levels of Aβ₁₋₄₂ or Aβ₁₋₄₂/Aβ₁₋₄₀ ratio between the two groups (Table 2). Due to the limited cross-sectional study and small number of HD participants, we could not ascertain whether the higher plasma levels of Aβ₁₋₄₂ and greater Aβ₁₋₄₂/Aβ₁₋₄₀ ratio observed in our HD participants would result in favorable cognitive function, given a longer observational duration. A similar uncertain situation also exists in discriminating between normal cognitive function and subtle cognitive function, CDR0 from CDR0.5, in HD participants using plasma Aβ measurements.

NfL is a component of neuronal axon cytoskeleton. HD participants are vulnerable to axon damage through several mechanisms. These pathogenic mechanisms include uremic toxins, reduced cerebral blood perfusion during an HD session, sympathetic hyperactivation, and diabetes.³⁷ Hou et al reported that the plasma NfL levels were marginally higher in HD participants with CI than in those without CI.³⁷ In our study, HD participants were considered to have normal cognitive function or subtle CI based on the CDR values. Thus, we did not find a significant difference in the plasma NfL levels between the two groups (Table 2). However, we are aware that a small number of participants may bias this result, and our results await further study.

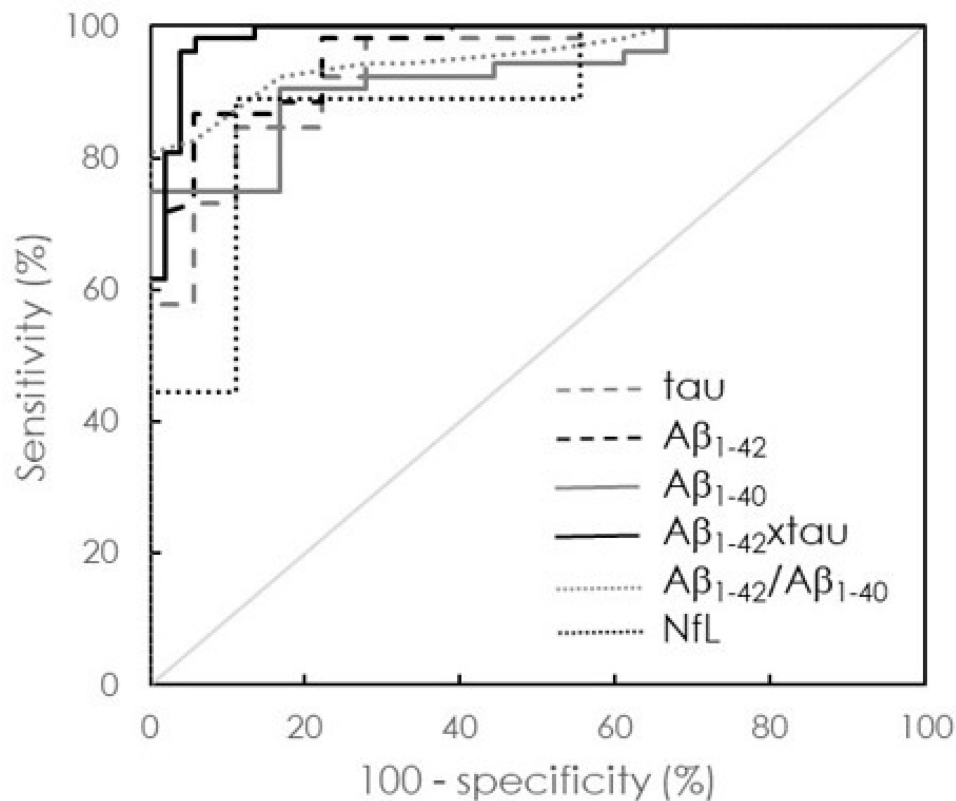


Figure 2 Receiver operating characteristic curve analysis for discriminating hemodialysis participants from controls.

An association between anemia and hypoalbuminemia with CI in adults has been reported.^{38,39} A correlation has also been reported between HD participants with CI and lower serum albumin and hemoglobin levels.³⁷ In addition, the hemoglobin level was negatively correlated with striatum function in HD participants.³⁸ Our HD participants were well nourished and presented with normal hemoglobin levels based on laboratory measurements. Thus, we suppose that this is one of the reasons why it was not possible to distinguish between normal and subtle CI in our HD participants. Through correlation analysis, we found that plasma tau protein was weakly negatively correlated with sex and Kt/V-urea and $A\beta_{1-40}$ were weakly negatively correlated with hypertension. In contrast, the $A\beta_{1-42}$ level was positively correlated with cholesterol levels (Table 3). In a healthy Taiwanese population study, men had more comorbidities than women.⁴⁰ Our HD participants had similar comorbidities and Kt/V-urea values in men and women (data not shown). Therefore, an exact explanation for the results of the correlation analysis cannot be drawn from our study. A larger sample size and longer observational period are necessary to address this epiphenomenon in the HD population.

Table 5 Spearman Correlation Between Orientation to Time, Orientation to Place, Attention and Calculation, Recall, Language and Plasma Biomarkers in Hemodialysis Subjects (n = 7)

Item	tau		$A\beta_{1-42}$		$A\beta_{1-40}$		$A\beta_{1-42} \times \text{tau}$		r
	r	p	r	p	r	p	r	p	
Orientation to time	0.4062	0.3659	-0.1878	0.6868	-0.2695	0.5589	0.3818	0.3981	0.2238
Orientation to place	0.4062	0.3659	-0.1878	0.6868	-0.2695	0.5589	0.3818	0.3981	0.2238
Attention and calculation	0.6079	0.1476	-0.2056	0.6583	0.1385	0.7672	0.5779	0.1741	-0.1248
Recall	-0.4431	0.3194	0.2653	0.5654	0.2832	0.5382	-0.4153	0.3541	-0.2141
Language	-0.01311	0.9778	-0.6876	0.0878	0.5357	0.2153	-0.1106	0.8133	-0.6301

Our study has a few limitations. First, we measured plasma biomarkers for cognitive function at one time point. Considering the time effect on cognitive function changes, a longitudinal observation is optimal for evaluating the patterns of these biomarkers between HD participants and healthy controls. Second, it is difficult to determine whether a participant is cognitively normal. Mental tests, such as the MMSE, are time-consuming and rely heavily on individual understanding. Therefore, it is reasonable to evaluate cognitive function using sufficient longitudinal data or consensus tests. Our HD participants were considered to have normal or subtle CI, based on their MMSE test and CDR values. Concerning the difficulty of completing the MMSE test in participants with advanced CI, underestimation cannot be completely ruled out in our study. However, it is worth investigating the clinical significance of nanoparticle-based plasma neurodegenerative biomarkers in HD participants with advanced CI to predict future outcomes. Third, the number of participants in our study was relatively small, and the study was conducted at one HD center. The study results may not be generalizable to other HD cohorts in individual HD centers. Our study also presents several hotspots for estimating CI using nanoparticle-based plasma neurodegenerative biomarkers in HD participants with grossly normal cognitive function. First, the levels of these plasma biomarkers were higher in HD participants with grossly normal cognitive function than in healthy controls, and they demonstrated a high AUC to distinguish them from healthy controls. These observations provide a possible strategy for using the plasma A β , tau, and NfL levels to monitor long-term cognitive function in the HD population. Second, it was difficult to distinguish normal cognitive function from subtle CI in HD participants using the plasma biomarkers tested in our study. Given this result, medical providers need to explore more neurodegenerative biomarkers for the early detection of subtle CI in HD participants. Finally, these plasma biomarkers cannot be correlated with MMSE parameters in HD participants with grossly normal cognitive function. At present, the MMSE is appointed as a formal tool by the Taiwanese government to estimate cognitive function in healthy reimbursement. Our study results raise a discussion on whether the application of other mental evaluation tools in HD participants could be linked to plasma neurodegenerative biomarker measurements. Therefore, a potential therapeutic strategy is possible to prevent CI in HD patients by medical providers.

Conclusions

HD participants demonstrated significantly higher plasma levels of A β ₁₋₄₂, tau protein, A β ₁₋₄₂ \times tau, NfL, and A β ₁₋₄₂/A β ₁₋₄₀ and significantly lower plasma A β ₁₋₄₀ levels than healthy controls. However, these plasma biomarkers cannot be used to distinguish between HD participants with normal cognitive function and those with subtle CI. The application of these plasma neurodegenerative biomarkers to evaluate long-term cognitive function in HD participants requires further investigation.

List of Abbreviations

AD, Alzheimer's disease; AUC, area under the curve; CDR, clinical dementia rating; CI, cognitive impairment; CKD, chronic kidney disease; HD, hemodialysis; IMR, immunomagnetic reduction; MMSE, Mini-Mental State Examination; NfL, neurofilament light chain; ROC, receiver operating characteristic.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

The study protocol was approved by the Committee on Human Research at Kaohsiung Chang Gung Memorial Hospital in Taiwan (number: 202100284B0). The study was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all subjects involved in the study.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Disclosure

All authors declare no conflicts of interest in this work.

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