Neurotoxicology 73 (2019) 85–91

Contents lists available at ScienceDirect

Neurotoxicology

journal homepage: www.elsevier.com/locate/neuro

Full Length Article

Indole-3 acetic acid increased risk of impaired cognitive function in patients receiving hemodialysis

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ARTICLE INFO

Keywords:
Indole-3 acetic acid
Cognitive function
Dementia
Chronic kidney disease
Hemodialysis

ABSTRACT

Patients receiving hemodialysis (HD) have a higher risk of cognitive impairment and dementia than the general population. The accumulation of uremic toxins in the brain causes uremic encephalopathy, however, limited data exists to elucidate the effect of protein-bound uremic toxins on cognitive function. Here we investigate the effect of indole-3 acetic acid (IAA) and hippuric acid (HA), two different protein-bound uremic toxins from amino acid derivatives, on cognitive function by *Silico* and in a clinical study. Prevalent HD patients were enrolled in two independent hospitals. Serum IAA and HA were measured using mass spectrometry. Cognitive performance was measured using Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), and Cognitive Abilities Screening Instrument (CASI) by trained psychologists. Using *silico* data to predict the effect of blood-brain barrier penetration was performed. The *silico* data demonstrated that IAA and HA had positive blood-brain barrier penetration ability. Amongst the 230 HD patients, serum IAA was associated with poor MMSE score (β = -0.90, 95% CI -1.61 to -0.19) and poor CASI score (β = -3.29, 95% CI -5.69 to -0.88) in stepwise multiple linear regression analysis. In logistic regression model, Serum IAA was also associated with cognitive impairment based on MMSE definition (OR, 1.96, 95% CI 1.10, 3.5) and CASI definition (OR, 2.09, 95% CI 1.21, 3.61). There was no correlation between Serum HA levels and cognitive function status. In conclusion, IAA, not HA, was associated with cognitive impairment in HD patients. Further large scale and prospective studies are needed to confirm our findings.

1. Introduction

End stage renal disease (ESRD) has higher rate of cognitive impairment in patients compared to the general population (Collins et al., 2007; O'Lone et al., 2016). The risk of hospitalization, disability, dialysis withdrawal and mortality rate increase with the presence of dementia (Kurella et al., 2006; Kurella Tamura et al., 2009). Patients with chronic kidney disease (CKD) have more nephrogenic related risk factors (Bugnicourt et al., 2013; Kurella Tamura and Yaffe, 2011) for dementia in addition to the common risk factors observed in the general population (Knopman et al., 2001; Kurella Tamura and Yaffe, 2011). Besides cerebrovascular disease and anemia, secondary hyperparathyroidism, dialysis disequilibrium, and uremic toxins (Bugnicourt et al., 2013; Kurella Tamura and Yaffe, 2011), are the major causes of

Abbreviations: IAA, indole-3 acetic acid; HA, hippuric acid; CKD, chronic kidney disease; HD, hemodialysis; MMSE, Mini-Mental State Examination; CASI, Cognitive Abilities Screening Instrument; MoCA, Montreal Cognitive Assessment; LC- MS/MS, Liquid chromatography tandem mass spectrometry; MRM, multiple reaction monitoring

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https://doi.org/10.1016/j.neuro.2019.02.019
Received 27 June 2018; Received in revised form 25 January 2019; Accepted 26 February 2019
Available online 28 February 2019
0161-813X/

cognitive impairment in ESRD patients. The accumulation of uremic solutes in the plasma and the brain (Chillon et al., 2016) causes severe organ damage, but the direct effect of uremic toxins on the brain remains unclear. Uremic toxins can be classified into three categories, such as small water-soluble solutes, protein-bound solutes, and middle molecules by molecular weight and protein binding property (Jourde-Chiche et al., 2009; Vanholder et al., 2008). One of the most important protein uremic toxins, indoxyl sulfate, is reportedly associated with cognitive impairment on CKD in both clinical (Yeh et al., 2016) and basic studies (Adesso et al., 2017). Indole is metabolized from tryptophan fermentation by gut microbiome. Indole is further metabolized into indoxyl sulfate, which can cross the blood brain barrier (BBB), accumulate in brains as a results of BBB transporter dysfunction (Watanabe et al., 2014), and is toxic to astrocytes and microglia (Adesso et al., 2017). However, limited studies have investigated the levels of other protein-bound uremic toxins on cognitive function. Indole-3-acetic acid (IAA) is also a protein-bound uremic solute from tryptophan metabolism (Fernandez-Prado et al., 2017; Jourde-Chiche et al., 2009). IAA was reported to predict mortality and cardiovascular events in CKD patients (Dou et al., 2015). In cultured human endothelial cells, IAA activated an inflammatory AhR/p38MAPK/NF-κB pathway that induced the proinflammatory enzyme cyclooxygenase-2, and increased ROS production and tissue factor expression (Dou et al., 2015; Gondouin et al., 2013). Oxidative stress and neuroinflammation are also known to contribute to the pathogenesis of neuronal degeneration (Hsieh and Yang, 2013). Therefore, we hypothesize that IAA is associated with cognitive function decline in patients with CKD. Additionally, urinary levels of hippuric acid (HA), another protein-bound uremic toxin are reportedly elevated in patients with major depressive disorders and in rat models of depression (Zheng et al., 2013). Thus, we aim to investigate the associations of IAA and HA with cognitive function among patients receiving HD.

2. Methods

2.1. Subjects

We enrolled participants from two hospital-based dialysis units (Kaohsiung Medical University Hospital and Kaohsiung Municipal Hsiao-Kang Hospital) from August 2016 through January 2017. Patients aged over than 30 years with maintenance dialysis for at least 90 days were recruited. Patients who had existing diagnosis of cerebrovascular disease (N = 39) and dementia (N = 6) were excluded. Neuropsychological tests were performed in a total of 275 patients by trained psychologists. The final analysis excluded patients who failed to complete the neuropsychological tests (N = 45) (Supplementary Fig. 1). All patients received regular HD with high efficiency dialyzers three times per week at 250 to 300 ml/min blood flow rate, 500 ml/min on dialysate flow, and lasted 3.5–4 hours per session. The study protocol was approved by the Institutional Review Board of Kaohsiung Medical University (KMUHIRB-E(I)-20160095). All subjects signed the informed consent form.

2.2. Cognitive function assessment

Three cognitive function tests were used in the study: Mini-Mental State Examination (MMSE) (Costa et al., 2014; Folstein et al., 1975; Sarnak et al., 2013), Montreal Cognitive Assessment (MoCA) (Costa et al., 2014; Foster et al., 2016; Nasreddine et al., 2005), and Cognitive Abilities Screening Instrument (CASI) (Hsieh et al., 2009; Lin et al., 2002; Teng et al., 1994) (Supplementary Table 1). The MMSE contains items on orientation, registration, attention and calculation, recall, language, and praxis and is the most widely used screening tool for cognitive function impairment. The maximum score was set as 30 and cognitive impairment was defined as a score of less than 24 (Fadili et al., 2014; Jung et al., 2013; Odagiri et al., 2011). The MoCA, a more sensitive 30-point screening tool. The cognitive impairment was defined as a score ≤ 24, which reported as the optimal cut-off score in detecting cognitive impairment (Tiffin-Richards et al., 2014). The tasks encompass multiple domains of cognition including short-term memory, visuospatial abilities and executive function, language, attention, concentration and working memory, and orientation to time and place. The CASI is designed to assess a broad range of cognitive domains with a 40-item global cognitive test, which contains nine cognitive evaluation domains including long-term memory, short term memory, orientation, attention, mental manipulation, list-generating fluency, language, abstraction/judgment, and drawing. The cognitive impairment cutoff value of CASI was based on age and educational status divided into three categories (Lin et al., 2002). The definition was no formal education using CASI < 50, received 1–5 years of schooling using CASI < 68, and received 6 or more years of education using CASI < 80. In addition, the Center for Epidemiologic Studies Depression Scale (CES-D) was used to determine depression symptoms score with scores ranging from 0 to 30 and 16/17 as cut-off point for depression screening positive outcome (Hedayati et al., 2006).

2.3. Comorbidity, biochemical measurements and blood-brain barrier prediction

Each HD unit maintains a registry that collects patient information longitudinally using an electronic healthcare record system. From this registry, it was possible to extract individual-level information on factors such as: sociodemographic data (age, sex, and years of education), primary cause of kidney failure (hypertension, diabetes, glomerulonephritis, and others), time on dialysis, dialysis access (fistula vs. graft), medical history, and biochemical data for all participants. Patients with HbA1C of 6.5% or higher, or taking antidiabetic drugs were defined as diabetes mellitus. Hypertension was defined by a blood pressure of 140/90 mmHg or higher or taking antihypertensive drugs. Blood samples were obtained at the beginning of the week after overnight fasting from patients through the arteriovenous fistula or graft immediately before their scheduled HD session. Biochemical data included serum values for hemoglobin, albumin, alanine transaminase, alkaline phosphatase, lipid profiles, uric acid, blood urea nitrogen, creatinine, C-reactive protein, sodium, potassium, ion calcium, phosphorous, parathyroid hormone, iron, aluminum, magnesium, and zinc levels from routine blood samples obtained within 30 days before cognitive evaluation at the middle of the HD session. Serum levels of IAA and HA were measured using tandem mass spectrometry method (Supplemental Material). The prediction of toxins' movement to cross BBB by using *silico* data prediction was also performed (Supplemental Material).

2.4. Statistical analysis

Continuous variables of demographic data were displayed as mean ± SD and ordinal and nominal variables were presented as percentages. The distribution of log transformed free IAA and free HA values in HD participants were plotted with a neuropsychiatric test score. The relationships among serum IAA and HA levels with scores on the MMSE were assessed by multiple linear regression models with adjustment for age, sex, education level, comorbidities, baseline laboratory data, depression scale, and hemodialysis duration as potential confounders. The same model was applied for MoCA and CASI tests. Relevant demographic parameters were also analyzed by a forward stepwise selection with p-values for independent variables to enter and to stay in the models set at 0.2 and subsequently, a final elimination step at $P < 0.05$. Results were reported as beta coefficient (β) and ORs with 95% CIs, and a two-tailed $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SAS version 9.4 and STATA version 14.

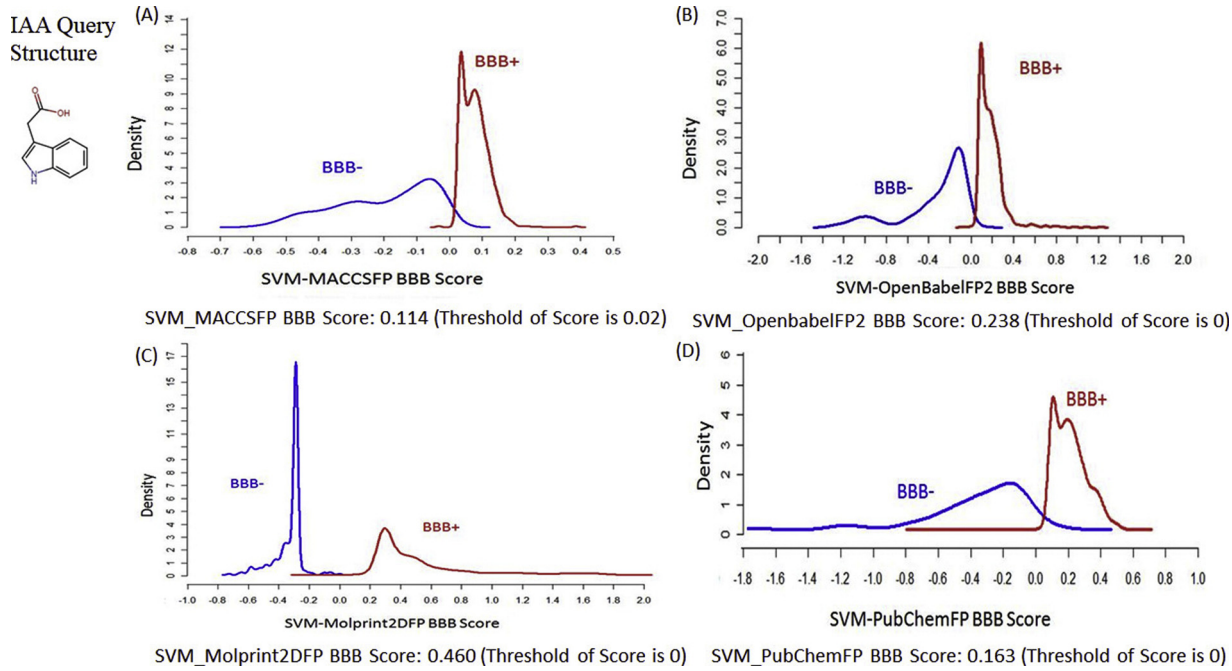

Fig. 1. *silico* data predict blood brain barrier penetrative effect of indole-3 acetic acid.

3. Results

3.1. Prediction of the blood-brain barrier permeably of Indole-3-acetic acid and Hippuric acid

The ability or inability of a compound to penetrate the BBB depends on the BBB-score in the selected algorithm. A score of more than zero rates a compound as BBB + . The online BBB predictor classifies IAA as a BBB + compound. SVM algorithms scores on four types of fingerprints were 0.114 using MACCSFP (threshold of score is 0.02), 0.238 using OpenbabelFP2 (threshold of score is 0), 0.460 using Molprint2DFP (threshold of score is 0), and 0.163 using PubChemFP (threshold of score is 0) (Fig. 1). Furthermore, using the ADMET prediction, the BBB penetration probability of IAA was 0.9852 and it also confirmed that IAA can easily cross the BBB. As for HA, SVM algorithms scores on four types of fingerprints were 0.078 using MACCSFP (threshold of score is 0.02), 0.146 using OpenbabelFP2 (threshold of score is 0), 0.681 using Molprint2DFP (threshold of score is 0), and 0.287 using PubChemFP (threshold of score is 0) (data not shown). Using ADMET prediction, the BBB penetration probability of HA was 0.9310. Using the integrated online ADMET predition, both IAA and HA have the ability of BBB penetration (Supplementary Fig. 2).

3.2. Multiple reaction monitoring chromatograms and mass spectra

The gradient condition of mobile phase was shown in Supplementary Table 2, Table 3. MRM transitions and conditions for analysis of IAA and HA were demonstrated in Supplementary Table 4. The protonated molecule of IAA at *m/z* 176.1 fragmented in MS-MS to give a major product ion at *m/z* 130.0 (used for quantification), and second abundant ion at *m/z* 77.1 (used for qualification). (Supplementary Figs. 6 and 7).

3.3. Demographic and clinical characteristics

In the HD cohort, 230 participants who completed the baseline cognitive assessments were included in this analysis. The mean age of the study cohort was 57.6 ± 11.2, 54% were male, 74% had hypertension, 38% had diabetes. The major cause of ESRD in the HD cohort was glomerulonephritis (39%) and diabetes (31%) (Table 1). The mean years of HD duration 7.02 ± 5.92 and most of the vascular access in these patients were arteriovenous fistula (89%). The mean dialysis duration was 7.02 ± 5.92 years and Karnofsky performance scale was 82.89 ± 11.33. Clinical laboratory data of HD patients were shown in Table 1. The mean serum levels of the data were 10.72 ± 1.22 mg/dl on hemoglobin, 3.9 ± 0.27 mg/dl on albumin, 2.58 ± 4.19 mg/dl on C-reactive protein, and 4.66 ± 1.09 mmole/l on phosphate (Table 1). The mean serum levels of IAA and HA were 1.09 ± 0.74 μg/ml and 36.65 ± 29.72 μg/ml. The mean scores of the neuropsychiatric test were 24.38 ± 4.80 in MMSE, 21.36 ± 5.81 in MoCA, and 81.26 ± 16.88 in CASI (Table 2). Cognitive impairment of the HD patients was observed in 55/230 (23.91%) as defined by a MMSE score < 24 and 51/230 (22.17%) as defined by a CASI score level with education.

3.4. Associations of serum IAA and HA levels and cognitive impairment in HD patients

The scatter plot with cubic spline demonstrated the association of IAA, HA and cognitive function on MMSE, CASI, and MoCA total scores. There was negative association between IAA and MMSE (r = -0.18, p = 0.005), MoCA (r = -0.13, p = 0.057), and CASI (r = -0.20, p = 0.003) (Fig. 2A–C). The association between HA and cognitive function was statisticaly insignificant on MMSE, MoCA, and CASI (Fig. 2D–F). In linear regression models adjusted for age, sex, education level, higher levels of IAA were associated with poorer MMSE scores (β coefficient -0.95, 95% confidence interval [CI] -1.68 to -0.22) and poorer CASI scores (β coefficient -3.29, 95% CI -5.77 to -0.82). As for CASI cognitive evaluation domain, IAA levels were associated with poorer long term memory (β coefficient -0.30, 95% CI -0.56 to -0.04), short term memory (β coefficient -0.64, 95% CI -1.07 to -0.2), and mental manipulation (β coefficient -0.64, 95% CI -1.03 to -0.26) (Supplementary Fig. 3B). Associations of serum IAA and HA levels with the composite scores of cognitive performances were evaluated with multivariable linear regression analyses. Serum IAA levels were negative associated with MMSE (β coefficient -1.31, 95% CI -2.14 to -0.49), MoCA (β coefficient -1.04, 95% CI -2.06 to -0.02), and CASI (β coefficient -4.75, 95% CI -7.42 to -1.86) in crude model (Table 3; model 1). Further adjustment

Table 1
Baseline characteristics of hemodialysis participants.

Patients (N = 230)	
Age (years)	57.6 ± 11.2
Male	124 (53.9%)
Education	
No	9 (3.9%)
Elementary school	44 (19.1%)
Junior high school	49 (21.3%)
Senior high school	70 (30.4%)
College	58 (25.2%)
Cause of ESRD	
Hypertension	22 (9.6%)
Diabetes Mellitus	71 (30.9%)
Glomerulonephritis	90 (39.1%)
Others[a]	47 (20.4%)
Arteriovenous shunt	
Arteriovenous fistula	205 (89.1%)
Arteriovenous graft	25 (10.9%)
Hemodialysis duration (years)	7.02 ± 5.92
Karnofsky performance scale	82.89 ± 11.33
Comorbidities	
Diabetes mellitus	87 (37.8%)
Hypertension	170 (73.9%)
Heart failure	50 (21.7%)
Dyslipidemia	78 (33.9%)
Coronary artery disease	48 (20.9%)
Clinical laboratory data	
Hemoglobin (mg/dl)	10.72 ± 1.22
Albumin (mg/dl)	3.9 ± 0.27
Alanine transaminase (U/L)	13.87 ± 8.14
Alkaline phosphatase (mg/dl)	211.67 ± 189.21
Total cholesterol (mg/dl)	167.03 ± 39.06
Triglyceride (mg/dl)	155.82 ± 96.55
Low-density lipoprotein (mg/dl)	95.86 ± 32.41
Uric acid (mg/dl)	7.5 ± 1.45
Blood urea nitrogen (mg/dl)	65.56 ± 13.98
Creatinine (mg/dl)	10.28 ± 1.92
C-reactive protein (mg/dl)	2.58 ± 4.19
Sodium (mmole/l)	138.49 ± 3.07
Potassium (mmole/l)	4.51 ± 0.61
Ion calcium (mmole/l)	4.62 ± 0.46
Phosphate (mmole/l)	4.66 ± 1.09
Parathyroid hormone (pg/ml)	36.19 ± 15.09
Iron (μg/dl)	63.43 ± 23.09
Aluminum (μg/l)	20.45 ± 14.93
Magnesium (mg/dl)	2.61 ± 0.40
Zinc (μg/dl)	95.65 ± 16.31
Single pool Kt/V	1.56 ± 0.24
Uremic toxins	
Indole-3 acetic acid (μg/ml)	1.09 ± 0.74
Hippuric acid (μg/ml)	36.65 ± 29.72

[a] Other causes of end-stage renal disease include polycystic kidney disease, tumor, IgA nephropathy, Systemic Lupus Erythematosus, gout, interstitial nephritis.

Table 2
Neuropsychiatric test of hemodialysis participants.

Patients (N = 230)	
Mini–Mental State Examination (MMSE)	24.38 ± 4.80
Montreal Cognitive Assessment (MoCA)	21.36 ± 5.81
The Cognitive Abilities Screening Instrument (CASI)	81.26 ± 16.88
Long term Memory	9.48 ± 1.62
Short term Memory	8.2 ± 2.91
Attention	6.82 ± 1.74
Mental manipulation	7.82 ± 2.55
Orientation	16.24 ± 3.75
Abstract thinking	8.12 ± 2.86
Language	9.16 ± 1.74
Spatial construction	7.96 ± 2.91
Name fluency	7.47 ± 2.56
Center for Epidemiological Studies Depression (CESD)	10.95 ± 7.34

for age, sex, education level, the correlation remained significant for MMSE and CASI (Table 3; model 2). We further applied forward stepwise variable selection in the multiple linear analysis, serum IAA was negative correlated to MMSE (β coefficient -0.90, 95% CI -1.61 to -0.19) and CASI (β coefficient -3.29, 95% CI -5.69 to -0.88) but not MoCA (β coefficient -0.70, 95% CI -1.54 to 0.14). Serum HA levels were not associated with cognitive performance in crude model (Table 3; model 1) and stepwise selection model (Table 3; model 3). Multiple linear regression analysis of all variables and cognitive function (MMSE, CASI), there were statistically significant negative association on IAA, age, and serum potassium level. Education levels were statistically significantly positive associated with cognitive function (Supplementary Table 5). Using logistic regression model with stepwise variable selection, higher serum IAA levels were related to increase risk of cognitive impairment based on MMSE definition (OR 1.96, 95% CI 1.10–3.50) and CASI definition (OR 2.09, 95% CI 1.21–3.61, Table 4). The serum HA levels were not correlated to the cognitive impairment (Table 4).

4. Discussion

In the present study, we identified IAA but not HA as being associated with cognitive impairment among ESRD patients. This is the first clinical study to elucidate the inverse association between IAA and cognitive functions measured by MMSE, MoCA and CASI based on both scatter plot and regression analysis. Higher serum IAA levels were related to increased risk of cognitive impairment, as defined by the MMSE and CASI assessments. Stratified analysis demonstrated the highest IAA levels were correlated to the worst cognitive function. The penetration of IAA through BBB was predicted by using *silico* data. These data supported the effect of protein-bound uremic toxins on cognitive function in HD patients.

Among patients receiving HD, the causes of cognitive impairment are multifactorial, which includes dialysis disequilibrium, secondary hyperparathyroidism, cerebrovascular disease, and uremic toxins accumulation (Bugnicourt et al., 2013; Kurella Tamura and Yaffe, 2011). As kidney function declines, plasmatic level of uremic toxins increase and these toxins are believed to be the major cause of cognitive impairment. Indoxyl sulfate (IS), which is the most well-known protein-bound uremic toxins, may have direct neurotoxic or vascular effects on cerebrovascular diseases and cognitive function (Chillon et al., 2016). IS activates the aryl hydrocarbon receptor (AhR), which is a ligand-activated transcriptional factor,(Watanabe et al., 2013) that may contribute to neurotoxicity through the mechanism of endothelial dysfunction (Gondouin et al., 2013; Schroeder et al., 2010). Clinical study demonstrated the association between higher IS levels and impaired executive function among patients with CKD (Yeh et al., 2016). However, no data is available regarding IAA and cerebro-renal interaction. Similar to IS, IAA which belongs to the family of indolic uremic solutes (Duranton et al., 2012) are metabolized from tryptophan fermentation by the gut microbiome (Evenepoel et al., 2009). IAA can also be metabolized in tissue from tryptamine (Coppen et al., 1974) and other tryptophan derivatives. The efflux of IAA from the brain to the blood stream across the BBB was regulated by OAT1 and OAT3 (Deguchi et al., 2006). IAA is an agonist of the transcription factor AhR (Gondouin et al., 2013) and also activated an inflammatory non-genomic AhR/p38MAPK/NF-κB pathway in cultured human endothelial cells (Dou et al., 2015). IAA induces endothelial inflammation and oxidative stress in *vitro* study and significant predictor of mortality and cardiovascular events in clinical study (Dou et al., 2015). The toxic mechanism of IAA has been reported to include tissue factor production in endothelial, peripheral blood mononuclear cells by AhR (Gondouin et al., 2013), inhibition of UDP-glucuronosyltransferase activity, and mitochondrial activity (Mutsaers et al., 2013). Administration of IAA to pregnant mice decreased neuron formation and induced microencephaly in the fetus (Furukawa et al., 2007). The decreased neuron

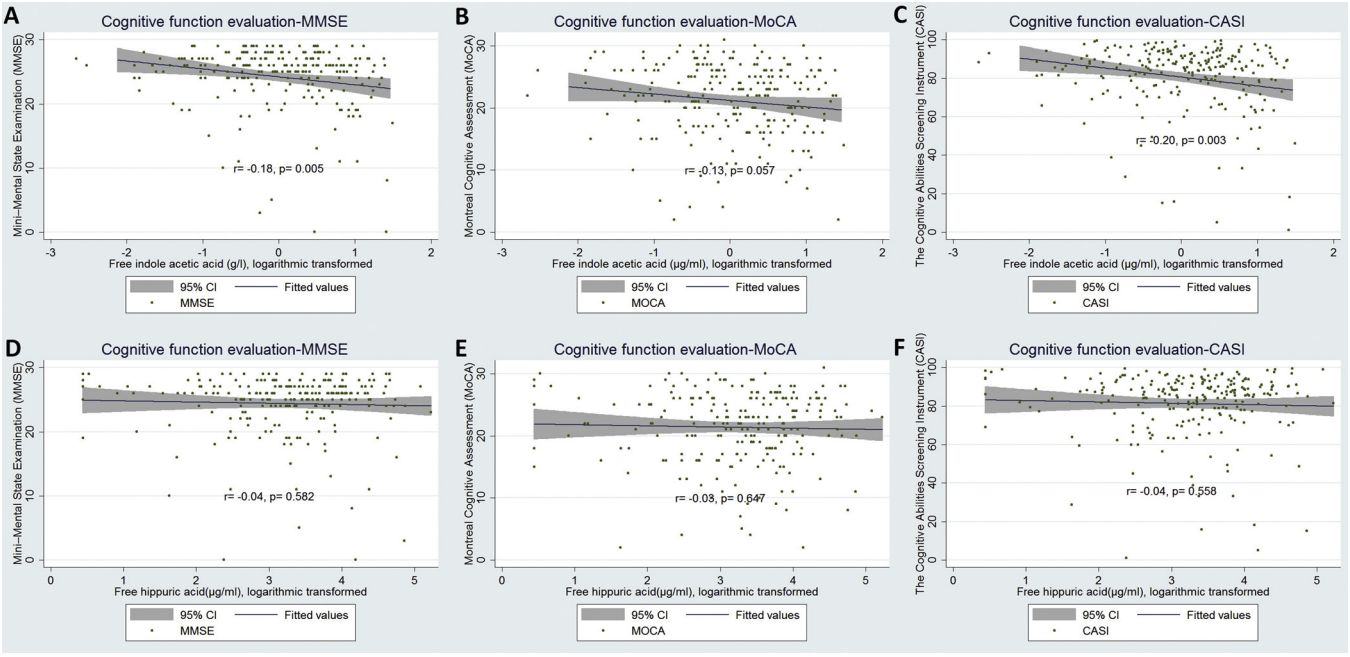

Fig. 2. A–F. Scatter plot with spline line demonstrated the association of indole-3 acetic acid and hippuric acid and cognitive function test (MMSE, MoCA, CASI).

formation and increased cell damage in animal or cell culture experiments (Watanabe et al., 2014) partially explained the present findings of cognitive impairment related to serum IAA levels. Besides, IAA was reported correlate with anxiety and depression in CKD patients (Karu et al., 2016). However, in the previous study, the cognitive function decline had statistically insignificant correlation with serum IAA levels because of study limitations on small sample size and cognitive function assessed via self-report of Kidney Disease Quality of Life short-form (Karu et al., 2016). In our study, we enrolled 230 HD patients and performed standardized clinical interviews of different cognitive function tests. As the results shown, the cognitive function was statistically negative associated with serum IAA levels.

As for HA, we demonstrated the ability of BBB penetration in *silica* data. In previous study, The serum and cerebrospinal fluid concentrations of HA are positively correlated with neurophysiological indices (Schoots et al., 1989), imply that HA may induce neurological symptoms, perhaps via dysfunction of the OAT system at the BBB (Bahn et al., 2005; Ohtsuki et al., 2002). However, we demonstrated that higher serum HA levels are insignificantly associated with poorer cognitive function. The different results of HA could be related to the dialyzer membrane, and the conditions under which the dialyzer is operated. Furthermore, HD is rather limited in improving the clearance of protein-bound uremic toxins depending on their conjugates and affinity to albumin. The protein binding ability is 86–94% in IAA but 42–48% in HA (Florens et al., 2018). Total removal depends on how rapidly the solute unbinds from its carrier protein as the free concentration decreases. Because of the different binding ability of IAA and HA, IAA can hardly be removed during HD session but parts of the HA may be removed by HD (Zimmerman et al., 1990). This may partly explain the different cognitive function results between IAA and HA.

Table 3
Association between indole-3 acetic acid, hippuric acid and cognitive function test in hemodialysis participants using multivariable linear regression analysis.

	Indole-3 acetic acid			Hippuric acid		
Cognitive test	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
MMSE	−1.31 (−2.14, −0.49)	−0.95 (−1.68, −0.22)	−0.90 (−1.61, −0.19)	−0.01 (−0.03, 0.01)	−0.02 (−0.04, −0.002)	−0.02 (−0.04 to −0.0008)
MoCA	−1.04 (−2.06, −0.02)	−0.51 (−1.37, 0.35)	−0.70 (−1.54, 0.14)	0.00 (−0.02, 0.03)	−0.01 (−0.04, 0.01)	−0.01 (−0.03 to 0.01)
CASI	−4.75 (−7.42, −1.86)	−3.29 (−5.77, −0.82)	−3.29 (−5.69, −0.88)	−0.01 (−0.09, 0.06)	−0.06 (−0.12, 0.01)	−0.04 (−0.10 to 0.02)

Note: MMSE, Mini-Mental State Examination; CASI, Cognitive Abilities Screening Instrument; MoCA, Montreal Cognitive Assessment.
Multivariable linear model demonstrated as Beta coefficient (β) with 95% Confidence intervals (CI).
Model 1 is crude estimation.
Model 2 is adjusted for age, sex, education level.
Model 3 is adjusted for stepwise procedure selected covariates.
In Indole-3 acetic acid analysis: Covariates selection of age, education, hyperlipidemia, albumin, sodium, potassium, blood urea nitrogen, and Low-density lipoprotein in MMSE, Covariates selection of age, education, depression scale, hyperlipidemia, heart failure, ion calcium, phosphate, magnesium, aluminum, blood urea nitrogen, alkaline phosphatase, C-reactive protein, and triglyceride in MoCA, Covariates selection of age, education, hyperlipidemia, diabetes, albumin, sodium, potassium, ion calcium, blood urea nitrogen, alkaline phosphatase, total cholesterol in CASI.
In Hippuric acid analysis: Covariates selection of age, education, hyperlipidemia, albumin, potassium, aluminum, blood urea nitrogen, and total cholesterol in MMSE, Covariates selection of age, education, depression scale, hyperlipidemia, heart failure, hypertension, aluminum, blood urea nitrogen, alkaline phosphatase, and triglyceride in MoCA, Covariates selection of age, education, hyperlipidemia, diabetes, albumin, sodium, potassium, aluminum, blood urea nitrogen, alkaline phosphatase, and total cholesterol in CASI.

Table 4
Association between indole-3 acetic acid, hippuric acid and cognitive function impairment in hemodialysis participants using multivariable logistic regression analysis. Cognitive impairment was defined by MMSE < 24 and CASI based on different education levels.

	Indole-3 acetic acid		Hippuric acid	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
MMSE	1.96 (1.10, 3.50)	0.023	1.00 (0.99, 1.02)	0.880
MoCA	1.04 (0.67, 1.59)	0.870	1.00 (0.99, 1.02)	0.432
CASI	2.09 (1.21, 3.61)	0.008	1.00 (0.98, 1.02)	0.994

Note: MMSE, Mini-Mental State Examination; CASI, Cognitive Abilities Screening Instrument; MoCA, Montreal Cognitive Assessment.
Model is adjusted for stepwise procedure selected covariates.
In Indole-3 acetic acid analysis: Covariates selection of age, education, depression scale, diabetes, hypertension, hyperlipidemia, coronary artery disease, heart failure, albumin, sodium, ion calcium, phosphate, alkaline phosphatase, uric acid, Kt/V, and hemodialysis duration in MMSE, Covariates selection of age, education, hypertension, hemoglobin, ion calcium, triglyceride, and hemodialysis duration in MoCA, Covariates selection of age, sex, education, depression scale, diabetes, hyperlipidemia, albumin, sodium, potassium, ion calcium, magnesium, alkaline phosphatase, blood urea nitrogen, alanine transaminase, and Kt/V in CASI.
In Hippuric acid analysis: Covariates selection of age, education, depression scale, diabetes, albumin, sodium, ion calcium, phosphate, uric acid, diabetes, alkaline phosphatase, Kt/V, and hemodialysis duration in MMSE, Covariates selection of age, education, hypertension, hemoglobin, ion calcium, triglyceride, and hemodialysis duration in MoCA, Covariates selection of age, sex, education, depression scale, diabetes, hypertension, heart failure, albumin, sodium, potassium, ion calcium, serum ion, zinc, blood urea nitrogen, alanine transaminase, alkaline phosphatase, and Kt/V in CASI.

The strength of the present study is to conduct several detailed neuropsychological assessments, including several timed tasks of attention and executive functions that appear to be especially sensitive to cognitive impairment in HD. Three different cognitive function tests of MMSE, MoCA and CASI were evaluated by well-trained psychologists to eliminate the bias from the diagnosis tools with various sensitivity and specificity. Additionally, we collected comprehensive data with complete baseline demographics, comorbidities, and clinical laboratory data to enable us to evaluate the independent effect of IAA on cognitive function by controlling for several confounders. However, some limitations still should be mentioned. Firstly, a causal relationship could not be inferred in this cross-sectional study. Due to convenience sampling, one cannot exclude the possibility of selection bias. Hence, longitudinal studies and longer follow-up should be conducted to clarify the issue. Secondly, not all possible parameters were included in this study such as dietary habits, genetic factors, and medications. Finally, no brain imaging was available to ascertain the etiology of cognitive impairment, so we excluded HD patients with existing diagnosis of dementia and cerebrovascular disease based on physician diagnosis and detailed medical records.

The etiology of cognitive impairment in HD patients is multifactorial. However, many risk factors contributing to cognitive decline are difficult to modify, such as pre-exist diabetes, hypertension, cardiovascular disease, or vascular calcification. The present study declared the association between IAA and cognition function. The toxicity has prompted efforts to lower the burden of protein-bound uremic toxins through dialytic and non-dialytic strategies. One alternative strategy to decrease circulating IAA level is to reduce the production. Since IAA is derived from the breakdown of dietary tryptophan by colon microbes, the production can be suppressed by restricting specific dietary protein intake, manipulating the colon microbial metabolism, or reducing intestinal absorption. For example, AST-120 treatment attenuated protein-bound uremic toxins accumulation in multiple organs, including brain tissue (Sato et al., 2017). There is potential treatment effect to modify or lower protein-bound uremic toxins, but further trials should be performed to verify the contribution of IAA to the cognitive impairment.

5. Conclusion

Serum levels of IAA but not HA were associated with cognitive impairment in patients receiving HD. This suggests that the effect of cognitive function by protein-bound uremic toxins. Thus, further studies to investigate the pathophysiology mechanism and investigate the removal of IAA from HD patients for improving the cognitive function are needed.

Author contribution statement

Dr. Yi-Ting Lin takes responsibility for the research design, the whole research process, and their analyses and interpretation. Dr. Ping-Hsun Wu takes responsibility for the research, data, and their analyses and interpretation. Dr. Mwenya Mubanga, Dr. Mei-Chuan Kuo, and Dr. Yi-Wen Chiu critically revised the manuscript for important intellectual content. Dr. Cheng-Sheng Chen takes responsibility for the evaluation of neuropsychological tests. Mrs. Hei-Hwa Lee takes responsibility for the measurement of indole-3 acetic acid and hippuric acid by tandem mass spectrometry. Prof. Shang-Jyh Hwang and Prof. Po-Lin Kuo take responsibility to the research design.

Conflicts of interest

I certify that there is no conflict of interest with any financial organization.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgements

The funding sources did not play any role in the design and conduct of the study, collection, management, analysis, and interpretation of the data, and preparation, review, or approval of the manuscript. The study was funded by grants from the Ministry of Science and Technology, Taiwan (NSC 106-2314-B-037 -054 and MOST 107-2314-B-037 -098 -MY3), Kaohsiung Medical University Hospital, Taiwan (KMUH105-5M11), KMU-KMUH Co-Project of key research grant from Kaohsiung Medical University, Taiwan (KMU-DK106004), and Kaohsiung Municipal Siaogang Hospital, Taiwan (Kmhk-105-006).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neuro.2019.02.019.

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