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Indoxyl sulfate, not p-cresyl sulfate, is associated with cognitive impairment in early-stage chronic kidney disease

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ABSTRACT

Backgrounds: Patients with chronic kidney disease (CKD) more commonly experience cognitive impairment, but the etiologies are not clear. Uremic toxins such as p-cresyl sulfate (PCS) and indoxyl sulfate (IS) have been shown to increase the risks of cardiovascular diseases and mortality; however, no study has investigated the associations of PCS and IS with cognitive function in patients with CKD.
Methods: Patients with CKD aged ≥50 years and age- and sex-matched non-CKD comparison subjects were recruited. CKD stage was defined according to the National Kidney Foundation guidelines. Cognitive function was evaluated using comprehensive neuropsychological tests. The associations between uremic toxins and cognitive function domains were examined using multiple linear regression analysis. The interaction between uremic toxins and CKD stages on cognitive functions were also examined.
Results: In total, 199 patients with CKD and 84 comparison subjects completed the study. The patients with CKD had poorer cognitive function and higher serum PCS and IS levels. A higher serum IS level was associated with poor executive function ($\beta = -0.31$, $P = 0.003$) only in stage 3 CKD patients after adjustment for age, sex and educational level. Serum PCS level was not associated with cognitive function in patients with CKD.
Conclusions: Our study showed that a higher serum IS level was associated with poor executive function in the early stage of CKD. It would be worthwhile to investigate the effect of IS removal in early-stage CKD on the prevention of cognitive impairment in future studies.

1. Introduction

Cognitive impairment in CKD is associated with poorer clinical outcomes (Radic et al., 2010; Sehgal et al., 1997; Kimmel et al., 1998; Murray and Knopman, 2010); thus, it has received a great deal of attention in recent years. Patients with CKD are at higher risk of cognitive decline and even dementia (Wang et al., 2010; Seliger et al., 2004). Poor cognitive function in various domains, such as memory, attention, executive function and visuospatial function, has been found in patients with CKD (Yang and Tsai et al., 2010; Elias et al., 2009; Tsai and Wang, 2010).

Uremic toxins levels increase as CKD progress, and they have been speculated to be one of causes of cognitive impairment in CKD. However, there is still little known about the role of uremic toxins. Among various uremic toxins, indoxyl sulfate (IS) and p-cresyl sulfate (PCS) were thought to be ones of most likely to affect this cerebro–renal interaction dysfunction (Watanabe et al., 2014). However, impacts and mechanisms of their effects on cognition are poorly understood.

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PCS and IS are protein-bound uremic toxins that originate via protein fermentation in the large intestine (Evenepoel et al., 2009). The levels of PCS and IS increase as CKD progresses, and the pre-dialysis concentrations of PCS and IS are 41-fold and 116-fold greater as compared with those of normal subjects (Sirich et al., 2014). Even on dialysis, tight protein binding severely limits solute clearance by dialysis (Meyer et al., 2004). Recent studies have shown direct associations of PCS and IS with cardiovascular diseases in CKD patients (Meijers et al., 2010; Barreto et al., 2009); meanwhile, cardiovascular diseases have been linked to an increased risk of dementia in the general population (Newman et al., 2005; Paciaroni and Bogousslavsky, 2013). Animal studies have also suggested that IS may have a direct impact on the central nervous system, being involved in the circadian rhythm in rats and murine spinal cord neurons (Iwata et al., 2007; D'Hooge et al., 2003). However, no study has directly investigated the associations between uremic toxins and cognitive function in humans. In this study, we will also explored which cognitive domains were affected by uremic toxins.

Cognitive impairment in CKD becomes apparent as CKD progresses (Yang and Tsai et al., 2010; Tamura and Yaffe et al., 2008), and the levels of PCS and IS also increase in inverse relationships with renal function (Meijers et al., 2010; Barreto et al., 2009). If uremic toxins are associated with cognitive impairment, another question is whether there exist interactions between PCS/IS levels, CKD stage and cognitive function. Thus, in this study, we aimed to investigate the associations of PCS and IS with cognitive function among patients with CKD and to examine whether PCS and IS have different effects on cognitive function at different stages of CKD.

2. Methods

2.1. Participants

Study participants who 1) were aged ≥50 years; 2) met the criteria for CKD according to the National Kidney Foundation Kidney Disease Outcome Quality Initiative (NKF KDOQI); and 3) had an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 consecutively treated in the Outpatients Department at a university teaching hospital were recruited. The exclusion criteria were patients who were on dialysis, had ever received renal transplantation, or had any other DSM-IV-TR Axis I diagnosis or any clinical history of major neurological disorders, including dementia. Advertisements were posted at the hospital to enroll the comparison group, and the inclusion and exclusion criteria were similar to those of the patient group, with the exceptions that they had no clinical history of CKD and an eGFR ≥60 mL/min/1.73 m^2. The eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) estimation equation (Levey et al., 2007), which is the most recently advocated formula for calculating the GFR and is assumed to be the gold standard of renal function. CKD stage was defined according to the National Kidney Foundation as follows: stage 3, eGFR 30–59 mL/min/1.73 m^2; stage 4, eGFR 15–29 mL/min/1.73 m^2; stage 5, eGFR <15 mL/min/1.73 m^2. The study protocol was approved by the Institutional Review Board of Kaohsiung Medical University. Informed consent was obtained from all subjects.

2.2. Measurement of serum PCS and IS levels

Human serum samples (50 μL) were pretreated with 1400 μL acetonitrile (ACN) to precipitate proteins. The serum samples were shaken by vortex for 5 min, followed by centrifugation at 13,400 × *g* for 20 min at 4 °C. Eventually, each supernatant of the serum samples was collected in a tube and evaporated using a spin vacuum instrument. The lyophilized samples were then redissolved in 200 μL 30% ACN aqueous solution with 0.1% formic acid (FA) and analyzed using a Mass Spectrometer Analytic System.

PCS and IS were detected by tandem mass spectrometry (Thermo Finnigan TSQ Quantum Ultra Mass Spectrometer, Thermo Fisher Scientific Inc., Waltham, MA, USA). Furthermore, the tandem MS system was equipped with a Micro ESI ion source, which was set at 3.0 kV, coupled with an Acella 1250 UHPLC analytical system (Thermo Fisher Scientific Inc.). The samples containing mixtures of PCS and IS were sequentially injected into the UHPLC via the Acella 1250 autosampler and were separated using a Shiseido HPLC CAPCELL PAK C18 MGII column (150 mm × 1.5 mm, 3.0 μm, Tokyo, Japan). The mobile phases were composed of (A) 0.1% (v/v) FA in water, and (B) 0.1% (v/v) FA in ACN, with a 250 μL/min flow rate, and the linear gradient was set as follows: 30% (B) in 2 min, 30–60% (B) in 6 min, 60–98% (B) in 3 min, 98% (B) in 2 min, 98–30% (B) in 0.1 min and 30% (B) in 6.9 min. The detection mode of the mass spectrometer was set up with an applied voltage of 2.5 kV in the negative ion mode, and the vaporizing and capillary temperatures were set at 300 °C and 350 °C, respectively. The sheath gas and aux gas pressures were set at 35 and 10, respectively, with a collision pressure of 1.5 and a collision energy adjusted to 22 V. The survey scan mode "multiple reaction monitoring (MRM)" was utilized, MRM transitions 187>80 and 187>107 belonging to PCS, and 212>80 and 212>132 belonging to IS for quantification. Xcalibur software (version 2.2, Thermo-Finnigan Inc., San Jose, CA, USA) was used to acquire the MS spectra and control the mass spectrometer.

2.3. Cognitive function assessment

Comprehensive neuropsychological testing included examination of six rational domains (Butters et al., 2004), for which several individual tests were administered. The six rational domains were executive function, memory, information-processing speed, language, visuospatial function, and attention. The tests used to assess executive function were the WAIS-similarity (Wechsler, 1987), Trail-making B (Benton and Hamsher, 1989), Frontal Assessment Battery (FAB) (Dubois et al., 2000), and Controlled Oral Word Association (COWA) tests (Albert, 1973); those used to assess memory were the total number of words learned in 12-item and 6-trial Selective Reminding Tests (SRT) (Buschke and Fuld, 1974) and the number of delayed recalls in the SRT following a 15-min delay after the last trial; information-processing speed was examined using the Wechsler Adult Intelligence Scale (WAIS)-digit symbol test (Wechsler, 1987) and the Trail-making *A* test (Reitan, 1978); language was assessed using the WAIS-vocabulary test (Wechsler, 1987); visuospatial function was measured using the Visual Discrimination Test (VDT) (Sunrise-Editors, 1995); and attention was examined using the WAIS-digit span test (Wechsler, 1987). We calculated the standard z-score of each test first in order to generate a composite score for each domain. This method of calculating domain scores that represent each cognitive domain has been described previously (Tsang et al., 2011).

2.4. Statistical analysis

Independent *t* tests were used to compare differences in continuous variables. Chi-square tests were used to analyze the distribution differences in categorical variables. One-way analysis of variance (ANOVA) was used to compare the differences in the PCS and IS levels between the comparison subjects and patients with different stages of CKD. The associations between serum PCS and IS levels and cognitive domain scores were examined by the liner regressions, adjusting for age, sex, educational level and CKD stage. Those that exhibited significant correlation in above regression model were further examined the interactions between PCS or IS levels and CKD stage. If an interaction existed between the