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RESEARCH ARTICLE

Indoxyl Sulfate Levels and Its Relation with Executive Function in Routine Hemodialysis Patients

Yuliarni Syafrita,[1] Restu Susanti,[1] Syarif Indra[1], Harnavi Harun[2]
[1]Department of Neurology, Faculty of Medicine, Universitas Andalas, Padang, Indonesia,
[2]Department of Internal Medicine, Faculty of Medicine, Universitas Andalas, Padang, Indonesia

Abstract

Executive function is a crucial cognitive domain that can be adversely affected by various toxic substances, including the uremic toxin indoxyl sulfate. Although it's known that indoxyl sulfate has harmful effects on intra or extra-kidney organs, its impact on executive function remains unclear. This study aimed to evaluate indoxyl sulfate levels in patients with chronic kidney disease (CKD) undergoing hemodialysis and to understand its correlation with executive function impairments. This observational cross-sectional study was conducted at the Dr. M. Djamil General Hospital Padang Hemodialysis Unit from March to July 2022; 60 patients undergoing hemodialysis and 20 healthy controls participated. Executive function was assessed using the Trail Making Test B (TMT-B). Indoxyl sulfate levels were quantified using an ELISA assay with the Assay Genie kit. The Mann-Whitney test set statistical significance at a $p<0.05$. The average age of the subjects was 51.4±11.4 years, with 53% being male. The mean indoxyl sulfate levels were considerably higher in the CKD group (118.79 ng/ml, range: 11–1,709 ng/ml) compared to the control group (6.028±1.829 ng/ml), with a significant difference ($p<0.001$). Impaired executive function was observed in 75% of the CKD patients. The average indoxyl sulfate level was 165.12 ng/ml (range: 29–1,709 ng/ml) in the impaired executive function group and 71.22 ng/ml (range: 11–333 ng/ml) in the group with normal executive function, indicating a significant difference ($p=0.013$). Patients with CKD undergoing hemodialysis exhibit elevated serum indoxyl sulfate levels compared to healthy individuals. Moreover, CKD patients with impaired executive function have notably higher indoxyl sulfate levels than those with normal executive function. Further research is needed to elucidate the mechanistic links between indoxyl sulfate and cognitive impairments.

Keywords: Executives renal functions, hemodialysis, indoxyl sulfate

Introduction

Chronic kidney disease (CKD) is often associated with a spectrum of neurological disorders, one of the most notable being cognitive impairment. It has been observed that CKD patients face a heightened risk of cognitive dysfunction when compared to the general population, with the likelihood of cognitive decline rising as renal function deteriorates.[1] One of the most critical aspects of cognitive processes is executive function, which is central to diverse problem-solving tasks and achieving complex goals. Given its rapid evolution as a cognitive domain, executive function becomes particularly susceptible to impairments from structural or metabolic abnormalities in the brain. A study by Sánchez-Fernández et al.[2] highlighted discernible differences in the executive functions between CKD patients on hemodialysis (HD) and healthy controls.

While CKD has many complications, these aren't solely attributable to underlying diseases. A significant contributor to these complications is the accumulation of uremic toxins owing to the kidney's compromised filtration capacity. Indoxyl sulfate stands out among these uremic toxins.[3] Indoxyl sulfate is primarily excreted via secretion in the kidney tubules in individuals with healthy kidney function. However, hemodialysis machines need to catch up in replicating this intricate process. Hemodialysis is chiefly effective in clearing indoxyl sulfate forms that remain soluble and unbound to plasma proteins, allowing them to traverse the dialysis membrane. Since the clearance rate of dialysis is significantly subpar compared to a fully functioning kidney, there's a marked buildup of indoxyl sulfate in the plasma over time.[4–6]

Indoxyl sulfate has been implicated in many pathologies, from kidney and vascular diseases to bone disorders and central nervous system ailments. Research by Kuo et al.[7] provided evidence suggesting that the unbound, accessible

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Correspondence: Yuliarni Syafrita. Department of Neurology, Faculty of Medicine, Universitas Andalas. Jln. Perintis Kemerdekaan No. 94, Padang 25127, West Sumatera, Indonesia. E-mail: ysyafrita@yahoo.com

form of indoxyl sulfate present in plasma might be linked to cognitive dysfunction. Yet, the influence of indoxyl sulfate when complexed with other proteins remains obscure.

While numerous studies have underscored the potentially harmful effects of uremic toxins, including indoxyl sulfate, on cognitive health,[8] the specific contribution of indoxyl sulfate to executive function anomalies in patients subjected to hemodialysis has yet to be extensively probed. This investigation sought to elucidate the association between serum indoxyl sulfate concentrations and compromised executive function in individuals routinely undergoing hemodialysis. A distinctive feature of this research was its focus on discerning how the concurrent presence or absence of depression in these patients could modulate the said relationship.

Methods

This comparative observational study was conducted at the Dr. M. Djamil General Hospital Padang Hemodialysis Unit. The study targeted chronic kidney disease patients who underwent hemodialysis from March to July 2022.

Sixty patients were recruited for the study and met the inclusion and exclusion criteria. Additionally, 20 healthy individuals were included as control subjects due to the absence of baseline data on indoxyl sulfate levels in a typical healthy population. Notably, all selected patients were beneficiaries of health insurance protection, ensuring that they did not bear any financial burdens due to their participation. The hemodialysis regimen was standardized, with each patient undergoing the procedure twice weekly.

The Research Ethics Committee of the Faculty of Medicine, Universitas Andalas, reviewed and approved the study protocol under reference number 888/UN.16.2/KEP-FK/2022.

The neuropsychological tool Trail Making Test B (TMT-B) evaluated participants' executive functions. This assessment was conducted one hour before their hemodialysis session to ensure patients were in an optimal state. In TMT-B, patients were instructed to sequentially connect numbers and corresponding letters displayed on the test sheet. Before the official test, participants underwent a brief practice session. Executive function was deemed normal if a participant could complete the TMT-B in under 80 seconds (for those below 55 years) or within 120 seconds (for those aged 55–69 years).

The depression status of the participants was ascertained using the Patient Health Questionnaire 9 (PHQ-9).[9] This tool generates a score between 0 and 27. A score exceeding 4 indicated the presence of depression, while a score of 4 or below was indicative of its absence.

Venous blood samples (approximately five ml) were collected from participants and placed in vacutainer tubes. These samples were then centrifuged at speeds between 2,000 and 3,000 rpm for a duration of 20 minutes. The resulting serum was transferred to microtubes and stored at –80°C. Once all samples were amassed, indoxyl sulfate concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) reader, with the Assay Genie ELISA kit employed for the assay.

Results

A total of 80 participants were involved in the study, consisting of 60 patients with chronic kidney disease undergoing routine hemodialysis and 20 healthy control subjects. The age range for hemodialysis patients was between 21 and 69 years. These patients had been on hemodialysis for a period ranging from 6 to 108 months. The average age of the hemodialysis patients was 51.4±11.4 years. Males constituted 53% of the patient group, slightly outnumbering the

Table 1 Baseline Data of Research Subjects

Characteristics	n=60 (%)
Age (years)	
≤50	26 (43)
≥50	34 (57)
Gender	
Male	32 (53)
Female	28 (47)
Underlying disease	
Hypertension	48 (80)
Diabetes mellitus	4 (7)
None	8 (13)
Result of PHQ-9	
Depression	37 (62)
Normal	23 (38)
TMT-B	
Impaired	45 (75)
Normal	15 (25)

Table 2 Relationship between Baseline Characteristics with Impaired Executive Function

Variables	Executive Function		p
	Impaired n=45	Not Impaired n=15	
Age (years)			
≤50	24	5	0.179
≥50	21	10	
Gender			
Male	23	9	0.550
Female	22	6	
Education level (years)			
≤9	14	0	0.013
≥9	31	15	
Hypertension			
Yes	35	13	0.456
Normal	10	2	
Diabetes mellitus			
Yes	3	1	1.000
Normal	42	14	
Depression			
Yes	26	11	0.283
Normal	19	4	

females. The most common underlying disease is hypertension. Depression was found in 61% of the patients, and impaired executive function in 75% (Table 1).

From Table 2, there was a significant relationship between educational level and impaired executive function (p=0.013, p<0.05), and there was no association of other baseline characteristics with executive dysfunction (>0.05).

The average indoxyl sulfate level for the patients was 118.79 ng/ml, with a range spanning from 11 ng/ml to 1,709 ng/ml. The intermediate indoxyl sulfate level in the healthy control group was 6.028±1.829 ng/ml). The difference in indoxyl sulfate levels between the CKD patients and healthy controls was statistically significant (p<0.001), where the level of indoxyl sulfate in the case group was around 19 times that of the control group (Table 3).

Impaired executive function was observed in 75% of the patient group, represented by 45 individuals (Table 1). When comparing the indoxyl sulfate levels in the impaired executive function group, the average level was 165.12 ng/ml, ranging from 29 ng/ml to 1,709 ng/ml. The average level in the not-impaired executive function group was 71.22 ng/ml, varying between 11 ng/ml and 333 ng/ml. There was a significant difference in indoxyl sulfate levels between the impaired and without executive function with p=0.013 (p<0.05, Table 4). The results indicate that hemodialysis patients exhibited significantly higher indoxyl sulfate levels than healthy

Table 3 Difference of Indoxyl Sulfate Level in Case and Control Groups

Variable	Groups		p
	Control n= 20	Case n=60	
Indoxyl sulfate	6.028±1.829	118.79 (11–1,709)	<0.001

Table 4 Differences Between Indoxyl Sulfate Level in Impaired and Without Executive Function in Hemodialysis Patients

Variable	Executive Function		p
	Impaired (n = 45)	Without Impaired (n = 15)	
Indoxyl sulfate	165.12±(29–1,709)	71.22 (11–333)	<0.013

controls. Furthermore, those with impaired executive function showed markedly elevated levels of indoxyl sulfate when contrasted with patients showcasing normal executive functions.

Discussion

Chronic kidney disease (CKD) is characterized by persistent renal abnormalities, such as a glomerular filtration rate (GFR) of below 60 ml/min/1.73 m^2, albuminuria lesser than 30 mg per 24 hours, or visible signs of kidney damage (like hematuria or structural anomalies) persisting for more than three months.[10]

Cognitive impairment is frequently observed in CKD patients.[11,12] The risk of cognitive deficits in CKD patients is notably higher than in the general population.[13] As kidney function deteriorates, the likelihood of cognitive dysfunction rises.[14] Numerous uremic toxins, including uric acid, parathyroid hormone, and indoxyl sulfate, have been implicated in cognitive anomalies.[8,15,16]

Executive function embodies an intricate interplay of goal-oriented behavioral processes, tightly interwoven with the prefrontal cortex's structure.[17] Among CKD patients, executive function is a particularly vulnerable cognitive domain, with significant deficits observed even in hemodialysis recipients. Research by Kurella Tamura et al.[18] in 2017 and corroborated by Murthy and Shukla[19] in 2020 underscores the vulnerability of executive function in CKD patients.

In our study, indoxyl sulfate levels in hemodialysis patients were markedly elevated—almost 19-fold compared to the control group. This elevation underscores the inefficiency of hemodialysis in removing indoxyl sulfate from the bloodstream. Indoxyl sulfate's extensive protein binding makes its clearance via hemodialysis exceptionally challenging.[20] Conventional treatments show that indoxyl sulfate clearance significantly lags behind ureas.[21]

These heightened levels of indoxyl sulfate in dialysis patients—sometimes reported to be up to 80 times higher than in healthy controls[22]—have grave implications for cognitive health. The impaired renal transport mechanisms in CKD facilitate the accumulation of indoxyl sulfate in the brain. Its neurotoxic effects, accelerated by vascular calcification and aging, are hazardous for dementia patients.

Extensive research has outlined indoxyl sulfate's harmful effects on renal and extrarenal organs. Elevated indoxyl sulfate has been linked to declining glomerular filtration rates,[23,24] enhanced coronary atherosclerosis,[25] and more.

A significant finding of our study was the correlation between heightened indoxyl sulfate levels and impaired executive function in hemodialysis patients. While several factors can influence executive function, our results suggest a significant role of indoxyl sulfate levels in shaping the cognitive landscape of CKD patients on hemodialysis.

Conclusions

CKD patients on routine hemodialysis exhibit substantially elevated serum indoxyl sulfate levels compared to a healthy cohort. Among these patients, those with compromised executive function have even higher indoxyl sulfate concentrations.

Conflict of Interest

All contributing authors confirm no conflict of interest associated with this investigation.

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