

Improved cardiovascular autonomic function and decreased protein-bound uremic toxins in patients with end-stage renal disease after peritoneal dialysis

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Abstract

Objective: Cardiovascular autonomic neuropathy is highly prevalent in patients with end-stage renal disease (ESRD), and it has a high fatality rate. This study aimed to determine whether peritoneal dialysis (PD) improves cardiovascular autonomic function (CAF) and decreases protein-bound uremic toxin (indoxyl sulfate [IS], p-cresyl sulfate [PCS]) levels.

Methods: IS and PCS levels, and parameters of CAF (heart rate response to deep breathing [HR_DB], the Valsalva ratio, baroreflex sensitivity, and the frequency domain) were prospectively evaluated in 26 patients with ESRD undergoing PD at two time points (pre-PD and 6 months post-PD). For comparison, 19 consecutive patients with pre-dialysis chronic kidney disease and 30 age- and sex-matched healthy volunteers were included as the disease and control groups, respectively.

Results: Baroreflex sensitivity, HR_DB, and the Valsalva ratio were significantly lower in the ESRD and disease groups than in the control group. IS and PCS levels were significantly higher in the ESRD group than in the control group. Sympathetic/parasympathetic activity was improved

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after PD. IS levels were significantly decreased after PD and IS level changes were correlated with the frequency domain.

Conclusions: IS may play a role in cardiovascular autonomic neuropathy, and decreased IS levels after dialysis are associated with sympathetic/parasympathetic activity imbalance.

Keywords

Cardiovascular autonomic function, end-stage renal disease, indoxyl sulfate, protein-bound uremic toxin, p-cresyl sulfate, peritoneal dialysis

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Background

Autonomic dysfunction is highly prevalent in chronic kidney diseases (CKD) and occurs in >50% of patients undergoing hemodialysis (HD).¹ Cardiovascular autonomic neuropathy (CAN), which is imbalance of sympathetic/parasympathetic activity, appears to be mainly due to uremia and its consequences. CAN is associated with worse outcomes of patients and a risk of cardiovascular disease (CVD) in a number of human pathologies.^{2,3}

CAN, which has a length-dependent pattern, is a common condition, but it is one of the most overlooked complications of end-stage renal disease (ESRD).^{3,4} The vagus nerve is responsible for approximately 75% of parasympathetic activity in humans and can be damaged in the early phase of CAN.⁵ In this situation, parasympathetic activity contributes to sympathetic predominance. Sympathetic hyperactivity plays an important and distinct role in hypertension associated with CKD. In addition to the underlying autonomic imbalance, the pathophysiological mechanism of development of CAN involves multiple factors, including cardiovascular risk factors,⁶ interaction between uremic toxins (e.g., indoxyl sulfate [IS], p-cresyl sulfate [PCS], and uric acid) and inflammation, biomarkers of oxidative stress and

endothelial dysfunction (e.g., interleukin-1 β , interleukin -6, and tumor necrosis factor- α),⁷ altered cerebral perfusion,^{8,9} renal ischemia, elevated levels of angiotensin II, and suppressed levels of brain nitric oxide, which all stimulate sympathetic activity.⁵ As CAN progresses, sympathetic denervation occurs in the late stage of this disease.

A growing number of studies have confirmed the toxicity of the protein-bound uremic retention solutes IS and PCS, and their role in progression of vascular and renal disease, as well as in increasing the risk of CVD.¹⁰⁻¹³ With regard to the relationship between CAN and uremic toxins in ESRD, there is a paucity of information on the role of uremic toxins in the severity of CAN. Exploration of this relationship could aid in developing therapeutic strategies that could prevent CAN or mitigate its severity in patients with ESRD.

Therefore, in this study, we tested the hypotheses that toxicity of the protein-bound uremic toxins IS and PCS contributes to the severity of CAN, and that peritoneal dialysis (PD) not only decreases the levels of protein-bound uremic toxins (IS and PCS), but also improves cardiovascular autonomic function. Successful translation of these approaches to the clinical setting offers the promise of reducing CVD and improving the quality of life of patients.

Materials and Methods

Study design

This single-center, prospective case-control study was conducted at Chang Gung Memorial Hospital-Kaohsiung, which is a medical center and the main referral hospital serving an area with 3 million inhabitants in southern Taiwan.

Participants

This prospective study recruited 26 consecutive patients (age, ≥ 20 years) with ESRD with a disease duration of >3 months who were evaluated for PD therapy. Patients were enrolled in this study only if full

written informed consent was obtained from the patients or their families. Informed consent was obtained from all of the study subjects. For comparison, 19 consecutive patients (age, ≥ 20 years) with pre-dialysis CKD (CKD stages 3–4) were included as disease controls and 30 age- and sex-matched healthy volunteers were included as healthy controls (Table 1). The exclusion criteria were as follows: 1) moderate-to-severe heart failure (New York Heart Association classes III and IV); 2) any type of arrhythmia that prevents analysis of heart rate variability (HRV) or pacemaker implantation; and 3) degenerative disorders known to affect the autonomic system. Patients were enrolled in this

Table 1. Baseline characteristics and laboratory data of patients and controls.

Characteristics	ESRD group (n = 26)	Disease group (n = 19)	Control group (n = 30)	P value
Age (years) (mean \pm SD)	56.4 \pm 9.6	66.8 \pm 8.0	59.9 \pm 6.5	0.12
Male/female	15/11	12/7	20/10	0.14
Underlying diseases				
Hypertension (n, %)	19 (73.1)	13 (68.4)	—	
Diabetes mellitus (n, %)	12 (46.1)	14 (73.7)	—	
Dyslipidemia (n, %)	8 (30.7)	6 (31.6)	—	
Presence of CVD history (n, %)	2 (7.7)	1 (5.2)	—	
Alcoholism	4 (15.4)	—	—	
Smoking	3 (11.5)	—	—	
Laboratory data				
Total cholesterol (mmol/L)	4.86 \pm 1.19	4.44 \pm 1.36	4.92 \pm 0.72	0.43
LDL-cholesterol (mmol/L)	2.61 \pm 0.91	4.05 \pm 0.79	2.90 \pm 0.67	0.59
Hemoglobin, g/L	99 \pm 15	123 \pm 20	133 \pm 15	0.41
hematocrit (%)	29.8 \pm 4.9	36.9 \pm 5.4	40.4 \pm 3.6	0.20
eGFR (mL/minute/1.73 m ²)	5.2 \pm 2.1	39.8 \pm 13.6	102.7 \pm 15.4	<0.001
Blood urea nitrogen (mmol/L)	35.1 \pm 17.9	12.0 \pm 11.6	5.2 \pm 1.2	<0.001
Creatinine (mmol/L)	1043.1 \pm 300.6	167.9 \pm 88.4	61.9 \pm 8.8	<0.001
Calcium (μ mol/L)	2.18 \pm 0.3	2.25 \pm 0.05	2.28 \pm 0.08	0.001
Phosphorus (mmol/L)	1.52 \pm 0.48	1.19 \pm 0.32	NA	
Protein-bound uremic toxin				
Indoxyl sulfate (μ g/mL)	16.0 \pm 8.7	NA	2.1 \pm 1.4	<0.001 ^{α}
p-Cresol sulfate (μ g/mL)	4.4 \pm 2.6	NA	2.7 \pm 2.7	0.026 ^{α}

Values are expressed as mean \pm SD unless otherwise indicated.

ESRD, end-stage renal disease; SD, standard deviation; CVD, cardiovascular disease; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate; NA, not available.

α = The difference in protein-bound uremic toxin was compared between the ESRD and control groups by the independent-t test.

study only if they or their families provided full written informed consent. Informed consent was obtained from all study subjects. The study protocol was approved by Chang Gung Memorial Hospital's Institutional Review Committee on Human Research (CGMH IRB 103-0367B).

Clinical and laboratory assessments

Demographic data, risk factors, and a history of previous vascular events (i.e., myocardial infarction and coronary artery disease) were obtained at baseline. In all subjects, blood pressure (BP) was checked using a mercury sphygmomanometer after 5 minutes of rest. For each patient, two BP readings, separated by 2 minutes, were made and the average was taken. Vascular risk factors included hypertension, which was defined as a systolic BP of >140 mm Hg and/or a diastolic BP of >90 mm Hg, or use of antihypertensive medications. Other vascular risk factors were diabetes mellitus, which was defined as elevated blood glycohemoglobin levels (glycated hemoglobin levels >6.5%) or current use of antidiabetic medications, and dyslipidemia, which was defined as total cholesterol levels >5.18 mmol/L, triglyceride levels >2.03 mmol/L, or current use of lipid-lowering medications. Current cigarette smoking was defined as smoking within the last 5 years, whereas former cigarette smoking was defined as abstention from smoking for >5 years.

Blood biochemical tests for hemoglobin, hematocrit, serum urea, serum creatinine, estimated glomerular filtration rate, calcium, phosphorus, and albumin levels were conducted in all patients with ESRD at baseline (on day 1 before stable PD treatment) and at 6 months after undergoing stable PD. No blood biochemical tests were conducted in the control group.

Assessment of cardiovascular autonomic function

All autonomic function tests were performed on day 1 before stable PD treatment and at 6 months after maintaining PD therapy. These tests were scheduled in the morning between 8 am and 12 pm on the day of the study. No coffee, food, alcohol, or nicotine was permitted 4 hours before testing. All subjects underwent standardized evaluation of cardiovascular autonomic function.

Heart rate was derived from a continuously recorded standard three-lead electrocardiogram (Ivy Biomedical, model 3000; Branford, CT, USA), while arterial BP was continuously measured using beat-to-beat photoplethysmographic recordings (Finameter Pro, Ohmeda; Englewood, OH, USA). The following parameters were obtained through tests computed by Testworks (WR Medical Electronics Company, Stillwater, MN, USA): the heart rate response to deep breathing (HR_DB), the Valsalva ratio (VR), and baroreflex sensitivity (BRS). The VR was defined as the maximum heart rate during the Valsalva maneuver divided by the lowest heart rate obtained within 30 s of the peak heart rate. BRS was evaluated as the slope of the regression line fitting the relationship between changes in heart rate and the change in systolic arterial pressure elicited by the Valsalva maneuver.¹⁴ HR_DB (beats/minute) is a measure for respiratory sinus arrhythmia. The detailed calculation of HR_DB and VR was performed as described by Low.¹⁵

Beat-to-beat R-R interval changes were interpolated using a third-order polynomial and were re-sampled with 0.5-second intervals. The signals were then transformed to the frequency domain with fast Fourier transform by using 512 samples. Spectral powers were divided into three frequency domains of high frequency (HF, 0.15–0.4

Hz), low frequency (LF, 0.04–0.15 Hz), and very low frequency (VLF, 0–0.04 Hz).¹⁶ The ratio between powers of LF and HF (LF/HF ratio) represented an index of sympatho-vagal balance.

Measurement of serum PCS and IS levels

Human serum samples (50 mL) were pre-treated with 1400 mL acetonitrile to precipitate proteins. The serum samples were shaken by vortex for 5 minutes, followed by centrifugation at $13,400 \times g$ for 20 minutes at 4°C. PCS and IS levels were detected using a tandem mass spectrometer (Thermo Finnigan TSQ Quantum Ultra Mass Spectrometer; Thermo Fisher Scientific Inc., Waltham, MA, USA). The detailed methodology used was according to a previous study.¹⁷

Statistical analysis

Data are expressed as mean \pm standard deviation or median (interquartile range). Categorical variables were compared with the χ^2 or Fisher's exact test. Continuous variables that were not normally distributed were logarithmically transformed to improve normality. Continuous variables were compared using the Student's t-test or one way analysis of variance followed by Bonferroni's multiple comparison for a post-hoc test. Three separate statistical analyses were performed. First, the demographic data and cardiovascular autonomic function parameters of the ESRD and control groups were compared. Second, changes between baseline and 6-month post-PD biomarker values (protein-bound uremic toxins) and parameters of cardiovascular autonomic function were compared using the paired t-test. Third, the changes in each parameter over 6 months were defined as 6-month follow-up data minus baseline data. Additionally, correlation analysis was used to determine the

relationships between changes in protein-bound uremic toxins and changes in parameters of cardiovascular autonomic function. All statistical analyses were conducted using the SAS software package, version 9.1 (2002; SAS Statistical Institute, Cary, NC, USA).

Results

Demographic characteristics of the subjects

The demographic data of the patients with ESRD, those with diseases, and the healthy controls are shown in Table 1. The 26 patients with ESRD (mean age, 56.4 ± 9.6 years) included 15 men and 11 women. Age and sex were similar between the three groups. Of the subjects, 28 had one or more underlying diseases or factors, including hypertension, diabetes mellitus, hyperlipidemia, coronary artery disease, and smoking. IS and PCS levels were significantly higher in the ESRD group than in the control group (both $P < 0.05$). Blood biochemical test results, such as hemoglobin, hematocrit, blood urea nitrogen, creatinine, calcium, and phosphorus levels, among the three groups are also shown in Table 1.

Comparison of autonomic function among the three groups

In terms of autonomic function, VR, HR_DB, and BRS were significantly different among the three groups (Table 2) (all $P < 0.01$). Post-hoc testing showed that VR, HR_DB, and BRS were significantly higher in the control group than in the ESRD and disease groups (all $P < 0.05$). However, there were no significant differences in VR, HR_DB, and BRS between the disease and ESRD groups. For parameters of spectral analysis, LF, HF, and the LF/HF ratio were significantly different among the three groups (all $P = 0.03$). Post-hoc testing

Table 2. Cardiovascular autonomic function in patients and controls.

	ESRD group (n = 26)	Disease group (n = 19)	Control group (n = 30)	P value
Spectral analysis				
LF, normalized unit	40.3 ± 21.3	41.9 ± 22.4	54.4 ± 17.7	0.03 ^α
HF, normalized unit	57.1 ± 20.1	57.8 ± 22.5	45.4 ± 17.7	0.034 ^β
LF/HF ratio	1.0 ± 0.93	1.1 ± 1.1	1.6 ± 1.1	0.03 ^γ
Cardio-vagal autonomic function				
Valsalva ratio	1.3 ± 0.3	1.3 ± 0.1	1.5 ± 0.2	0.002 ^δ
HR_DB (beats/minute)	5.3 ± 3.9	5.4 ± 3.7	10.6 ± 7.3	<0.0001 ^ε
Baroreflex sensitivity	4.8 ± 2.5	4.3 ± 2.8	7.6 ± 3.2	<0.0001 ^ζ

All values are mean ± standard deviation.

LF, low frequency; HF, high frequency; HR_DB, heart rate response to deep breathing, ESRD, end-stage renal disease
Bonferroni's multiple comparisons for post-hoc tests:

α = ESRD group vs. control group, P = 0.042.

β = ESRD group vs. control group, P = 0.047.

γ = ESRD group vs. control group, P = 0.045.

δ = ESRD group vs. control group, P = 0.0008; disease group vs. control group, P = 0.011.

ε = ESRD group vs. control group, P < 0.0001; disease group vs. control group, P = 0.001.

ζ = ESRD group vs. control group, P < 0.0001; disease group vs. control group, P < 0.0001.

showed that LF was significantly higher, the HF ratio was significantly lower, and the LF/HF ratio was significantly higher in the control group than in the ESRD group (all P < 0.05).

Comparisons of autonomic parameters and uremic toxins before and after PD therapy

With regard to the parameters of spectral analysis, LF, HF, and the LF/HF ratio were significantly different after PD. Mean LF was significantly higher after PD compared with before PD (P = 0.03). Mean HF was significantly lower after PD compared with before PD (P = 0.045). The mean LF/HF ratio was significantly higher after PD compared with before PD (P = 0.03). With regard to cardiovascular autonomic parameters, parameters of cardiovagal function, including VR, HR_DB, and BRS, were all increased after PD compared with before PD, although this did not reach statistical significance (Table 3). With regard to

protein-bound uremic toxin levels, the mean IS level was significantly decreased after PD compared with before PD (P = 0.004), with no change in the mean PCS level (Table 3).

Correlations between uremic toxins and autonomic function parameters in patients with ESRD undergoing PD therapy

Correlation analysis was conducted to test the association between changes in autonomic function parameters and uremic toxins (Table 4). We found a significant negative correlation between changes in the LF/HF ratio and IS (P = 0.036) at 6 months after PD therapy.

Discussion

Our study confirmed the hypothesis that 6 months of PD therapy not only improves sympathetic/parasympathetic activity (LF/HF ratio) imbalance, but also decreases

Table 3. Changes in protein-bound uremic toxins and cardiovascular autonomic parameters before and after PD in patients with end-stage renal disease.

	PD treatment		P value
	Before	After	
Autonomic function testing			
Frequency domain			
LF, normalized unit	40.3 ± 21.3	55.3 ± 20.7	0.03
HF, normalized unit	57.1 ± 20.1	43.1 ± 20.3	0.045
LF/HF	1.1 ± 0.9	2.0 ± 1.4	0.03
Valsalva ratio	1.2 ± 0.3	1.3 ± 0.2	0.97
HR_DB (beats/minute)	5.1 ± 3.9	5.8 ± 3.6	0.26
Baroreflex sensitivity	4.5 ± 2.5	5.6 ± 3.7	0.26
Protein-bound uremic toxin			
Indoxyl sulfate (μg/mL)	17.6 ± 8.7	11.7 ± 5.3	0.004
p-Cresol sulfate (μg/mL)	4.6 ± 2.7	3.7 ± 1.9	0.17
Clinical and biochemical characteristics			
Systolic blood pressure(mmHg)	157.4 ± 40.9	134.2 ± 14.9	0.192
Diastolic blood pressure (mmHg)	87.0 ± 17.9	77.6 ± 8.4	0.191
Hemoglobin (g/L)	98 ± 11	105 ± 13	0.054
Albumin (g/L)	35 ± 5	35 ± 4	0.587

All values are mean ± standard deviation.

PD, peritoneal dialysis; LF, low frequency; HF, high frequency; HR_DB, heart rate response to deep breathing.

Table 4. Correlations between uremic toxins and cardiovascular autonomic parameters in patients with end-stage renal disease during the study period.

Pearson correlation	Δ Indoxyl sulfate		Δ p-Cresol	
	r	P	r	P
Δ Indoxyl sulfate	—	—	0.653	0.001
Δ p-Cresol	0.653	0.001	—	—
Δ BRS_seq	0.357	0.175	-0.340	0.198
Δ LF/HF ratio	-0.510	0.036*	-0.435	0.081
Δ HR_DB	-0.294	0.237	-0.263	0.291
Δ VR	-0.021	0.965	0.078	0.867

LF, low frequency; HF, high frequency; HR_DB, heart rate response to deep breathing; VR, Valsalva ratio; BRS_seq, sequence of baroreflex sensitivity; Δ, mean change during treatment (6-month follow-up minus baseline data).

protein-bound uremic toxins (IS). We examined serial changes in biomarkers, including protein-bound uremic toxins (IS and PCS) and cardiovascular autonomic parameters in patients with ESRD before and after PD, and observed five major findings. First, cardiovascular autonomic function

(HR_DB, VR, and BRS) were significantly lower in the ESRD and disease groups than in the control group. Second, protein-bound uremic toxins (IS and PCS) were significantly higher in the ESRD group than in the control group. Third, IS levels were significantly decreased after PD compared

with before PD, while PCS levels were unchanged. Fourth, there was significant improvement in sympathetic/parasympathetic activity (LF/HF ratio) after PD. The parameters of cardiovagal function, including VR, HR_DB, and BRS were all increased after PD, although this increment did not reach statistical significance. The non-significant statistical results of these cardiovascular functional parameters may be related to the small sample size of the study patients. Additionally, the lack of significance may be due to the severe reduction of parasympathetic activity in the late stage of CAN so that improvement was not obvious after short-term renal replacement therapy. Finally, the change in the protein-bound uremic toxin IS was negatively correlated with a parameter of cardiovascular autonomic function (LF/HF ratio).

Role of cardiac autonomic dysfunction in uremic complications

The clinical features of CAN are variable. CAN can present with decreased parasympathetic activity contributing to sympathetic predominance, and sympathetic hyperactivity plays an important and distinct role in hypertension associated with CKD. As CAN progresses, sympathetic denervation occurs in the late stage of the disease. Impairment of BRS tends to result in sympathetic hyperactivity, which in turn, leads to an increased risk of cardiac arrhythmia, hypertensive crisis, and associated cardiac events. In addition to the imbalance of sympathetic/parasympathetic activity, CAN is also associated with an increased risk of cardiac arrhythmia and sudden cardiac death in patients with CKD. Furthermore, impairment of BRS results in instability of BP, which is likely to have a negative effect on systemic blood perfusion, especially in patients with anemia and impaired renal autoregulation, such as those with uremia.¹⁸ These

mechanisms may possibly explain the poor prognosis of patients with impaired cardiovascular autonomic function.

Effects of renal replacement therapy on cardiovascular autonomic dysfunction

Cardiovascular morbidity and mortality remain significant problems within the ESRD population, accounting for approximately 50% of all deaths in patients undergoing dialysis and in recipients of renal transplants.^{19,20} Published studies on the effect of renal replacement therapy on the change of CAN in advanced renal disease are limited.^{21–23} One retrospective study enrolled 32 patients with chronic uremia who were managed with either HD (16 cases) or PD (16 cases) therapy.²⁰ These 32 patients had CAN and peripheral neuropathy, as assessed using HRV time domain indices and peripheral sensory nerve conduction studies, respectively. This previous study showed the adequacy of HD and continuous ambulatory PD in improving cardiac autonomic nervous function in patients with chronic uremia. Another study compared the effect of an icodextrin-based dialysis solution and a glucose-based dialysis fluid on sympathetic and parasympathetic activity in the heart, as assessed using HRV, in patients with diabetes undergoing PD.²¹ This previous study showed partial recovery of sympathetic activity in the icodextrin-based dialysis group. The Frequent Hemodialysis Network Daily Trial randomized 245 patients to receive 12 months of either six times per week or three times per week in-center HD.²² In this trial, the HRV values were calculated from 24-hour Holter electrocardiograms at baseline and 12 months in 131 patients, and included LF power (a measure of sympathetic modulation) and HF power (a measure of parasympathetic modulation). This trial showed that the six times per week HD group (daily

HD) group had an increased LF component of HRV and reduced left ventricular mass, which indicated that daily HD was associated with increased vagal modulation of heart rate (HF power) and increased HRV.²² Another study investigated 20 patients with ESRD who had undergone either HD ($n=13$) or PD ($n=7$), and assessed cardiac autonomic function by using HRV parameters (time and frequency domain analysis parameters).²³ This previous study showed that 12 months of renal replacement therapy caused considerable improvement of CAN and that the ameliorative effect of continuous ambulatory PD was better than that of HD. In our study, the LF/HF ratio was significantly increased and became similar to that of the control group after adequate PD therapy for 6 months. However, the parameters of cardiovagal autonomic function (HR_DB, VR, and BRS) showed a tendency to increase, but this was not significant. A low LF/HF ratio in CKD patients has been previously reported.^{24,25} Furthermore, patients with a low LF/HF ratio have a worse prognosis.

The underlying mechanism of sympathetic/parasympathetic imbalance in patients with CKD is not completely known. A previous study showed that uremic toxins act on brain stem neurons in the rostral ventrolateral medulla, which is an important center of autonomic regulation and also involved in the baroreflex pathway.²⁶ Such findings may help in understanding the reduced LF/HF ratio, as well as blunting of BRS in patients with CKD. Our study also showed that IS levels were negatively correlated with sympathetic/parasympathetic balance (LF/HF ratio). This finding indicates that a lower IS toxin level is associated with a higher LF/HF ratio. The change in IS levels was positively correlated with that of BRS, although this did not reach statistical significance ($P=0.175$). These results suggest that IS plays a significant role in

cardiovascular autonomic dysfunction in patients with ESRD. A high IS level is associated with a prolonged QTc interval,²⁷ and has been suggested as a novel cardiovascular risk factor in CKD.²⁸ Cardiovascular autonomic dysfunction caused by high IS levels may partly explain the underlying associations among a prolonged QTc interval, increased cardiovascular risk, and high IS levels. Various studies have confirmed the toxicity of protein-bound uremic retention solutes and their role in vascular and renal disease progression, as well as in increasing the risk of CVD.^{29,30} This finding is expected because the underlying autonomic imbalance involves multiple factors, including cardiovascular risk factors.

Correlations of uremic toxins and autonomic function in patients with ESRD

The pathophysiological mechanism of development of CAN could be multifactorial and complicated. In fact, the mechanism involved in autonomic impairment in patients with ESRD is not completely known. IS and PCS are among uremic toxins that are solely derived from colonic bacterial fermentation of protein, which may negatively affect the kidneys.³¹⁻³³ Circulating IS could be associated with CKD and CVD mortality by increasing oxidative stress and activating inflammatory pathways, resulting in increased expression of intracellular adhesion molecules.³⁴⁻³⁶ Furthermore, IS and PCS have been considered to be the most likely factors to influence cerebro-renal interaction dysfunction.⁷ However, the effects and mechanisms of the effects of IS and PCS on uremia-related CAN are still being investigated. Animal and *in vitro* studies have shown that IS is a vascular toxin and can stimulate proliferation of vascular smooth muscle cells and induce oxidative stress in endothelial cells.^{37,38} High levels of IS might lead to

more severe microvascular complications and affect the severity of CAN.

Study limitations

This study has several limitations. First, the sample size was not large, and we did not exclude patients who had diabetes and did not compare non-diabetic and diabetic groups. We also did not control for medications that can reduce sympathetic or parasympathetic outflow, such as beta-blockers and calcium channel blockers, which can affect HRV. Beta-blockers, which have a direct and prominent effect on autonomic function, were ceased on the day of the study and resumed after the test. Therefore, there is uncertainty in assessing the effects of chronic glycemic impairment in unselected patients with type 2 diabetes. Second, although there was a close relationship between removal of uremic solutes (e.g., IS) and CAN in this prospective observational study, whether the role of the association was causal is unclear. Third, the follow-up period was not long. Therefore, effects of PD on end points were absent in this study. Studies with a large size and longitudinal design are necessary to evaluate the role of uremic toxins on the severity of CAN during clinical follow-up. Finally, there were no clinical manifestations or clinical scores for correlating the findings of cardiovascular autonomic function. Other quantitative autonomic clinical scores (e.g. the composite autonomic symptom scale-31) to assess clinical outcome should be considered for future studies.

Conclusion

Our study suggests that the protein-bound uremic toxin IS may play a role in CAN. Additionally, decreased IS levels after renal replacement therapy can contribute to

improvement of sympathetic/parasympathetic activity (LF/HF ratio) imbalance.

Authors' contributions

BCC participated in the design of the study and drafted the manuscript. YRL and CCH participated in clinical evaluation of patients. CCH performed the statistical analysis. CHL conceived the idea of the study, participated in its design and coordination, and helped draft the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data from this study can be acquired from the corresponding author upon reasonable request.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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