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The Association between Serum Indoxyl Sulfate, P-Cresyl Sulfate and Cardiovascular Risk Factors in Peritoneal Dialysis Patients

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Abstract

Background: Indoxyl Sulfate (IS) and p-Cresol Sulfate (*p*CS) are two important gut-derived uremic toxins accumulated in patients with Chronic Kidney Disease (CKD). They have been reported contributing greatly to Cardiovascular Disease (CVD) both in epidemiological studies and animal models. The present study was conducted to explore whether IS and *p*CS contribute to CVD by accelerating cardiovascular risk factors in Peritoneal Dialysis (PD) patients.

Methods: A cross-sectional study was conducted on 119 PD patients. Serum IS and *p*CS were measured by Ultra-High-Performance LC–tandem MS (UPLCMS/MS), and metabolic parameters involved in cardiovascular risk were measured by auto-biochemistry analyzer machine. Univariate and multivariate linear regression models were performed to determine the association between the independent variables (IS and *p*CS) and clinical indexes. Multivariable-adjusted logistic regression models were used to estimate the odds ratios (ORs, 95% confidence intervals (CIs)).

Results: The median BMI of PD patients was 20.10 (18.95, 22.90) kg/m^2. The median serum IS and *p*CS concentrations were 22.46 (13.45, 29.92) mg/L and 12.41 (5.29, 24.45) mg/L, respectively. Positive significant associations were observed between serum IS concentration and PD duration, creatinine, pre-albumin, phosphorus, magnesium and β2-microglobulin (β2-m) with the corresponding correlation coefficient r and p value of 0.22 (P=0.020), 0.48 (P<0.001), 0.32 (P<0.001), 0.34 (P<0.001), 0.28 (P=0.002), 0.50 (P<0.001). Also, statistically negative significant associations were observed between IS and estimated Glomerular Filtration Rate (eGFR)-0.46 (P<0.001). Besides, a significant positive association between *p*CS and albumin 0.32 (P<0.001) was indicated.

Conclusions: Serum clinical indexes were dependent cardiovascular risk factors for increasing IS and *p*CS levels, which contribute to CVD in peritoneal dialysis patients. Mechanism research should be conducted in the future to explore causality.

Keywords

Chronic kidney disease • Peritoneal dialysis • Indoxylsulfate • p-Cresol sulfate • Cardiovascular risk factors.

Introduction

Chronic Kidney Disease (CKD) was a serious public healthcare issue, affecting about 10% of the population worldwide [1-3]. Cardiovascular Disease (CVD) is significant cause of high morbidity and mortality in CKD patients [4,5]. In addition to traditional cardiovascular risk factors, uremic toxins have also been reported contributing greatly to CVD [6]. Protein-Bound Uremic Toxins (PBUTs) are solutes with molecular weight <500 Da, and retained in CKD patients because of high plasma protein affinity [7]. Among uremic toxins, Indoxylsulfate (IS) and p-Cresyl Sulfate (*p*CS) are two of the most vital PBUTs, with nearly 90% bonding to serum proteins [8]. High serum IS concentration has been reported to be correlated closely with the progression of CKD and a range of complications, including CVD, endothelial dysfunction, thrombosis and genomic damages [9]. The high level of serum IS also upregulates the gene expression of TGF-β1, TIMP-1, and pro-α1 collagen, which affect nephrons, particularly proximal tubular cells, and activate the tubulointerstitial fibrosis, glomerular sclerosis, and the advance of CKD [10]. High level of serum *p*CS contributes to inflammatory, stimulates polymorphonuclear leukocytes and endothelial activation to cytokines in patients with CKD [11]. Furthermore, the elevated serum *p*CS upregulates mRNA of inflammatory cytokines, actives TGF-b1 protein secretion, and increases oxidative stress by enhancing NADPH oxidase, leading to fibrosis and endothelial dysfunction [12]. When tryptophan exists in the diet, it is metabolized to indole by gut microbiota, which is absorbed by the gut and then circulates to the liver. After hydroxylation and sulfation process in the liver, indole converts into IS and then enters circulating system [13]. Under the healthy renal function condition, serum IS passes in the renal tubular cells through Organic Anion Transporter (OAT1 and OAT3) confined in the basolateral membrane and is later excreted into the kidney tubules via OAT4 contained at the apical membrane of renal tubular cells [13]. Clearance of IS via the native kidney is much higher than the dialytic removal, which does not replicate tubular secretion. Therefore, the plasma level of IS rises to a higher degree in CKD patients relative to healthy one [14]. P-cresol (4-methyl phenol) is another uremic toxin originating from aromatic amino acids tyrosine and phenylalanine metabolism by bacterial microbiota fermentation in the gut. In the colonic and the liver, it is metabolized by sulfation and glucuronidation to form *p*CS and p-cresyl glucuronidated. These two derivatives of p-cresol can be found both in the conjugated and unconjugated form in CKD patients [15,16].

Although uremic retention solutes data on Peritoneal Dialysis (PD) patients in China are scarce, it is crucial for clinicians to both recognize and enhance the cardiovascular health of patients undergoing dialysis. Thus, the study aims to explore whether IS and *p*CS contribute to CVD by accelerating cardiovascular risk factors in PD patients.

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Materials and Methods

Study participants

A cross-sectional study was conducted between April 2013 and May 2018 in Tongji Hospital, Wuhan, China. The research was approved by the Tongji Hospital Ethics Committee (TJ-IRB20171110), and registered at clinicaltrials.gov (ChiCTR1900024905). Informed consent was obtained from all patients before the study.

Of the 167 PD patients, those with missing data were excluded. Thus, a total of 119 CKD patients receiving PD (71 women, 48 men) were recruited. Patients were excluded if they have: 1. Diabetic nephropathy, 2. Have a recent incidence of peritonitis or other infectious complication within 30 days before the data collection. The inclusion criteria were as follows: patients aged older than 18 years and used PD for more than three months.

Basic characteristics data

Basic characteristics including sex, age, Body Mass Index (BMI), Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), estimated Glomerular Filtration Rate (eGFR), 24-h urine volume and starting date of dialysis treatment were collected for all subjects. The duration of dialysis (months) was computed as the period between dialysis initiation and the time of data collection.

Sample collection and storage

The 24-h urine, serum, and spent dialysate were collected from PD patients and immediately stored at −80°C. All samples were gathered and stored in consistent with the laboratory working procedures. Total IS and *p*CS in serum.

We measured serum total IS and *p*CS by Ultra-High-Performance LC–tandem MS (UPLCMS/MS), with Acquity UPLCTQD (Waters) and an Acquity UPLC HSS T3 column (2.1 mm × 100 mm, 1.8 μm; Waters). Serum samples (20 μL) were added to 160μL acetonitrile and 20 μL internal standard (2 μg/mL mixture of Indoxyl Sulfate-d4 and p-cresol sulfate-d7 with acetonitrile), then vortex (60 s) and centrifuged at 10000 r/min for 5 min. Finally, 50 μL supernatant was extracted and mixed with 950 μL ultra-pure water, and injected for UPLCMS/MS analysis. Nine known IS and *p*CS concentrations from 1ng/mL to 1 μg/mL were tested in duplicate to construct standard curves. The mobile phase A was 5 mmol/L ammonium acetate, and mobile phase B was 100% acetonitrile. The elution gradient was optimized as follows: 95%A (0-1.5 min), 95%A-5%A (1.5-3.0 min), and 5% A (3.0-4.5 min), 5%-95%A (4.5-5.0 min). The standards IS and Indoxyl Sulfate-d4 were obtained from Sigma Aldrich Trading Corporation, the chromatographic grade *p*CS and p-cresol sulfate-d7 were obtained from Toronto.

Serum biochemistry measurements

Serum biochemical parameters, including albumin, pre-albumin, blood glucose, Total Cholesterol (TC), Triglyceride (TG), Low-Density Lipoprotein Cholesterol (LDL-C), High-Density Lipoprotein Cholesterol (HDL-C), Parathyroid Hormone (PTH), β2-microglobulin (β2-m), magnesium, calcium, phosphorus, and hemoglobin, were measured. Fasting blood glucose was determined to evaluate metabolism status. Creatinine, uric acid, and urea nitrogen were measured to assess renal function. Metabolic parameters involved in cardiovascular risk were measured by auto-biochemistry analyzer machine at the central laboratory of Tongji Medical Hospital.

Urine parameters and dialysis adequacy indexes

We conducted analyses of the fluid, urea nitrogen, albumin, pre-albumin, and creatinine in 24-h urine and PD solution. By applying the standard methods and laboratory working procedures, weekly total urea clearance (Kt/V) and Creatinine clearance (Ccr), as parameters reflecting Residual Renal Function (RRF), were estimated based on the daily collection of urine and dialysate [17]. The residual eGFR was measured as an average of the 24h urinary urea and creatinine clearances. By applying Watson's formula, total body water was derived [18]. All analyses were conducted based on the national criteria.

Statistical analysis

The descriptive statistics were presented as frequency (%) for categorical data and as mean and standard deviation for continuous variables that are normally distributed or median and Interquartile Range (IQR) for skewed variables. P values were derived from student's t-tests or Kruskal-Wallis tests for continuous variables and chi-square tests for categorical variables. We also applied Spearman's rank correlation coefficients to determine the association between the dependent variables and Clinical indexes r>0 means the corresponding variables were positively associated with IS or pCS, otherwise, negatively associated with IS or pCS. We used univariate linear regression models to estimate associations of clinical indexes with serum IS and *p*CS levels. We conducted a generalized linear model, stepwise regression analyses based on the Akaike Information Criterion (AIC) by taking IS and *p*CS as the independent variables, and the clinical indicators as the dependent variables. The stepwise regression procedure selected all explanatory variables in the tables. These variables establish the current optimal equation. We applied generalized linear models to estimate the association of CVD risk factors with IS and pCS. The fully adjusted model was adjusted for age, sex, BMI, PD duration, eGFR, SBP (except SBP and DBP), blood glucose (except itself), TG (except itself), HDL-C (except TC and HDL-C) and LDL-C (except TC and LDL-C), albumin, pre-albumin, calcium, phosphorus and Alkaline Phosphatase (ALP). We also used multivariable-adjusted logistic regression models to estimate the odds ratios (ORs, 95% Confidence Intervals (CIs)) for hypertension and hyperlipidemia according to groups of IS and pCS. Since the sample size is too small to perform the tertile or quartile, the median is used as the cut-off point in the analysis. The outputs were reported based on the adjusted ORs with 95% CI. We performed all analyses on R software (version 3.5.0; R Core Team), and a two-sided p-value<0.05 was set as a statistical significance.

Results

Baseline characteristics of patients

Table 1 describes the essential characteristics of the study population. Among 119 patients included in the study, 71 (59.7%) were women (all of the Chinese origin). The mean age was 43.03 ± 12.10 years. The median BMI of PD patients was 20.10 (18.95, 22.90) kg/m^2. The median serum IS and *p*CS concentrations were 22.46 (13.45, 29.92) mg/L and 12.41 (5.29, 24.45) mg/L.

Spearman's rank correlation coefficients between IS, *p*CS, and clinical indexes

The associations between serum IS, *p*CS concentrations and clinical indexes were examined using Spearman's rank correlation analysis (Table 2). Positive significant associations were observed between serum IS concentration and PD duration, creatinine, pre-albumin, phosphorus, magnesium and β2-m with the corresponding correlation coefficient r and p value of 0.22 (P=0.020), 0.48 (P<0.001), 0.32 (P<0.001), 0.34 (P<0.001), 0.28 (P=0.002), 0.50 (P<0.001). Also, statistically negative significant associations were observed between IS and eGFR-0.46 (P<0.001). Besides, a significant positive association between *p*CS and albumin 0.32 (P<0.001) was indicated.

The associations between serum IS, *p*CS concentrations and clinical indexes were examined using Spearman's rank correlation analysis. Spearman's rank correlation coefficients are presented in Table 2. Serum IS level was positively associated with Peritoneal dialysis duration, serum creatinine concentration, pre-albumin, phosphorus, Magnesium and β2-microglobulin and negatively associated with eGFR. Serum *p*CS concentration was positively associated with albumin level.

Table 1. Basic characteristics of the study population.

Variables	Total population	Groups of IS concentration (mg/L)		*P* value[a]	Groups of *p*CS concentration (mg/L)		*P* value[a]
		Low IS (<22.46)	High IS (≥ 22.46)		14 (1.16)	14 (1.16)	
Number of participants	119	59	60		58	59	
Female, *n* (%)	71 (59.7)	33 (55.9)	38 (63.3)	0.525	33 (56.9)	37 (62.7)	0.651
Age (years)	43.03 ± 12.10	44.97 ± 11.22	41.12 ± 12.71	0.082	44.59 ± 11.39	40.93 ± 12.26	0.098
BMI (kg/m^2)	20.10 (18.95, 22.90)	20.60 (19.20, 22.95)	19.95 (18.40, 22.90)	0.165	20.24 (19.05, 22.08)	20.10 (18.90, 23.30)	0.825
Hypertension, *n* (%)	90 (76.3)	46 (78.0)	44 (74.6)	0.829	43 (74.1)	45 (77.6)	0.828
Diabetes, *n* (%)	5 (4.2)	5 (8.5)	0 (0.0)	0.065	1 (1.7)	3 (5.1)	0.623
Hyperlipidemia, *n* (%)	58 (48.7)	31 (52.5)	27 (45.0)	0.522	26 (44.8)	31 (52.5)	0.516
SBP (mmHg)	146.29 ± 26.46	148.85 ± 26.43	143.73 ± 26.46	0.295	148.86 ± 26.02	143.91 ± 27.00	0.317
DBP (mmHg)	87.53 ± 14.68	88.08 ± 14.56	86.97 ± 14.90	0.681	89.60 ± 14.92	85.83 ± 14.11	0.164
Blood glucose (mmol/L)	5.16 (4.95, 5.57)	5.30 (4.90, 5.89)	5.12 (4.95, 5.35)	0.093	5.22 (4.87, 5.56)	5.15 (4.98, 5.63)	0.527
TC (mmol/L)	4.87 ± 1.10	5.00 ± 1.21	4.74 ± 0.98	0.206	4.95 ± 1.06	4.81 ± 1.16	0.518
TG (mmol/L)	1.42 (1.11, 2.12)	1.40 (1.17, 2.27)	1.46 (1.07, 1.99)	0.475	1.38 (1.03, 2.11)	1.49 (1.17, 2.09)	0.38
LDL-C (mmol/L)	2.69 ± 0.84	2.72 ± 0.92	2.66 ± 0.77	0.697	2.72 ± 0.79	2.68 ± 0.90	0.767
HDL-C (mmol/L)	1.16 (0.97, 1.40)	1.20 (0.99, 1.42)	1.16 (0.96, 1.39)	0.46	1.24 (0.99, 1.44)	1.06 (0.92, 1.35)	0.046
eGFR (ml/ min/1.73m^2)	4.50 (3.65, 5.40)	5.10 (4.20, 6.20)	4.00 (3.38, 4.82)	<0.001	4.45 (3.45, 5.40)	4.50 (3.85, 5.40)	0.448
Creatinine (umol/L)	951.69 ± 271.95	838.75 ± 236.31	1062.75 ± 260.23	<0.001	973.33 ± 288.87	933.64 ± 250.05	0.428
Uric acid (umol/L)	396.00 (353.50, 434.00)	382.00 (320.55, 416.90)	410.65 (374.45, 453.70)	0.006	390.65 (355.68, 417.40)	403.00 (350.80, 436.40)	0.506
Urea nitrogen (mmol/L)	17.12 (13.71, 20.46)	16.20 (13.71, 18.80)	18.50 (13.83, 21.00)	0.123	17.19 (12.27, 20.02)	17.88 (14.41, 21.00)	0.247
Albumin (g/L)	40.17 ± 3.86	39.84 ± 4.03	40.50 ± 3.70	0.358	39.13 ± 3.40	41.40 ± 3.79	0.001
Pre-albumin (mg/L)	402.35 ± 76.13	383.88 ± 72.80	419.60 ± 75.69	0.011	389.38 ± 65.86	417.69 ± 82.29	0.045
Transferrin (g/L)	2.11 (1.87, 2.47)	2.06 (1.87, 2.44)	2.12 (1.92, 2.52)	0.48	2.09 (1.87, 2.45)	2.12 (1.90, 2.51)	0.693
Ferritin (ug/L)	109.90 (47.37, 251.52)	127.95 (63.50, 264.10)	101.70 (43.55, 241.17)	0.258	107.85 (50.80, 220.03)	106.70 (45.26, 250.63)	0.794
Hemoglobin (g/L)	107.00 (98.25, 121.00)	106.00 (99.25, 123.00)	107.50 (95.00, 119.00)	0.554	106.00 (97.00, 123.00)	108.00 (101.00, 119.50)	0.834
Potassium (mmol/L)	4.43 (4.02, 4.97)	4.38 (4.02, 4.96)	4.50 (4.02, 5.00)	0.684	4.43 (4.09, 5.03)	4.50 (3.99, 4.89)	0.622
Calcium (mmol/L)	2.48 (2.39, 2.58)	2.44 (2.38, 2.57)	2.49 (2.41, 2.58)	0.262	2.49 (2.40, 2.59)	2.45 (2.37, 2.56)	0.277
Phosphorus (mmol/L)	1.63 (1.35, 2.00)	1.43 (1.17, 1.71)	1.77 (1.48, 2.30)	<0.001	1.64 (1.34, 1.97)	1.55 (1.36, 2.00)	0.595
Magnesium (mmol/L)	0.91 ± 0.15	0.86 ± 0.14	0.95 ± 0.15	0.003	0.91 ± 0.15	0.90 ± 0.15	0.712
Sodium (mmol/L)	140.30 (138.30, 141.85)	140.30 (138.40, 142.00)	140.40 (138.30, 141.35)	0.947	140.10 (138.50, 141.30)	140.50 (138.30, 142.30)	0.343
HCO_3 (mmol/L)	25.14 ± 2.72	25.26 ± 2.88	25.01 ± 2.58	0.617	25.08 ± 2.89	25.16 ± 2.54	0.881
Iron (mmol/L)	12.66 (9.27, 16.93)	13.04 (8.35, 17.33)	12.28 (10.19, 16.05)	0.916	12.66 (9.24, 16.73)	12.61 (10.07, 17.50)	0.515
Parathyroid hormone (pg/ml)	345.05 (190.00, 568.90)	361.80 (206.25, 494.58)	325.75 (153.62, 605.42)	0.628	339.50 (183.60, 515.23)	388.80 (200.90, 604.33)	0.401
β2-microglobulin (mg/L)	28.95 (23.81, 40.85)	25.43 (21.21, 31.04)	37.06 (28.09, 47.12)	<0.001	31.48 (23.91, 40.95)	26.53 (22.52, 40.58)	0.392
ALP (u/L)	74.00 (59.00, 90.00)	78.00 (58.50, 92.00)	73.00 (62.75, 87.50)	0.968	68.50 (56.50, 92.00)	76.00 (63.50, 90.00)	0.264
IS (mg/L)	22.46 (13.45, 29.92)	13.04 (8.42, 18.54)	29.92 (26.14, 37.81)	<0.001	19.43 (8.85, 26.19)	26.00 (18.52, 32.81)	0.001
*p*CS (mg/L)	12.41 (5.29, 24.45)	8.42 (3.00, 19.70)	17.19 (6.88, 26.07)	0.029	5.21 (2.41, 8.01)	24.27 (18.04, 34.29)	<0.001

Abbreviations: ALP: Alkaline phosphatase; BMI: body mass index; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; HDL-C: high density lipoprotein cholesterol; IS: Indoxyl sulfate; LDL-C: low density lipoprotein cholesterol; *p*CS: *p*-cresyl sulfate; SBP: Systolic Blood Pressure; TC: total cholesterol; TG: triglyceride.

Continuous variables were presented as mean ± SD or median (interquartile range), categorical variables were presented as frequency (%).

[a]*P* values were derived from student's t tests or Kruskal-Wallis tests for continuous variables and chi-square tests for categorical variables.

Table 2. Spearman's rank correlation coefficients between IS, pCS and clinical indexes.

Clinical indexes	IS		*p*CS	
	r	*P* value	r	*P* value
Age	-0.16	0.08	-0.14	0.13
BMI	-0.13	0.16	-0.11	0.23
Peritoneal dialysis duration	0.22	0.02	-0.07	0.47
SBP	-0.1	0.27	-0.15	0.12
DBP	-0.05	0.62	-0.19	0.04
Blood glucose	-0.05	0.58	0.07	0.46
TC	-0.05	0.57	-0.01	0.94
TG	-0.08	0.39	0.02	0.82
LDL-C	0.03	0.77	0.02	0.86
HDL-C	-0.09	0.31	-0.14	0.13
eGFR	-0.46	<0.001	0.1	0.27
Creatinine	0.48	<0.001	-0.07	0.46
Uric acid	0.13	0.15	-0.01	0.91
Urea nitrogen	0.16	0.08	0.05	0.56
Albumin	0.1	0.29	0.32	<0.001
Pre-albumin	0.32	<0.001	0.15	0.11
Transferrin	0.09	0.35	0.01	0.95
Ferritin	-0.09	0.34	0.1	0.31
Hemoglobin	-0.1	0.28	0	0.99
Potassium	-0.02	0.81	-0.07	0.48
Calcium	0.12	0.18	-0.07	0.43
Phosphorus	0.34	<0.001	-0.11	0.25
Magnesium	0.28	0.002	-0.02	0.82
Sodium	-0.03	0.71	0.03	0.77
HCO_3	0.03	0.75	0.07	0.47
Iron	-0.01	0.96	0.04	0.67
Parathyroid hormone	0.05	0.6	-0.05	0.58
β2-microglobulin	0.5	<0.001	-0.09	0.33
ALP	-0.02	0.83	-0.03	0.72

IS and *p*CS according to clinical indexes using univariate general linear models

In Table 3, we used univariate linear regression models to estimate associations of clinical indexes with serum IS and *p*CS levels. Positive direct associations were observed between serum IS concentration and PD duration, creatinine, pre-albumin, phosphorus, magnesium, and β2-m, with the corresponding β and 95% CIs of 1.19 (0.09, 2.29), 0.02 (0.01, 0.03), 0.05 (0.03, 0.08), 6.62 (3.00, 10.23), 20.12 (6.37, 33.87), and 0.47 (0.33, 0.62). Also, statistically negative associations were observed between IS and eGFR [-2.84 (-3.93, -1.74)]. Besides, the positive association between *p*CS and albumin [1.28 (0.61, 1.95)] was indicated.

General linear model, stepwise regression based on Akaike Information Criterion (AIC)

Taking IS and *p*CS as the independent variables and the clinical indicators as the dependent variables, we conducted stepwise regression analyses. Finally, sex, TG, HDL-C, creatinine, pre-albumin, and β2-m entered the equation of IS. BMI, SBP, DBP, blood glucose, albumin, and phosphorus entered the equation of pCS, as presented in Tables 4 and 5. The results indicated that those variables were dependent risk factors for increasing IS and *p*CS levels. Sex, creatinine, pre-albumin, and β2-m were significantly positive associated with serum IS. BMI and DBP were significantly negative associated with serum *p*CS while blood glucose and albumin were significantly positive associated with serum pCS.

Association of serum IS and *p*CS concentration with CVD risk factors

Taking CVD risk factors as dependent variables, we examined the associations of IS and *p*CS with blood pressure, glucose, and lipids using multivariate-adjusted general linear models as described in Table 6. We found that an IQR increase in serum IS concentration was significantly associated with HDL-C, with the corresponding β of-0.10 (95% CI: -0.19, -0.006) in the fully adjusted model l. An IQR increase in serum *p*CS concentration was significantly associated with DBP [-3.68 (-7.08, -0.27)] in model 2; however, after further adjusted for albumin, pre-albumin, calcium, phosphorus and ALP, the association was no longer significant. Besides, an IQR increase in serum *p*CS concentration was significantly associated with blood glucose, with β of 0.22 (95% CI: 0.02, 0.41) in model 1, however, after further adjusted for SBP, blood glucose, TG, HDL-C and LDL-C, the association was no longer significant.

Adjusted odds ratios for hypertension and hyperlipidemia according to IS and *p*CS concentration

In Figure 1, we showed multivariable-adjusted odds ratios and 95% CI for hypertension and hyperlipidemia with the low IS and *p*CS concentration groups as reference groups. Model 1 was adjusted for age, sex, BMI, PD duration, and eGFR. Model 2 was further adjusted for hypertension, diabetes, and hyperlipidemia, except itself. Model 3 was further adjusted for albumin, pre-albumin, calcium, phosphorus, and ALP. With the confounding factors mentioned above adjusted, no significant association were observed between serum IS and *p*CS levels with hypertension and hyperlipidemia.

Table 3. (95 % CI) for IS and pCS according to clinical indexes using univariate general linear models.

Variables	IS		*p*CS	
	β (95 % CI)	*P* value	(95 % CI)	*P* value
Age	-0.16 (-0.34, 0.014)	0.07	-0.13 (-0.35, 0.10)	0.27
Sex	2.16 (-2.23, 6.54)	0.34	0.22 (-5.23, 5.67)	0.94
BMI	-0.49 (-1.27, 0.29)	0.22	-0.59 (-1.54, 0.36)	0.23
Peritoneal dialysis duration	1.19 (0.09, 2.29)	0.04	-0.92 (-2.29, 0.45)	0.19
SBP	-0.06 (-0.14, 0.02)	0.17	-0.08 (-0.18, 0.02)	0.11
DBP	-0.05 (-0.20, 0.10)	0.52	-0.17 (-0.36, 0.008)	0.06
Blood glucose	-0.55 (-3.07, 1.98)	0.67	2.72 (-0.46, 5.89)	0.1
TC	-0.52 (-2.48, 1.45)	0.61	0.18 (-2.24, 2.59)	0.89
TG	-0.11 (-2.01, 1.79)	0.91	1.20 (-1.16, 3.57)	0.32
LDL-C	-0.11 (-2.68, 2.46)	0.93	-0.06 (-3.24, 3.13)	0.97
HDL-C	-3.88 (-9.75, 1.98)	0.2	-4.19 (-11.60, 3.22)	0.27
eGFR	-2.84 (-3.93, -1.74)	<0.001	0.94 (-0.55, 2.44)	0.22
Creatinine	0.02 (0.01, 0.03)	<0.001	-0.01 (-0.02, 0.01)	0.33
Uric acid	0.02 (-0.01, 0.04)	0.29	-0.02 (-0.06, 0.02)	0.39
Urea nitrogen	0.33 (-0.05, 0.70)	0.09	-0.18 (-0.66, 0.29)	0.45
Albumin	0.41 (-0.15, 0.96)	0.15	1.28 (0.61, 1.95)	<0.001
Pre-albumin	0.05 (0.03, 0.08)	<0.001	0.03 (-0.01, 0.06)	0.15
Transferrin	2.07 (-2.88, 7.03)	0.41	-0.34 (-6.54, 5.85)	0.91
Ferritin	-0.002 (-0.01, 0.004)	0.61	0.002 (-0.005, 0.01)	0.52
Hemoglobin	-0.07 (-0.18, 0.03)	0.16	0.02 (-0.11, 0.15)	0.77
Potassium	-0.64 (-3.68, 2.41)	0.68	-1.78 (-5.58, 2.02)	0.36
Calcium	10.86 (-0.26, 21.98)	0.06	-3.43 (-18.71, 11.84)	0.66
Phosphorus	6.62 (3.00, 10.23)	<0.001	-3.87 (-8.70, 0.96)	0.12
Magnesium	20.12 (6.37, 33.87)	0.005	-2.30 (-20.40, 15.80)	0.8
Sodium	0.16 (-0.06, 0.39)	0.16	0.01 (-0.28, 0.29)	0.97
HCO_3	0.12 (-0.68, 0.92)	0.77	0.52 (-0.47, 1.51)	0.31
Iron	0.14 (-0.22, 0.50)	0.44	0.16 (-0.28, 0.60)	0.48
Parathyroid hormone	0.004 (-0.001, 0.009)	0.1	-0.003 (-0.009, 0.004)	0.4
β2-microglobulin	0.47 (0.33, 0.62)	<0.001	-0.01 (-0.22, 0.20)	0.94
ALP	0.02 (-0.05, 0.08)	0.6	-0.04 (-0.12, 0.03)	0.27

Table 4. (95 % CI) for IS according to explanatory variables chosen by stepwise regression.

	β (95 % CI)	*P* value
Sex	6.09 (1.83, 10.35)	0.006
TG	-1.43 (-3.16, 0.30)	0.11
HDL-C	-5.78 (-11.73, 0.17)	0.06
Creatinine	0.02 (0.01, 0.023)	<0.001
Pre-albumin	0.03 (0.01, 0.06)	0.01
β2-microglobulin	0.21 (0.04, 0.38)	0.02
ALP	ALP	ALP

Table 5. (95 % CI) for pCS according to explanatory variables chosen by stepwise regression.

	β (95 % CI)	*P value*
BMI	-1.06 (-1.95, -0.17)	0.02
SBP	0.12 (-0.03, 0.27)	0.12
DBP	-0.30 (-0.55, -0.04)	0.03
Blood glucose	3.55 (0.62, 6.48)	0.02
Albumin	1.53 (0.83, 2.23)	<0.001
Phosphorus	-4.24 (-8.66, 0.19)	0.06

Table 6. Association of serum IS and pCS concentration with CVD risk factors among the study participants.

CVD risk factors	(95 % CI) for an IQR increase of IS concentration			β (95 % CI) for an IQR increase of *p*CS concentration		
	Model 1[a]	Model 2[b]	Model 3[c]	Model 1[a]	Model 2[b]	Model 3[c]
SBP	-2.78 (-10.10, 4.54)	-2.11 (-9.30, 5.08)	-3.89 (-11.15, 3.37)	-5.82 (-11.96, 0.33)	-4.79 (-10.94, 1.35)	-2.02 (-8.42, 4.39)
DBP	-2.63 (-6.66, 1.40)	-2.09 (-6.10, 1.92)	-2.94 (-7.16, 1.29)	-3.81 (-7.16, -0.45)	-3.68 (-7.08, -0.27)	-3.01 (-6.65, 0.62)
Blood glucose	-0.06 (-0.30, 0.18)	-0.04 (-0.29, 0.21)	-0.04 (-0.31, 0.22)	0.22 (0.02, 0.41)	0.20 (-0.003, 0.39)	0.23 (0.01, 0.44)
TC	-0.11 (-0.40, 0.17)	-0.07 (-0.34, 0.21)	-0.07 (-0.35, 0.22)	-0.02 (-0.26, 0.22)	-0.03 (-0.27, 0.21)	0.03 (-0.22, 0.28)
TG	-0.07 (-0.38, 0.25)	-0.18 (-0.46, 0.10)	-0.21 (-0.50, 0.09)	0.21 (-0.05, 0.47)	0.04 (-0.20, 0.29)	-0.03 (-0.29, 0.24)
HDL-C	-0.08 (-0.18, 0.01)	-0.09 (-0.17, -0.001)	-0.10 (-0.19, -0.006)	-0.07 (-0.14, 0.01)	-0.03 (-0.11, 0.04)	-0.04 (-0.11, 0.04)
LDL-C	-0.02 (-0.25, 0.20)	0.01 (-0.22, 0.24)	0.04 (-0.20, 0.27)	-0.04 (-0.24, 0.15)	0.01 (-0.19, 0.20)	0.07 (-0.13, 0.27)

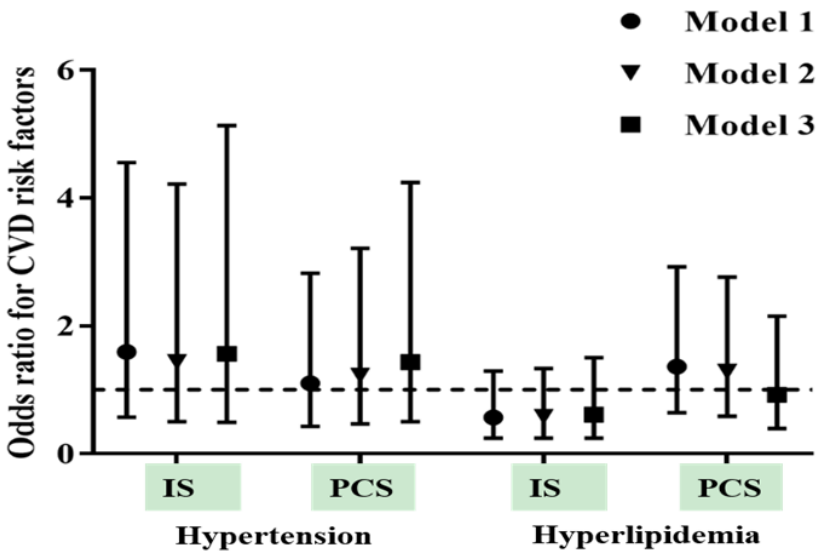

Figure 1. Adjusted odds ratios for hypertension and hyperlipidemia according to IS and pCS concentration.

Discussion

In our study, we found that serum IS and *p*CS were associated closely with cardiovascular risk factors, which contribute to CVD in PD patients. In the study, as shown in Table 1, serum levels of albumin, pre-albumin, and IS of the high *p*CS group were significantly higher than low *p*CS group. Furthermore, serum HDL-C of high *p*CS group was significantly lower than low *p*CS group. Similarly, serum creatinine, uric acid, pre-albumin, phosphorus, magnesium, β2-m, and *p*CS were significantly higher in the high IS group. Moreover, eGFR was significantly lower in the high IS group, supporting the idea that RRF are more effective in decreasing the serum level of IS and *p*CS and their high serum level of these uremic toxins contribute to heart failure and CVD [19]. Similarly, in a recent study, in the high-IS group, serum normalized Protein Nitrogen Appearance (nPNA), albumin, pre-albumin, Blood Urea Nitrogen (BUN), and creatinine were significantly higher [20]. In another finding, correlation analysis depicted that IS was positively associated with serum albumin, BUN, and creatinine levels [21]. Furthermore, Xue-Sen et al. in Kaplan–Meier analysis, confirmed that the incidence of first heart failure was significantly higher in the high IS group [20]. The above finding supports the idea that IS may be comprised in the pathogenesis of CVD [20]. Barrios et al. [22], also showed that IS, and *p*CS are primary risk factors for the decline of renal outcome.

In our finding, serum IS level was significantly associated with the *p*CS level, which was consistent with previous research; serum IS had a positive association with serum *p*CS [23]. Similarly, another study also showed that, for the first time, a significant positive association between IS and *p*CS [21], which have similar origin and clearance depending on the renal function. However, one previous study, recruited 75 Hemodialysis (HD) patients, revealed that serum IS was not associated with the *p*CS level [24]. This discrepancy might be due to the difference of PD and HD. Further studies are essential to clarify this inconsistency.

High serum IS level was positively associated with peritoneal dialysis duration (osteoarticular disorders [25]), serum creatinine concentration (muscle mass, nutrition, RRF and urinary protein losses [26]), pre-albumin (atherosclerotic vascular disease [26]), phosphorus (inducing endothelial dysfunction and stimulate vascular calcification [27,28]) and β2-m (vascular calcification and cardiovascular events [29]) and negatively associated with eGFR. High serum *p*CS concentration was also positively associated with albumin level. Similar results were reported [30-32], in which serum levels of IS, pCS, hippurate, adipic acid, and cinnamoyl glycine were high in subjects with kidney failure.

In the study, we revealed that serum concentration of IS was high when eGFR declined in PD patients, which was in line with functional studies done by former researches [33,34], in which secretory clearances declined in PD patients with the eGFR. Our data reinforced the finding done by Liesbeth et al. [34] that conservation of eGFR also provided to the inverse relationship with IS. Hence elevated serum IS in PD patients may be assumed to be on the causal pathway between eGFR and CVD [34]. However, in our study, serum *p*CS level was not significantly associated with eGFR. Similarly, Evelien et al. [35], in his earlier outcome, showed that *p*CS was not associated with eGFR. Of note, the inconsistency of uremic toxin concentrations among subjects was considerable, especially for the PBUTs, and was in line with the study done by Eloot et al. [36].

In our PD patients, a high level of median serum IS (22.5 mg/L) was significantly associated with an elevated level of median β2-m (29.0 mg/L). Similarly, in a recent study, the mean serum IS and β2-m levels, 2.7 mg/L, and 5.1 mg/L, respectively, were both significantly increased when CKD was diagnosed versus with baseline [37]. A recent mean follow-up study for 969 days revealed that the serum concentration of β2-m in chronic dialysis patients was associated with vascular calcification, together with CVD [29]. In PD patients, serum β2-m level is conversely associated with RRF [38].

In the study, a high concentration of IS was positively associated with increased serum concentration of creatinine, which was in line with a previous finding [39]. Similarly, in one earlier study, IS was significantly associated with crea¬tinine level, undoubtedly signify the characteristics of retention molecules in renal failure [21].

In our PD patients, we observed a high level of IS was significantly associated with an elevated serum level of pre-albumin. Prior studies have found that PD patients, on average, have higher pre-albumin levels versus HD patients. This might be alterations in pre-albumin metabolism due to the loss of a regulatory molecule through PD, analogous to changes in cholesterol metabolism [40].

In the study, a high level of serum IS in PD patients were significantly related to an elevated level of serum phosphorous, which are implicated in the pathogenesis of accelerated adynamic bone disease and CVD [34]. Similarly, in recent findings, CKD-Mineral Bone Disorder (MBD) is associated with persistent hyperphosphatemia, elevated PTH that leads to deformities of bone turnover and systemic bone vascular disease. Skeletal resistance to PTH induces low turnover adynamic bone disease in CKD patients, which is a crucial mechanism of CKD-MBD [41]. Similarly, RRF helps to control phosphate and β2-m [42], which was elevated in our PD patients. A previous finding depicted that serum IS concentrations are significantly associated with phosphate concentration in CKD patients [43].

Kidney function, which is the crucial excretion route of magnesium, [44] is essential when considering the cause of hypermagnesemia [45]. In the study, a high level of serum IS in PD patients was correlated with an elevated concentration of magnesium with eGFR of 4.50 (3.65, 5.40) mL/min/1.73 m^2. Similarly, In the recent finding, hypermagnesemia was caused by eGFR ≤ 30 mL/min/1.73m^2 [44,46]. It was described that eGFR of <30 mL/min/1.73 m2 in aged Japan's patients was related to a risk of hypermagnesemia due to the ingestion of magnesium oxide [47]. A high level of serum magnesium is a risk factor for CKD patients [48]. Kontani et al., in his previous finding, showed that hypermagnesemia frequently results from iatrogenic causes and procedure-related retroperitoneal or peritoneal leakage of magnesium-containing preparations [49]. In a recent study, hypermagnesemia is depicted by neuromuscular, respiratory, and CVD [50].

In our finding, increasing levels of serum *p*CS significantly associated with a high concentration of blood glucose. Similarly, previous studies confirmed that blood glucose could intrinsically cause an elevated serum *p*CS concentration [51,52]. A similar result was confirmed [53], in patients treated with PD. Szeto et al. [54] also depicted that for every 10 g/day higher glucose exposure, there was a 2.5% increase in the risk of CKD. This result supported the fact that the recommendations of the International Society for peritoneal dialysis that PD glucose dialysates with neutral pH and lower Glucose Degradation Products (GDP) are favored to preserve RRF [55]. One study also showed that *p*CS played a role in insulin resistance, in abnormal adipose tissue metabolism, and reallocation of fat in the body [56]. In the recent study, both IS, and *p*CS must be regarded as vital damaging vascular toxins and promoters of insulin resistance in CKD rats [57].

In our study, a high serum concentration of pCS was significantly associated with a decrease in DBP. Both low and high DBP are risk factors for the decline of RRF in dialysis patients [58]. A similar finding was done by Tian et al. [53]; the existence of CKD can confound the effect of maintaining blood pressure on RRF during episodes of intradialytic hypotension. The impact of DBP on RRF is not clear, but care is needed to avoid prolonged hypotension [53].

In the study, a high IS level was significantly associated with low serum HDL-C, which is a risk factor for vascular calcification and stiffness and is linked to an enhanced marker of progressing CVD [59]. Moreover, the concentration of IS has been associated with pentosidine and HDL-C, a marker of atherosclerosis in dialysis patients [60]. A similar study was done by Barreto et al. [61], in which a high level of IS was related to elevated aortic calcification and vascular stiffness in CKD patients. Lin et al. [62] also reported that higher total IS levels were associated with an enhanced risk of CVD in CKD patients. In another finding, a higher risk of coronary artery calcification was associated with serum IS [63]. In the previous study, IS facilitates atrial fibrillation [64]. Recent research suggests that IS may be related to impaired exterior arterial disease and neovascularization in CKD [65].

Our study had limitations. First, the relatively small number of subjects limited the statistical power for finding potential confounding factors, which is leading to large CI. Second, the study was done at a single center; therefore, center-specific effects cannot be excluded, although the patients were from various districts of central China. Selection biases cannot be avoided because of the small number of patients, so it may be challenging to extrapolate the results to all PD patients, which may limit the representativeness of the outcomes to other populations. Thus, the case-mix characteristics may not have been representative of the general PD population. Lastly, among eligible patients, some variables were missing. This cross-sectional study was conducted only in PD patients. Therefore, we believe that more extensive studies performed in all pre-dialysis patients with CKD are required. Interventional studies that include large groups of patients are necessary to evaluate whether therapeutic interferences can enhance the prognosis of dialysis patients.

Conclusion

This study investigated serum clinical indicators were dependent cardiovascular risk factors for increasing IS and pCS levels, which contribute to CVD in peritoneal dialysis patients. Even though most studies have proposed the advantage of therapeutic intervention on PBUT levels, most of these data came from post hoc analysis or small studies. Therefore, a more extensive, randomized control trial aimed to identify the impact of decreasing the levels of uremic toxins on hard outcomes, such as overall and cardiovascular mortality, is essential.

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