Original Article

Increased Levels of Total *p*-Cresylsulfate are Associated with Anxiety in Patients with Concomitant Coronary Artery Disease and Chronic Kidney Disease

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Objective: Psychosocial factors including anxiety, depression, and lower social support are common in patients with chronic kidney disease (CKD). Indoxyl sulphate (IS) and *p*-cresylsulfate (PCS) are uremic toxins with similar protein binding, dialytic clearance, and proinflammatory features. In addition, the associations between total IS and depression have also been established in previous research. We thus investigated the association of total IS and PCS with anxiety in the clinical setting.

Methods: The serum levels of total IS and PCS concentrations were measured in 813 consecutive patients with concomitant coronary artery disease (CAD) and CKD by using the Ultra Performance LC System. Anxiety levels were evaluated using the Beck Anxiety Inventory.

Results: Among the 813 patients with both CAD and CKD, 40% had anxiety. Compared with patients without anxiety, those with anxiety were older and had higher frequency of hypertension and female gender, higher BAI score, diastolic blood pressure, phosphorus, high-sensitivity C-reactive protein, total IS, and PCS levels as well as a lower frequency of current smoking and lower body mass index. Increased concentrations of total PCS were independently and significantly associated with anxiety. Multiple logistic regression analysis revealed total PCS as an independent association factor for anxiety, even after full adjustment of known biomarkers. Furthermore, serum total PCS levels were positively associated with serum levels of phosphorus, calcium, blood urea nitrogen, creatinine, and white blood cell count, and negatively associated with estimated glomerular filtration rate, hemoglobin concentration, and hematocrit.

Conclusions: Our findings provide evidence that total PCS may be independently associated with anxiety in patients with CAD and CKD. Whether total PCS plays a role in the pathogenesis of anxiety requires future investigation.

Key words: anxiety, chronic kidney disease, indoxyl sulfate, total *p*-cresylsulfate

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Introduction

Anxiety and depression disorders are of major importance in public health. These disorders are associated with a marked impairment of quality of life,[1] increased cardiovascular morbidity and mortality,[2,3] and a high rate of recurrence and chronicity.[4] The pathogenesis of anxiety is multifactorial, including a number of psychiatric illnesses such as anxiety disorders,[5] genetic and environmental factors[6,7] as well as increased expression of inflammatory markers such as interleukin (IL)-6, C-reactive protein (CRP), and tumor necrosis factor (TNF)-α.[8,9] The causes of the higher prevalence of anxiety in chronic kidney disease (CKD) patients are uncertain and may be related to a high prevalence of comorbid chronic diseases, complications of anemia and vitamin B12 deficiency, medications, genetic factors, greater mental stress and inflammatory status.[6-8] The presence of uremic products may also contribute to anxiety. Although the significant relationship between CKD and renal toxins has been studied and reviewed,[10] the relationship between CKD and anxiety remains unclear. CKD simply reflects renal functional status, unlike protein-bound uremic toxins which has been reported to trigger a meaningful and predominant direct reaction causing anxiety.[11,12]

The major renal toxins are protein-bound, especially *p*-cresylsulfate (PCS) and indoxyl sulfate (IS), and are absorbed through the gastrointestinal tract. High serum IS and PCS levels have been found to be associated with renal disease progression and mortality in CKD patients,[13] and were newly investigated compounds in connection with cardiovascular risk factors.[14-18] P-cresol (PC) is derived from ingested phenylalanine and plant phenols. In humans, it exists predominantly as conjugated PCS.[19] IS is an organic anion originating from indole, produced by intestinal bacteria as a metabolite of tryptophan, and metabolized in the liver.[20] In uremic patients, serum levels of PC and IS are elevated approximately 10 and 50 folds,[21,22] respectively, because of their strong protein-binding ability and poor clearance from the kidney during conventional hemodialysis.[23] Inflammation and immune function are principally associated with depressive and anxiety disorders in CKD patients, and patients with anxiety have higher interleukin 6 levels compared to those without.[8] Other studies have indicated that several inflammatory cytokines promote depression and anxiety, such as TNF-α or interleukin 8 (IL-8).[24,25] PCS and IS both have well-known pro-inflammatory properties that contribute to the pathogenesis of systemic inflammation.[26-28] Previously, human subjects with anxiety trait showed a distinct metabolic profile indicative of a different energy homeostasis (lactate, citrate, succinate, trans-aconitate, urea, proline), hormonal metabolism (adrenaline, DOPA, 3-methoxytyrosine) and gut microbial activity (methylamines, PCS, hippurate).[29] Taken together, these observations indicate that total IS and PCS may play a role in anxiety. However, until now, little data has been made available on the role of total IS and PCS in human subjects with anxiety. To investigate the association of anxiety with total IS and PCS, we measured serum levels of total IS and PCS in a Chinese population with CKD.

Materials and Methods

Participants

We prospectively enrolled 937 consecutive coronary artery disease (CAD) patients with concomitant CKD who were admitted to E-Da Hospital Cardiovascular ward between June 2006 and June 2010. Estimated glomerular filtration rate (eGFR) of each patient was calculated according to the formula in the extended Modification of Diet in Renal Disease (MDRD) Study within 3 − 6 months of admission.[30] The CKD status was confirmed by following the eGFR at three months after hospital discharge. Patients with CKD stage 1

were eligible for inclusion in the study. In the present study, the underlying renal diseases of the enrolled population were diabetic nephropathy (n = 303), hypertension (n = 491), obstructive nephropathy (n = 11), and glomerulonephritis (n = 8) according to their personal health records and laboratory findings. The exclusion criteria of the study were (1) active infection, (2) psychotic illness or other communication problems, (3) active malignancy, (4) age under 20 years, (5) patient refusal, and (6) acute kidney injury. After excluding 124 patients, a total of 813 CKD patients (442 with acute coronary syndrome, 371 with elective percutaneous coronary intervention for known CAD) were included in this study. The diagnosis of type 2 DM was based on the World Health Organization criteria.[31] Renal function of the patients was classified into categories based on the general filtration rate (GFR) according to the 2012 KDIGO guideline: Stage 1: eGFR ≥ 90 with albuminuria (Alb), Stage 2: eGFR 60 − 89 + with albuminuria, Stage 3a: eGFR 45 − 59, Stage 3b: eGFR 30 − 44, Stage 4: eGFR 15 − 29, Stage 5: eGFR < 15. The study protocol and procedure were approved by the Ethics Committee of E-Da Hospital (EDAH IRB No. EMRP-105-053). Informed consents were obtained from all subjects.

Laboratory measurements

Peripheral blood samples were taken from the antecubital vein after admission to the hospital. Blood samples collected for total IS and PCS determination were centrifuged and stored at −80°C for subsequent assay. After an overnight fast exceeding 8 hours, complete blood counts as well as serum creatinine and serum lipid profiles were determined in all patients. Plasma triglycerides, total cholesterol, low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), uric acid, creatinine, phosphorus, calcium, and glucose were measured with standard commercial methods using a parallel, multi-channel analyzer (Hitachi 7170A, Tokyo, Japan) as we previously reported.[14] Urinary albumin concentration was measured by immunoturbidimetry (Beckman Instruments, Galway, Ireland). The detection limit was 2 mg/L, and the interassay and intraassay coefficients of variance were less than 8%.

Serum samples were deproteinized by adding 3 parts methanol to 1 part serum for determination of total PCS and IS. All analyses were performed using a Waters Acquity Ultra Performance Liquid Chromatography (UPLC) system (Milford, MA, USA). UPLC assays with detection wavelength set at 280 nm for IS and 260 nm for PCS were performed at room temperature on an Acquity UPLC BEH phenyl column of 2.1 × 100 mm. The column temperature was maintained at 30°C. The buffer flow was 0.4 mL/min using 10 mM $NH_4H_2PO_4$ (pH = 4.0) (A) and acetonitrile (B) with a gradient from 82.5% A/17.5% B to 55% A/45% B over 9 minutes. Under these conditions, IS and PCS appeared at 1.4 min and 1.7 min, respectively. The limits of detection of this assay were 0.225 mg/L for IS and 1 mg/L for PCS. Calibration curves were constructed by plotting the peak areas against the concentrations of each analysate, and the curves had average r^2 values of 0.999 ± 0.001. Quantitative results were obtained and calculated as concentrations (mg/L). Intra- and inter-assay coefficients of variation (relative standard deviation) were 0.4% and 0.05% for IS and 5.50% and 7.48% for PCS, respectively. Furthermore, parallel comparisons of serum total PCS and IS levels obtained from UPLC and mass spectrometry (MS) in 10 randomly selected patients did not reveal a significant disagreement in the Bland-Altman plots (Pitman's test of difference in variance: r = −0.263 and P = 0.493 for serum IS; r = −0.765 and P = 0.124 for serum PCS).[14-18] In addition, the concentration of plasma CRP was measured using a high-sensitivity method (IMMAGE; Beckman Coulter, Immunochemistry Systems, Brea, CA, USA). The intra-

assay coefficients of variation were 4.2 − 8.7% for hs-CRP. Samples were measured in duplicate in a single experiment.

Anxiety assessment

The patients were evaluated using a detailed interview questionnaire based on the 21-item self-report Beck Anxiety Inventory (BAI; range 0 − 63) to assess various characteristics of anxiety.[32] The internal and test-retest reliability and validity of the BAI are well-established.[32,33] Individual items on the BAI are rated from 0 (absent) to 3 (severe symptoms; almost unbearable).[34] A score of 0 − 7 indicates minimum level of anxiety; 8 − 15 mild anxiety, 16 − 25 moderate, and 26 − 63 severe anxiety. In this study, patients with all of their BAI scores ≥ 8 were defined as having anxiety, whereas patients with all their BAI scores ≤ 7 were defined as those without anxiety.

Statistical analysis

Data normality was analyzed using the Kolmogorov-Smirnov test. Continuous, normally distributed variables are presented as mean ± SD, and non-normally distributed variables as medians (interquartile range). Statistical significance of differences among multiple means across different groups was determined by one-way analysis of variance (ANOVA), whereas unpaired Student's t-test was used to compare the differences between normally distributed variables. Categorical variables were recorded as frequencies and/or percentages, and inter-group comparisons were analyzed with the chi-square test. Since the distributions of serum total PCS, IS, hs-CRP, and triglyceride were skewed, logarithmically transformed values were used for statistical analysis.

The association of total IS and PCS with anxiety was examined by multivariate logistic regression analysis on 1) total IS or PCS, age, gender, body mass index (BMI), and albumin; 2) total IS or PCS, age, gender, BMI albumin, hypertension, smoking, and diastolic blood pressure; 3) total IS or PCS, age, gender, BMI, albumin, hypertension, smoking, diastolic blood pressure, phosphorus, hs-CRP, and creatinine; 4) total IS or PCS, age, gender, BMI, albumin, hypertension, smoking, diastolic blood pressure, phosphorus, hs-CRP, and eGFR. We further divided the distribution of total PCS in pooled data into tertiles and used general linear and logistic regression models to estimate the significant trends across increasing tertiles and to estimate the odds ratio (OR) of anxiety in each tertile using the lowest tertile as a reference category. Multivariate adjusted ORs are presented with 95% confidence interval (CI).

Multiple linear regression analyses were used to examine the correlations and independence between serum total PCS and the values of other parameters. Statistical significance was accepted if $p < 0.05$. All of the statistical analyses were performed using SAS statistical software, version 8.2 (SAS Institute Inc.; Cary, NC, USA).

Results

Of the 813 patients screened, 328 fulfilling the selection criteria of being diagnosed as having both CKD and anxiety were assigned to the "anxiety" group. The other 485 patients meeting the criteria except for the absence of anxiety were assigned to the "non-anxiety" group. In the present study, we found that the median serum total IS and PCS levels in patients with anxiety were significantly higher than those in 31 randomly selected healthy subjects with normal renal function (defined as eGFR ≥ 90 mL/min/1.73 m^2 without urine albumin). The median serum total IS level of the anxiety group and the non-anxiety healthy group was 0.6 mg/L (interquartile range 0.2 − 1.3) vs. 0.2 mg/L (interquartile range 0.1 − 0.5), respectively ($p = 0.001$). The median

serum total PCS level was 1.7 mg/L (interquartile range 1.0 − 6.1) vs. 1.0 mg/L (interquartile range 1.0 − 1.6), respectively (p = 0.002). In addition, there was no statistically significant difference in serum total IS and PCS levels between men and women. Patient groups with and without hypertension and/or hyperlipidemia did not have significantly different levels

Table 1. Baseline clinical and biochemical characteristics of the study population

Variable	Total	Anxiety	*No anxiety*	*p value*
No	813	328	485	
Sex (male/female)	603/210	219/109	384/101	< 0.0001
Age (yrs)	65.6 ± 11.2	67.2 ± 11.5	64.5 ± 10.9	0.001
Body mass index (kg/m^2)	25.9 ± 4.1	25.4 ± 3.8	26.2 ± 4.3	0.011
Hypertension (n, %)	597 (73.4)	258 (78.7)	339 (69.9)	0.006
Hyperlipidemia (n, %)	557 (68.5)	229 (69.8)	328 (67.6)	0.510
Diabetes mellitus (n, %)	303 (37.3)	128 (39.0)	175 (36.1)	0.395
Current smoking (n, %)	385 (47.4)	131 (39.9)	254 (52.4)	0.001
Cause of admission				
Acute coronary syndrome	442 (54.4)	177 (54.0)	265 (54.6)	0.850
Elective PCI	371 (45.6)	151 (46.0)	220 (45.4)	0.850
Chronic kidney disease				
Stage 1: eGFR ≥ 90 + Alb	48 (5.9)	8 (2.4)	40 (8.2)	0.055
Stage 2: eGFR 60-89 + Alb	372 (45.8)	147 (44.8)	225 (46.4)	0.795
Stage 3a: eGFR 45-59	217 (26.7)	87 (26.5)	130 (26.8)	0.974
Stage 3b: eGFR 30-44	96 (11.8)	48 (14.6)	48 (9.9)	0.629
Stage 4: eGFR 15-29	45 (5.5)	19 (5.8)	26 (5.4)	0.754
Stage 5: eGFR < 15	35 (4.3)	19 (5.8)	16 (3.3)	0.241
Beck anxiety inventory	7.6 ± 6.9	13.8 ± 6.9	3.5 ± 2.2	< 0.0001
SBP (mmHg)	133 ± 22	133 ± 22	131 ± 22	0.233
DBP (mmHg)	77 ± 14	78 ± 14	76 ± 13	0.024
Fasting glucose (mg/dL)	139.0 ± 66.1	136.2 ± 60.0	141.0 ± 69.9	0.313
HbA1C (%)	6.9 ± 1.7	6.9 ± 1.6	6.9 ± 1.7	0.713
Total-cholesterol (mg/dL)	178.1 ± 40.3	175.0 ± 39.9	180.1 ± 40.4	0.074
Triglyceride (mg/dL)	122.0 (87.5 − 180.0)	123.0 (88.0 − 179.0)	121.0 (87.0 − 182.0)	0.516
HDL-cholesterol (mg/dL)	39.9 ± 12.1	39.8 ± 12.9	40.0 ± 11.6	0.853
LDL-cholesterol (mg/dL)	104.9 ± 34.0	103.0 ± 34.6	106.3 ± 33.5	0.173
Uric acid (mg/dL)	6.6 ± 2.0	6.6 ± 1.9	6.6 ± 2.0	0.585
Phosphorus (mg/dL)	2.6 ± 1.0	2.8 ± 1.0	2.4 ± 0.9	0.022
Calcium (mg/dL)	8.9 ± 0.7	9.0 ± 0.8	8.9 ± 0.7	0.640
Albumin (g/dL)	4.0 ± 0.4	3.9 ± 0.4	4.0 ± 0.4	0.084
Blood urea nitrogen (mg/dL)	21.3 ± 14.3	21.1 ± 14.3	21.5 ± 14.4	0.743
Creatinine (mg/dL)	1.5 ± 1.4	1.6 ± 1.6	1.5 ± 1.3	0.728
eGFR (ml/min/1.73m^2)	56.5 ± 19.5	55.9 ± 20.0	57.0 ± 19.2	0.463
Hs-CRP (mg/L)	2.6 (1.0 − 6.7)	2.7 (1.2 − 6.6)	2.3 (0.8 − 7.2)	0.024
WBC (× 10^9/L)	8.175 ± 3.253	8.354 ± 3.463	7.906 ± 2.898	0.055
Indoxyl sulphate (mg/L)	0.4 (0.2 − 1.2)	0.6 (0.2 − 1.3)	0.2 (0.2 − 1.0)	0.020
Total *p*-cresylsulfate (mg/L)	1.1 (1.0 − 3.8)	1.7 (1.0 − 6.1)	1.0 (1.0 − 2.7)	0.001

Data are expressed as mean ± SD, number (percentage), or median (interquartile range); PCI: Percutaneous coronary intervention; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; eGFR: Estimated glomerular filtration rate; Hs-CRP: High-sensitivity C-reactive protein; WBC: White blood cell.

of serum total IS and PCS. Patient groups with and without diabetes showed a significant difference in serum total PCS levels (data not shown).

Table 1 presents the clinical and biochemical data of the study participants with and without anxiety. Compared with patients without anxiety, serum levels of total IS and PCS were significantly higher among those with anxiety symptoms (0.6 mg/L [interquartile range 0.2 to 1.3] vs. 0.2 mg/L [interquartile range 0.2 to 1.0], p = 0.020; 1.7 mg/L [interquartile range 1.0 to 6.1] vs. 1.0 mg/L [interquartile range 1.0 to 2.7], p = 0.001). Furthermore, patients with anxiety were older and had a higher frequency of female gender and hypertension, higher BAI score, diastolic blood pressure, phosphorus, and hs-CRP, as well as a lower frequency of current smoking and body mass index, while the frequency of hyperlipidemia and diabetes mellitus, causes of admission, CKD stages, systolic blood pressure, levels of fasting glucose, HbA1c, total-cholesterol, triglyceride, HDL-C, LDL-C, uric acid,

Table 2. Association of serum indoxyl sulfate and total p-cresylsulfate with anxiety in fully adjusted models

Model adjusted for	Anxiety		
	OR	95% CI	*P value*
Serum indoxyl sulfate			
Age, gender, BMI, albumin	1.37	0.93 − 2.00	0.110
Age, gender, BMI, albumin, hypertension, smoking, diastolic blood pressure	1.37	0.91 − 2.05	0.129
Age, gender, BMI, albumin, hypertension, smoking, diastolic blood pressure, phosphorus, Hs-CRP, creatinine	1.82	0.92 − 3.61	0.085
Age, gender, BMI, albumin, hypertension, smoking, diastolic blood pressure, phosphorus, Hs-CRP, eGFR	1.69	0.88 − 3.26	0.116
Serum total p-cresylsulfate			
Age, gender, BMI, albumin	1.12	1.02 − 1.22	0.013
Age, gender, BMI, albumin, hypertension, smoking, diastolic blood pressure	1.11	1.02 − 1.22	0.023
Age, gender, BMI, albumin, hypertension, smoking, diastolic blood pressure, phosphorus, Hs-CRP, creatinine	1.11	1.00 − 1.23	0.045
Age, gender, BMI, albumin, hypertension, smoking, diastolic blood pressure, phosphorus, Hs-CRP, eGFR	1.26	1.01 − 1.59	0.042

Results of multivariate logistic regression analysis are presented as the odd ratio (OR) of having an anxiety status and increased serum indoxyl sulfate and total *p*-cresylsulfate levels; CI: Confidence interval; BMI: Body mass index; Hs-CRP: High-sensitivity C-reactive protein; eGFR: Estimated glomerular filtration rate.

Table 3. Univariate and multivariate analysis of the impact of serum total p-cresylsulfate level on anxiety

Factor	Tertiles of total *p*-cresylsulfate			
	Q1 (95% CI)	Q2 (95% CI)	*Q3 (95% CI)*	*p value*
All subjects				
No. of cases/reference	119/183	97/158	112/144	0.016
Cut-off total PCS concentration (ng/mL)	≤ 1.0	1.0 − 3.6	3.6 − 82.9	
Univariate	1.00	0.77 (0.39 − 1.48)	2.12 (1.12 − 4.08)	0.023
Multivariate model 1*	1.00	1.26 (0.46 − 3.49)	2.87 (1.05 − 8.26)	0.044
Multivariate model 2†	1.00	1.15 (0.37 − 3.63)	3.42 (1.01 − 12.52)	0.043

Values shown are cut-offs of serum total *p*-cresylsulfate levels of all subjects, and odds ratios (ORs) with 95% confidence intervals (CIs); *Adjusted for age, gender, body mass index, diastolic blood pressure, serum albumin, phosphorus, high-sensitivity Creactive-protein, creatinine, hypertension, and smoking; †Adjusted for age, gender, body mass index, diastolic blood pressure, serum albumin, phosphorus, high-sensitivity Creactive-protein, estimated glomerular filtration rate, hypertension, and smoking; PCS: *P*-cresylsulfate.

calcium, albumin, blood urea nitrogen, creatinine, eGFR, and white blood cell count were not significantly different between the two groups (Table 1).

Total PCS concentration was significantly associated with anxiety, even after controlling for anthropometric variables, diastolic blood pressure, hypertension, smoking, albumin, phosphorus, hs-CRP, creatinine, and eGFR (Table 2). The association between serum IS levels and anxiety disappeared after further adjustments for anthropometric variables, diastolic blood pressure, hypertension, smoking, albumin, phosphorus, hs-CRP, creatinine, and eGFR (Table 2).

Increasing levels of total PCS showed a significant linear trend and were independently associated with anxiety, especially when concentrations were analyzed both by tertile and a continuous variable (Tables 2 and 3). Fully adjusted ORs in the second and third tertile were 1.26 (95% CI 0.46 − 3.49) and 2.87 (95% CI 1.05 − 8.26) in model 1, and 1.15 (95% CI 0.37 − 3.63) and 3.42 (95% CI 1.01 − 12.52) in model 2, respectively, using multiple logistic regression analysis. Moreover, serum total PCS levels showed significant positive association with anxiety severity ($p = 0.002$; Fig. 1).

Multiple linear regression analysis, adjusted for gender and age, revealed that total PCS was positively associated with calcium, phosphorus, blood urea nitrogen (BUN), creatinine, and white blood cell (WBC) count. In addition, levels of hemoglobin, hematocrit, and eGFR were negatively associated with the levels of total PCS (Table 4).

Table 4. Associations between serum total p-cresylsulfate level and clinical and biochemical characteristics

Variable	Total *p*-cresylsulfate level	
	β coefficient (95% CI)*	*p* value
Body mass index	-0.00 (-0.26 − 0.26)	0.987
Systolic blood pressure	-0.07 (-0.08 − 0.02)	0.284
Diastolic blood pressure	-0.10 (-0.15 − 0.02)	0.135
Fasting sugar	0.00 (-0.01 − 0.01)	0.997
Total-cholesterol	-0.10 (-0.04 − 0.01)	0.125
Triglyceride	-0.01 (-0.01 − 0.01)	0.848
HDL-cholesterol	-0.01 (-0.11 − 0.10)	0.917
LDL-cholesterol	-0.09 (-0.05 − 0.01)	0.198
Uric acid	0.11 (-0.13 − 1.04)	0.124
Phosphorus	0.30 (0.72 − 2.35)	< 0.0001
Calcium	0.26 (1.03 − 7.49)	0.042
Blood urea nitrogen	0.49 (0.24 − 0.40)	< 0.0001
Creatinine	0.60 (4.33 − 6.26)	< 0.0001
eGFR	-0.44 (-0.27 − -0.14)	< 0.0001
Albumin	-0.03 (-3.82 − 2.42)	0.660
Hemoglobin	-0.47 (-2.51 − -1.28)	< 0.0001
Hematocrit	-0.48 (-0.98 − -0.52)	< 0.0001
White blood cell count	0.16 (0.05 − 0.63)	0.022
Hs-CRP	0.05 (-0.02 − 0.05)	0.469
Current smoking	0.05 (-1.82 − 3.24)	0.582

*Adjusted for age and gender by multiple linear regression analysis; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; eGFR: Estimated glomerular filtration rate; Hs-CRP: High-sensitivity C-reactive protein.

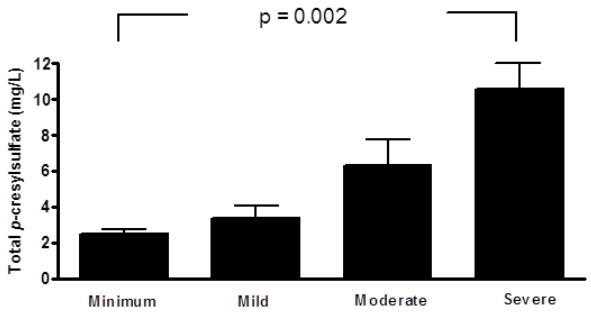

Fig. 1 Changes in total serum p-cresylsulfate levels with anxiety severity measured by the Beck Anxiety Inventory score.

Discussion

In the current study, we found that serum total PCS levels were independently associated with anxiety in CAD patients with CKD. The associations between total PCS and anxiety still persisted after controlling for conventional risk factors, including age, gender, BMI, hypertension, smoking, diastolic blood pressure, albumin, phosphorus, hs-CRP, creatinine,

and eGFR. Furthermore, in the present study, there was no statistically significant difference in causes of admission between patients with and without anxiety. Moreover, we also demonstrated that hs-CRP was increased in patients with anxiety, and that total WBC count positively correlated with total PCS levels. These findings are in agreement with the current evidence regarding the association between inflammation and anxiety.[8,9]

Previous studies demonstrated that serum levels of free and total PCS were elevated in patients with advanced CKD. Although free PCS has recently been reported to be the predictor of survival in CKD patients,[35] the free PCS concentrations were difficult to detect because of measuring limitation in such a low concentration (< 5%) in the non-hemodialysis subjects in our study.[35,36] Previous studies have shown that uremic toxins exist mostly in protein-bound forms.[37] As a result, we measure total IS and PCS rather than 'free-form' IS and PCS per se in the present study.

Anxiety is a common finding among CKD patients.[38,39] Furthermore, moderate/severe anxiety is associated with a worse outcome not only in quality of life, but also in the overall survival in this population.[1-4,39] Though the pathophysiology of anxiety is still largely obscure, the causes of anxiety in patients with renal disease may be different from those in the general population. In particular, as renal function deteriorates, the levels of protein-bound uremic toxins and pro-inflammatory cytokines increase.[40] Anxiety is considered to be an inflammatory systemic disease. Biomarkers of inflammation are increased in patients with anxiety and an imbalance in the endogenous opioidergic system might be involved in the complex pathogenesis of the disease.[41] PCS, a protein-bound uremic toxin, has been found to enhance free radical production through increased oxidative burst activity in leucocytes at baseline. The pro-inflammatory propensity of PCS may contribute to anxiety in patients with CKD.[8] Consistently, previous studies have shown that uremic toxins are associated with anxiety in CKD patients.[11,12] It is therefore reasonable to propose that PCS may act as a pro-inflammatory cytokine that plays a role in chronic inflammation, thereby contributing to the pathogenesis of anxiety.[8,24,25] The results of the present study support the idea in the literature[8,24,25] that PCS may play an important role in the pathophysiology of anxiety in CKD patients through perpetuation of inflammatory response. However, this speculation must be substantiated by further evidence.

Pro-inflammatory cytokines (eg, hs-CRP, TNF-α, IL-6, and IL-8) have been previously reported to be associated with anxiety through inflammation[8,42,43] and involvement of inflammatory mediators in the central nervous system in patients with anxiety symptoms.[41] In this study, we showed that patients with anxiety had significantly higher serum total PCS and IS levels and also had significantly higher serum hs-CRP concentration than those without. Additionally, the results of a previous study also support our finding. Using mass spectrometric analysis on the urine samples of testing subjects having consumed dark chocolate for two weeks, a previous study revealed metabolic changes in both endogenous and gut microbial metabolism.[29] Interestingly, these metabolic changes, which were found to be associated with an additional decreased level of PCS compared to the baseline, were statistically significant only in subjects with inherent high anxiety trait.[29]

In addition, our results revealed that there were significant trends in the association of total PCS level with anxiety severity, as measured by the BAI score. Firstly, PCS accumulates in serum after mild deterioration of renal function with the serum levels approximately 10 − 50 times higher in uremic patients than the normal level. In our previous studies, we found that total PCS and/or IS were

associated with cardiovascular disease such as coronary artery disease, severity of coronary atherosclerosis, and prolonged corrected QT interval, as well as major adverse cardiac events in patients with early CKD.[14-18] These associations indicate that PCS may be associated with cardio-vascular damage in patients at the early stage of CKD. In addition, the development of anxiety or depression has been independently associated with various clinical factors, including female gender, CKD, markers of inflammation, high serum calcium and magnesium concentrations, and intact parathyroid hormone levels.[44-47] In concert with the results of previous reports, we also found that total PCS, together with calcium, phosphorus, BUN, creatinine, and WBC count, were independently associated with anxiety. Furthermore, levels of hemoglobin, hematocrit, and eGFR were negatively associated with the levels of total PCS. These findings suggest that total PCS is a true uremic retention toxin and that its serum concentration increases with the progression of anxiety, implicating its role in the pathogenesis of anxiety syndromes in CKD patients. Moreover, researchers have provided strong evidence for the view that a network among CKD, anxiety, sleep disturbance, inflammation and mortality may be interactive.[46,48,49] However, this is still not well understood and requires elucidation.

Hsu et al. indicated that depression in patients with CKD was significantly and independently associated with a lower total IS level.[50] In our present study, we found that the association between serum IS and anxiety disappeared after adjustment for conventional risk factors in patients with CKD. The lack of association between serum concentrations of total PCS and IS in patients receiving dialysis in previous studies suggests different metabolic pathways and non-interchangeable risk markers for the two uremic toxins.[51,52] In addition, Hsu et al. also demonstrated no association between the total PCS concentration and depression in patients undergoing hemodialysis. On the other hand, the authors found that patients with lower IS and PCS levels with depression had lower body weight and poorer oral intake,[50] which could influence the serum total PCS level as total PCS is primarily from dietary tyrosine ingestion.

Some limitations of this study need to be considered. Firstly, our study population was relatively small. Further studies with larger populations are needed. The cross-sectional design also limited our ability to infer any causal relationship between total PCS levels and anxiety. Secondly, our analyses were based on single measurements of serum total PCS, which may not have reflected the relationship over time. It would be interesting to measure serial changes of serum total PCS levels in anxiety or depression subjects to further clarify the role of total PCS in the pathogenesis of anxiety or depression. Thirdly, in the present study, not all clinical and biochemical parameters involved in the pathogenesis of anxiety were investigated. Fourthly, patients enrolled in the current study were mainly from the ward of the Division of Cardiology and none from the ward of the Division of Nephrology. Therefore, the etiology of CKD may not match the real world disease distribution pattern of Taiwan. However, we believe that this did not affect our observation of the association between serum total PCS and anxiety in CAD patients with CKD. Fifthly, because our study population was relatively small, the effect of total PCS on anxiety at different CKD stages may need to be investigated. Moreover, from the present study, it remains unclear regarding the role of PCS in anxiety without clarifying the underlying mechanisms. Further investigations are thus warranted.

In conclusion, total PCS was shown to be positively associated with the levels of inflammatory biomarkers and the development of anxiety, suggesting that total PCS may act through inflammatory reactions to play an

important role in the pathogenesis of anxiety. However, the mechanisms by which total PCS and anxiety are linked remain to be investigated.

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