Indoxyl sulfate has a dominant role regarding the risks during different stages of chronic kidney disease

Cheng-Hsu Chen

Taichung Veterans General Hospital

Shih-Chien Huang

Chung Shan Medical University

Pei-Chih Lin

Chung Shan Medical University

Shang-Feng Tsai

Taichung Veterans General Hospital

Yi-Chia Huang (✉ ych@csmu.edu.tw)

Chung Shan Medical University <https://orcid.org/0000-0003-2234-4464>

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Abstract

Background: Increased levels of uremic toxins and decreased antioxidant capacities have a significant impact on the progression of chronic kidney disease (CKD). However, it is unclear whether they interact with each other in order to mediate the damage of renal function. The purpose of this study was to determine whether uremic toxins [i.e., homocysteine and indoxyl sulfate (IS)] and glutathione-dependent antioxidant enzyme activities are dependently or independently associated with each other in affecting renal function during different stages of CKD patients.

Methods: One hundred thirty-two patients diagnosed with CKD stage 1 to 5 participated in this cross-sectional study.

Results: Patients who had reached an advanced CKD stage experienced a gradual increase in plasma uremic toxin levels, along with decreased glutathione peroxidase (GSH-Px) activities. Plasma homocysteine, cysteine and IS concentrations were positively associated with each other, but negatively correlated to GSH-Px activity levels after adjusting potential confounders in all CKD patients. Although plasma homocysteine, cysteine, IS and GSH-Px levels were significantly associated with renal function, only plasma IS levels still had a significant association with renal function after these parameters were simultaneously adjusted.

Conclusions: IS plays a more dominant role than other factors in affecting renal function, where a higher IS concentration needs to be controlled in order to defer the progressive loss of renal function.

Background

In patients with chronic kidney disease (CKD), solutes are not excreted by the kidneys and linger during the circulation to cause negative biologic function, and are called uremic toxins [1]. Uremic toxins are classified into smaller water soluble moleculars (< 500 Da) (i.e., urea, creatinine, phosphorous), middle molecules (≥ 500 Da) (i.e., advanced glycation end-products), and protein-bound molecules [i.e., homocysteine, indoxyl sulfate (IS), *p*-cresol sulfate (PCS)] based upon their biochemical and physical properties [1, 2]. Amongst the different types of uremic toxins, protein-bound uremic toxins have attracted much attention, as they have cytotoxic effects and are less efficiently removed during dialysis treatment [3].

One of the protein-bound uremic toxins, homocysteine, is a sulfur-containing amino acid biosynthesized from methionine which can be metabolized through either remethylation or transsulfuration pathways. IS is another representative protein-bound uremic toxin which originates from bacterial amino acid (i.e., tryptophan, tyrosine, phenylalanine) fermentation into indole and *p*-cresol in the large intestine mucosa, before circulating in the blood to the liver. Indole would then be hydroxylated and sulfated to IS in the liver, before re-entering the blood's circulation [4]. Plasma homocysteine and IS are removed on passage through the kidney, with their concentrations having been observed gradually increasing during the progression of renal dysfunction [2, 5–8]. Elevated levels of homocysteine [9, 10] and IS [11, 12] have

been shown to induce oxidative stress, which would accelerate the deterioration of renal function, and therefore be associated with the risk of CKD [13–16]. Restoring the balance between oxidative stress and antioxidant defense capacities would aid in protecting the kidneys from further damage and potentially limit the progression of renal dysfunction.

A glutathione (GSH)-dependent antioxidant system consisting of reduced GSH, oxidized glutathione (GSSG) and functionally dependent enzymes [i.e., glutathione peroxidase (GSH-Px), glutathione reductase and glutathione *S*-transferase (GSH-St)], plays a fundamental role in the cellular defense against reactive free radicals and other oxidant species in the human body. Under normal conditions, GSH and GSH-Px are abundant in the kidney [17, 18]. GSH status and its dependent antioxidant enzyme activities may be depleted in order to cope with increased oxidative stress via kidney damage, or their synthesis could be reduced in keeping with the loss of renal function. Previous studies have indicated that plasma or erythrocyte GSH concentration, GSH-Px and/or glutathione reductase activities decreased with loss of renal function [19–21]. GSH and its dependent antioxidant enzyme activities, and the GSH/GSSG redox ratio have thus been considered to be more informative markers of oxidative stress and antioxidant capacities in CKD patients [22–24].

Although increased levels of homocysteine and IS, and decreased GSH-dependent antioxidant capacities have been shown to be associated with renal function loss, it remains unclear whether they interact with each other to mediate the damage of renal function. The purpose of this study was to determine whether homocysteine, IS, GSH and its dependent antioxidant enzyme activities are dependently or independently associated with each other in affecting renal function during different stages of CKD patients.

Methods

Study design and sample size calculation

This was a cross-sectional study. A significant relationship between IS and estimated glomerular filtration rate (eGFR) levels ($r = -0.7$, $p < 0.001$) was observed in a previous study [6]. We then calculated sample size based on the detection of a significant correlation coefficient of 0.35 between GSH and eGFR levels with a power of 90%, and a 2-sided test with an α of 0.05. A total of 82 patients was required in order to match the calculation criteria. The final recruitment number came to a total of 132 CKD patients, which was greater than our original calculation.

Subjects

Patients with CKD were recruited from the outpatient clinic of the Division of Nephrology at Taichung Veterans General Hospital, Taiwan. The 5 CKD stages were classified based upon the eGFR levels (stage 1: ≥ 90 mL/min/1.73 m²; stage 2: 60 – 89 mL/min/1.73 m²; stage 3a: 45 – 59 mL/min/1.73 m²; stage 3b: 30 – 44 mL/min/1.73 m²; stage 4: 15 – 29 mL/min/1.73 m²; stage 5: <15 mL/min/1.73 m²) [25]. Patients were recruited when their ages were within the 20 to 80 year range, and their CKD staging was confirmed by an experienced nephrologist. Patients were excluded if they were either pregnant or

lactating, had received a renal transplantation, or had a history of liver disease, cancer or alcoholism. This study was approved by the Institutional Review Board of Taichung Veterans General Hospital (IRB TCVGH No. SF15019A and No. CF17272A). Each patient signed an informed consent prior to taking part in the study.

Data collection and biochemical measurements

Data on each subject's age, gender, smoking and drinking habits were recorded. Each subject's height and weight were measured and their body mass index (BMI, kg/m²) was calculated. Systolic and diastolic blood pressures were measured after a resting period of at least 5 minutes.

Fasting blood samples were drawn on an appointed day. Blood specimens were collected in vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA) either containing an anticoagulant or not, as is required when estimating the patient's hematological and biochemical status. Serum albumin, glucose, creatinine, phosphorus, alanine and aspartate aminotransferase (ALT and AST), along with blood urea nitrogen were all measured using an automated biochemical analyzer. Plasma homocysteine and cysteine concentrations, along with plasma IS levels were quantified by high performance liquid chromatography using fluorescence detection, following the modified method of Araki and Sako [26] and Cheng et al. [27], respectively. Plasma malondialdehyde (MDA) and oxidized low-density lipoprotein (ox-LDL) levels were assessed as indicators of oxidative stress. Plasma MDA concentration was measured along with thiobarbituric acid reactive substances at an excitation wavelength of 515 nm and an emission wavelength of 555 nm using a fluorescence spectrophotometer [28]. The plasma ox-LDL level was measured using a commercial kit (Mercodia AB, Sylveniusgatan 8A, SE-754 50 Uppsala, Sweden). Trolox equivalent antioxidant capacity (TEAC) was analyzed according to the previous method [29]. Plasma GSH and GSSG concentrations, along with GSH-Px activities were determined using the respective commercial kits (Cayman Chemical Company, Ann Arbor, MI, USA).

Statistical analysis

All data analyses were performed by using the SAS statistical software package (version 9.4; Statistical Analysis System Institute Inc., Cary, NC, USA). A Shapiro-Wilk test was performed to test the normal distribution. Demographic characteristics and biochemical data were compared for significance among groups using a one-way analysis of variance or the Kruskal-Wallis one way analysis of variance on ranks. Chi-square or Fisher's exact tests were implemented for the analyses of categorical variables. Partial Spearman's correlation analysis was used to assess the association of homocysteine, cysteine and IS concentrations with levels of oxidative stress indicators and GSH-dependent antioxidant activities after adjusting for potential confounders. Multiple linear regression analyses with either eGFR levels or CKD stage as the dependent variable, were used to examine the association of renal function with homocysteine, IS, oxidative stress indicators and GSH-dependent antioxidant activities after adjusting for potential confounders. The value of $p < 0.05$ was considered as having a statistical significance.

Results

A total of 132 CKD patients, with 21 stage 1, 31 stage 2, 31 stage 3a, 20 stage 3b, 16 stage 4, and 13 stage 5 were recruited for this study. As the number of patients were too small in both stage 4 and stage 5, and the CKD patients within these stages were checked as having similar characteristics, we then combined these 2 stages into one group. The mean and median age of all CKD patients was 57.6 years and 59 years, respectively, with a range of 20–78 years. There were no significant differences in the values of age, gender, height, weight, BMI, systolic and diastolic blood pressures, ALT, AST, albumin, or glucose (Table 1).

Table 1
Demographic characteristics and hematological measurements in patients with different stages of chronic kidney disease

Characteristics	Stage 1 (n = 21)	Stage 2 (n = 31)	Stage 3a (n = 31)	Stage 3b (n = 20)	Stage 4–5 (n = 29)
Age (y)	55.19 ± 14.75	56.48 ± 13.64	61.90 ± 11.20	58.10 ± 14.08	55.48 ± 12.45
Gender (male / female)	10 / 11	22 / 9	25 / 6	12 / 8	21 / 8
Height (cm)	162.37 ± 6.94	162.69 ± 9.95	164.72 ± 10.21	161.36 ± 5.73	164.09 ± 10.68
Weight (kg)	69.59 ± 13.98	66.10 ± 17.27	71.88 ± 13.38	67.12 ± 8.89	68.19 ± 11.90
BMI (kg/m ²)	26.44 ± 5.30	24.77 ± 5.05	26.30 ± 3.08	25.74 ± 2.73	25.28 ± 3.58
SBP (mmHg)	134.48 ± 17.42	132.74 ± 15.98	137.84 ± 14.58	135.85 ± 12.06	134.72 ± 13.70
DBP (mmHg)	81.33 ± 12.69	76.68 ± 9.59	79.03 ± 11.29	76.25 ± 10.58	75.90 ± 12.01
eGFR (mL/min/1.73 m ²)	106.81 ± 15.49 ^a	72.52 ± 9.90 ^a	51.92 ± 5.06 ^b	36.85 ± 4.76 ^{b,c}	16.82 ± 5.95 ^c
ALT (U/L)	28.90 ± 19.49	25.80 ± 19.12	22.55 ± 11.13	22.06 ± 10.70	19.03 ± 9.05
AST (U/L)	26.37 ± 9.46	27.03 ± 21.86	23.10 ± 6.42	24.24 ± 10.32	22.69 ± 14.30
Albumin (mg/dL)	4.03 ± 0.70	4.22 ± 0.32	4.33 ± 0.25	4.22 ± 0.43	4.12 ± 0.35
Glucose (mg/dL)	106.28 ± 18.06	105.03 ± 25.05	112.66 ± 32.39	136.29 ± 60.50	108.12 ± 31.63
Current smoking habits (n, %)	3, 14.29%	3, 9.68%	4, 12.90%	3, 15.0%	3, 10.34%

Values are means ± standard deviation. BMI, body mass index; SBP systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated

glomerular filtration rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; hs-CRP, high sensitivity C-reactive protein; BUN,

blood urea nitrogen.

^{a,b,c}Values in a row with different superscript letters are significantly different, $p \leq 0.05$.

Characteristics	Stage 1 (n = 21)	Stage 2 (n = 31)	Stage 3a (n = 31)	Stage 3b (n = 20)	Stage 4–5 (n = 29)
Current drinking habits (n, %)	2, 9.52%	6, 19.35%	4, 12.90%	0	3, 10.34%
Values are means $\pm$ standard deviation. BMI, body mass index; SBP systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated					
glomerular filtration rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; hs-CRP, high sensitivity C-reactive protein; BUN,					
blood urea nitrogen.					
^{a,b,c} Values in a row with different superscript letters are significantly different, $p \leq 0.05$.					

The levels of uremic toxins and indicators of oxidative stress, and GSH-dependent antioxidant activities during different stages of CKD patients are listed in Table 2. Following the progression of renal dysfunction, patients at a more advanced CKD stage had gradually increased plasma uremic toxin levels and decreased GSH-Px activities. Stage 4–5 CKD patients apparently had the highest serum BUN and plasma IS concentrations among the 5 groups. On the other hand, the levels of MDA, ox-LDL, TEAC, GSH, GSSG, and the GSH/GSSG ratio were similar among groups.

Table 2

Plasma uremic toxins, indicators of oxidative stress and antioxidant capacities in patients with different stages of chronic kidney disease

Characteristics	Stage 1 (n = 21)	Stage 2 (n = 31)	Stage 3a (n = 31)	Stage 3b (n = 20)	Stage 4–5 (n = 29)
<i>Uremic toxins</i>					
Blood urea nitrogen (mg/dL)	13.57 ± 3.01 ^c	15.26 ± 4.32 ^c	20.77 ± 5.78 ^b	26.35 ± 6.52 ^b	53.17 ± 14.67 ^a
Creatinine (mg/dL)	0.74 ± 0.13 ^c	1.06 ± 0.19 ^c	1.43 ± 0.17 ^b	1.81 ± 0.33 ^{a,b}	4.09 ± 1.42 ^a
Phosphorus (mg/dL)	3.60 ± 0.44 ^{a,b}	3.50 ± 0.48 ^b	3.43 ± 0.40 ^b	3.48 ± 0.47 ^{a,b}	4.04 ± 0.69 ^a
Homocysteine (μmol/L)	12.90 ± 5.97 ^c	16.89 ± 9.82 ^{b,c}	18.63 ± 7.36 ^{a,b}	18.44 ± 7.15 ^{a,b}	23.68 ± 11.67 ^a
Cysteine (μmol/L)	235.52 ± 63.10 ^b	234.05 ± 43.97 ^{a,b}	237.61 ± 41.04 ^{a,b}	234.28 ± 40.15 ^{a,b}	273.80 ± 66.39 ^a
Indoxyl sulfate (μmol/L)	3.19 ± 1.66 ^c	4.19 ± 2.59 ^c	11.53 ± 5.21 ^b	13.24 ± 6.93 ^b	37.17 ± 18.21 ^a
<i>Oxidative stress indicators</i>					
MDA (μmol/L)	0.73 ± 0.26	0.74 ± 0.21	0.76 ± 0.22	0.72 ± 0.20	0.72 ± 0.44
ox-LDL (U/L)	33.11 ± 16.50	29.78 ± 13.73	43.98 ± 31.14	42.96 ± 21.26	38.07 ± 21.95
GSH/GSSG ratio	0.16 ± 0.05	0.15 ± 0.04	0.16 ± 0.05	0.14 ± 0.03	0.14 ± 0.03
<i>Antioxidant capacities</i>					
TEAC (μmol/L)	3780.29 ± 362.40	3713.25 ± 495.80	3768.95 ± 382.98	3929.99 ± 276.37	3888.96 ± 339.50
GSH (μmol/L)	108.76 ± 32.00	99.38 ± 24.22	108.14 ± 40.93	96.09 ± 23.09	95.92 ± 26.75
GSSG (μmol/L)	672.70 ± 49.76	675.60 ± 53.75	676.17 ± 73.20	679.15 ± 70.75	671.29 ± 72.37

Values are means ± standard deviation. MDA, malondialdehyde; ox-LDL, oxidized low density lipoprotein cholesterol; TEAC, trolox equivalent

antioxidant capacity; GSH, glutathione; GSSG, oxidized glutathione; GSH-Px, glutathione peroxidase.

^{a,b,c}Values in a row with different superscript letters are significantly different, $p \leq 0.05$.

Characteristics	Stage 1 (n = 21)	Stage 2 (n = 31)	Stage 3a (n = 31)	Stage 3b (n = 20)	Stage 4–5 (n = 29)
GSH-Px activity (nmol/mL/min)	154.03 ± 55.27 ^a	165.55 ± 106.18 ^{a,b}	136.63 ± 42.94 ^{a,b}	145.58 ± 42.29 ^{a,b}	110.48 ± 33.08 ^b
Catalase activity (nmol/mL/min)	54.06 ± 32.83	55.22 ± 48.81	52.18 ± 27.12	43.65 ± 26.58	49.06 ± 25.18
Values are means ± standard deviation. MDA, malondialdehyde; ox-LDL, oxidized low density lipoprotein cholesterol; TEAC, trolox equivalent					
antioxidant capacity; GSH, glutathione; GSSG, oxidized glutathione; GSH-Px, glutathione peroxidase.					
^{a,b,c} Values in a row with different superscript letters are significantly different, $p \leq 0.05$.					

We performed partial Spearman's correlation coefficient analyses to assess the association of homocysteine, cysteine and IS levels with oxidative stress indicators and GSH-dependent antioxidant activities after adjusting for age, gender, BMI, albumin, smoking and drinking habits (Table 3). Plasma homocysteine, cysteine and IS concentrations were associated with each other after adjusting for potential confounders. These 3 uremic toxins did not correlate with levels of TEAC, GSSG, or the GSH/GSSG ratio, but negatively correlated to GSH-Px activity levels in all CKD patients.

Table 3
Partial Spearman's correlation coefficients (r_s) of homocysteine, cysteine, and indoxyl sulfate with indicators of oxidative stress and glutathione-dependent antioxidant capacities in patients with chronic kidney disease¹

	Homocysteine ($\mu\text{mol/L}$)	Cysteine ($\mu\text{mol/L}$)	Indoxyl sulfate ($\mu\text{mol/L}$)
MDA ($\mu\text{mol/L}$)	-0.115	-0.331 [†]	-0.127
ox-LDL (U/L)	0.087	-0.073	0.171
GSH/GSSG ratio	0.168	0.215*	-0.081
Indoxyl sulfate ($\mu\text{mol/L}$)	0.339 [†]	0.240**	–
Homocysteine ($\mu\text{mol/L}$)	–	0.571 [†]	0.339 [†]
Cysteine ($\mu\text{mol/L}$)	0.571 [†]	–	0.240**
TEAC ($\mu\text{mol/L}$)	0.146	0.097	0.078
GSH ($\mu\text{mol/L}$)	0.159	0.221*	-0.054
GSSG ($\mu\text{mol/L}$)	0.085	0.144	0.059
GSH-Px activity (nmol/mL/min)	-0.496 [†]	-0.363 [†]	-0.219*
¹ n = 132. Values are r_s , correlation coefficient. Adjusting for age, gender, body mass index, albumin, smoking and drinking habits. * $p < 0.05$, ** $p < 0.01$, [†] $p < 0.001$.			
MDA, malondialdehyde; ox-LDL, oxidized low density lipoprotein cholesterol; TEAC, trolox equivalent antioxidant capacity; GSH, glutathione; GSSG, oxidized glutathione; GSH-Px, glutathione peroxidase.			

Multiple linear regression models were analyzed to understand the effects of homocysteine, IS, indicators of oxidative stress, and GSH-dependent antioxidant activities on renal function (i.e., CKD stage and eGFR levels) after adjusting for age, gender, BMI, albumin, smoking and drinking habits (Table 4). Plasma levels of MDA, ox-LDL, TEAC, GSH, GSSG, and the GSH/GSSG ratio had no association with renal function in all CKD patients. However, plasma homocysteine, cysteine and IS levels had significantly negative impacts on renal function; whereas GSH-Px activity had a significantly positive effect on CKD stage and eGFR levels. In order to investigate whether homocysteine, cysteine, IS, and GSH-Px activity are dependent or independent with one another when associated with renal function, the levels of homocysteine or cysteine, IS and GSH-Px activity were then further simultaneously adjusted in the multiple linear regression models (Table 4). Plasma IS levels were independent of the levels of homocysteine, cysteine

and GSH-Px activity to be associated with CKD stage and the eGFR levels. On the other hand, the associations of plasma homocysteine, cysteine, and GSH-Px activity levels with renal function had disappeared after being additionally adjusted for plasma IS levels.

Table 4

Multiple linear regression of various parameters with stage of chronic kidney disease or estimated glomerular filtration rate as the dependent variable after adjusting for potential confounders¹

	Stage	eGFR (mL/min/1.73 m ²)
MDA + confounding factors	-0.168 (0.403)	3.026 (10.400)
ox-LDL + confounding factors	0.006 (0.005)	-0.173 (0.124)
Homocysteine + confounding factors	0.042 (0.011) [†]	-1.099 (0.286) [†]
Cysteine + confounding factors	0.005 (0.002) [*]	-0.137 (0.054) [*]
IS + confounding factors	0.058 (0.004) [†]	-1.384 (0.124) [†]
TEAC + confounding factors	0.035 · 10 ⁻² (0.029 · 10 ⁻²)	-0.010 (0.007)
GSH + confounding factors	-0.003 (0.004)	0.105 (0.090)
GSSG + confounding factors	-0.001(0.002)	0.019 (0.044)
GSH/GSSG ratio + confounding factors	-3.178 (2.683)	96.852 (68.993)
GSH-Px activity	-0.002 (0.001)	0.003 (0.032)
+ IS + homocysteine + confounding factors		
Homocysteine	0.004 (0.008)	-0.290 (0.238)
+ IS + GSH-Px + confounding factors		
Cysteine	-0.003 (0.002)	0.017 (0.043)
+ IS + GSH-Px + confounding factors		

¹n = 132. Values are β , regression coefficient (standard error). Adjusted for confounding

factors (i.e., age, gender, body mass index, albumin, smoking and drinking habits). * $p < 0.05$,

** $p < 0.01$, [†] $p < 0.001$.

MDA, malondialdehyde; ox-LDL, oxidized low density lipoprotein cholesterol; IS, indoxyl

sulfate; TEAC, trolox equivalent antioxidant capacity; GSH, glutathione; GSSG, oxidized

glutathione; GSH-Px, glutathione peroxidase.

	Stage	eGFR (mL/min/1.73 m ²)
IS + homocysteine + GSH-Px + confounding factors	0.056 (0.005) [†]	-1.328 (0.135) [†]
IS + cysteine + GSH-Px + confounding factors	0.059 (0.005) [†]	-1.394 (0.135) [†]
GSH-Px activity + homocysteine + IS + confounding factors	-0.002 (0.001)	0.003 (0.032)
[†] n = 132. Values are β , regression coefficient (standard error). Adjusted for confounding factors (i.e., age, gender, body mass index, albumin, smoking and drinking habits). * $p < 0.05$, ** $p < 0.01$, [†] $p < 0.001$.		
MDA, malondialdehyde; ox-LDL, oxidized low density lipoprotein cholesterol; IS, indoxyl sulfate; TEAC, trolox equivalent antioxidant capacity; GSH, glutathione; GSSG, oxidized glutathione; GSH-Px, glutathione peroxidase.		

Discussion

Increased oxidative stress is well recognized as being an important metabolic accompaniment in CKD patients. Previous studies have indicated that CKD patients had a higher oxidative stress status than healthy subjects [8, 13–16, 20]; however, oxidative stress status (i.e., MDA, ox-LDL, GSH/GSSG ratio) of our CKD patients not only remained no fluctuation among patients at different CKD stages, but there was also no effect on renal function in the present study. It is worth noting that antioxidant capacities (i.e., TEAC, GSH, GSSG) also remained steady among different CKD stage patients. Cysteine is a major, yet limiting substrate for the synthesis of GSH within cells. Although we did not measure other substrates of GSH synthesis (i.e. glycine and glutamate), plasma cysteine concentration was not reduced following renal function loss. In our CKD patients, sufficient cysteine concentration may help maintain GSH synthesis during different CKD stages. As long as CKD patients, including those at an advanced disease stage, still possess capable antioxidant capacities to cope with increased oxidative stress, GSH and its dependent antioxidant capacities may not be the significant factor affecting their oxidative stress status during renal function loss.

Under an increased oxidative stress condition, GSH is oxidized to GSSG, and along with GSH-Px, reduces hydroperoxides. Plasma GSH-Px is synthesized primarily in the proximal renal tubular cells [17], an early consequence of active nephron mass reduction which might lead to the reduction of plasma or erythrocyte GSH-Px activity in CKD patients [19, 20, 30, 31]. Therefore, it was not surprising to us when we observed that plasma GSH-Px activity experienced a significant reduction following the progressive loss

of renal function in our CKD patients. Plasma GSH-Px activity seemed to deplete when catalyzing the reduction of hydrogen peroxide and other organic hydroperoxides to water at the advanced stage of the disease. In spite of GSH being a substrate of GSH-Px, GSH-Px is a selenium-containing enzyme, so its selenium status was thus recognized to be another key factor affecting plasma GSH-Px activity in CKD patients [32], although not all studies agreed with this [20, 33]. Since selenium concentration was not analyzed in our study, the relationship between plasma GSH-Px activity and selenium cannot be discussed further. GSH-Px is the first line of cellular defense in the human body, and even though the first line of antioxidant defense system may have been exhausted, the secondary antioxidant enzymes in the antioxidant defense system, such as GSH-St, may be expected as being capable of coping with oxidative stress in CKD patients. Unfortunately, we did not measure GSH-St activity, otherwise the overall picture of GSH-dependent antioxidant capacities could be better understood during different stages of CKD patients.

Even though many factors have been mentioned in association with increased oxidative stress during renal function loss, the accumulation of uremic toxins in the circulation is an important contributing factor for increased oxidative stress in CKD patients [9–12]. However, elevated uremic toxins (i.e., homocysteine and IS) had no relationship with oxidative stress indicators, but did have a direct contribution on renal dysfunction in our CKD patients. It seemed that uremic toxins could directly ruin renal function without regulating other mechanisms to affect renal function. In a similar way with our previous study [8], a high homocysteine concentration was independent of oxidative stress when associated with the risk of CKD. Although the pathogenesis of hyperhomocysteinemia in CKD patients is not fully understood, a progressive increase in homocysteine levels was associated with decreasing eGFR in patients with CKD [34, 35]. In spite of the significant role which homocysteine plays in the risk of CKD, IS (a gut-derived uremic toxin) not only had a significant association with renal function in our CKD patients and others [6, 36], but it also played a more dominant role than homocysteine when associated with renal function. Increased IS concentration has been shown to cause nephrovascular toxicity and damage to vascular smooth muscle cells, while enhancing inflammatory gene expression, and promoting the degeneration of renal tubular epithelial cells and renal interstitial cells [4, 11, 37]. The change in plasma IS concentration should be regularly monitored for patients at any stage of CKD, in order to reduce or prevent the gradual loss of renal function.

In line with the study from Yu et al., [38], IS was not correlated with GSH, GSSG and the GSH/GSSG ratio in our CKD patients. Dou et al. [39] indicated that high IS concentrations (497.4 and 994.8 $\mu\text{mol/L}$) would decrease total GSH concentrations by 37% and 67% in human umbilical vein endothelial cells, respectively; while lower IS concentrations (99.5 and 199 $\mu\text{mol/L}$) had no effect on total GSH levels. The IS levels of CKD patients were between 6.4 ~ 72.4 $\mu\text{mol/L}$ in the study of Yu et al. [38], and between 1.5 ~ 93.7 $\mu\text{mol/L}$ in the present study; these IS levels were significantly less than the concentrations which were treated in endothelial cells by Dou et al. [39]. This may explain why our team and Yu et al. [38] did not observe the association between plasma IS concentration and an oxidative stress indicator (GSH/GSSG ratio) in CKD patients. Uremic toxin adsorbents (i.e., oral carbonaceous adsorbent AST-120) [4, 38], along with increasing dietary fiber intake [40, 41] have been shown to effectively reduce IS

concentrations. Therefore, we might postulate that the effective treatment of lowering IS would not exhaust GSH utilization and could maintain adequate GSH dependent antioxidant capacity for any stage of CKD patients.

The strength of this study was that patients from all stages of CKD were recruited, therefore the changes in uremic toxins, along with indicators of oxidative stress and antioxidant capacity could be compared among all CKD stages. However, the cross-sectional study design was lacking a longer observation period and more repeated measurements at defined intervals, thus it could not reflect a long-term status and changes in uremic toxins, oxidative stress and antioxidant capacities in CKD patients. The other limitation within this study was that PCS, another important uremic toxin, was not measured in this study. However, IS has been shown to be highly correlated with PCS levels in Asian CKD patients, and displays a higher level than those in the Caucasian population [6]. We believed that the IS level could reflect uremic status even though we did not measure PCS levels in our CKD patients.

Conclusions

In conclusion, our results revealed that plasma IS concentration was independent of oxidative stress indicators, homocysteine, GSH and its dependent antioxidant capacities were associated with the loss of renal function. IS plays a dominant role in regulating renal function, and higher IS concentrations need to be controlled in order to defer the progressive loss of renal function in CKD patients.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

CHC assisted with the study design, was responsible for the screening and recruitment of subjects, and interpreting the results; SCH assisted with the concept generation, interpretation of results and critically revised the manuscript; PCL analyzed biochemical measurements and performed data coding; SFT assisted with the screening and recruitment of subjects; YCH was responsible for the study design, interpretation of results, and preparation of the manuscript. All authors read and approved the final revision of the manuscript

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Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Taichung Veterans General Hospital (IRB TCVGH No. SF15019A and No. CF17272A). Each patient signed an informed consent prior to taking part in the study.

Consent for publication

Not applicable.

Competing interests

The authors have no conflicts of interest.

Abbreviations

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; CKD: chronic kidney disease; eGFR: estimated glomerular filtration rate; GSH: glutathione; GSSG: oxidized glutathione; GSH-Px: glutathione peroxidase; GSH-St: glutathione S-transferase; IS: indoxyl sulfate; MDA: malondialdehyde; ox-LDL: oxidized low-density lipoprotein; TEAC: Trolox equivalent antioxidant capacity.

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