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Albuminuria and Endothelial Dysfunction in Patients with Non-Diabetic Chronic Kidney Disease

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Albuminuria has been associated with cardiovascular events, but whether such an association can be explained by endothelial dysfunction is not fully understood. In this study, we examined the relationship between the urine albumin-to-creatinine ratio (UACR) and biomarkers of endothelial function in patients with chronic kidney disease (CKD).

Material/Methods: The cross-sectional associations of renal dysfunction and UACR with procoagulant and inflammatory factors were evaluated for 151 consecutive CKD (stage 3–5) patients. Subjects were grouped by UACR (≤300 mg/g or >300 mg/g) and estimated glomerular filtration rate (eGFR) (30≤ eGFR <60, 15≤ eGFR <30, or eGFR <15 ml/min per 1.73 m^2).

Results: A higher UACR level was associated with an increase in von Willebrand factor antigen (vWF: Ag) levels, vWF activity, factor VIII, interleukin-2, and log (interleukin-6), even after adjustment for risk factors. Linear regression analysis indicated that for every 88.5 mg/g increase in UACR, the vWF activity and factor VIII were elevated by 8.3% and 6.3%, respectively. The factorial design ANOVA data showed no statistically significant interaction between UACR and CKD stage with procoagulant and inflammatory factors.

Conclusions: Our study shows an eGFR-independent association of higher UACR with elevations in markers of endothelial dysfunction and inflammatory factors in CKD patients.

MeSH Keywords: Endothelial Cells • Proteinuria • Renal Insufficiency, Chronic

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Background

Cardiovascular disease (CVD) is the major factor affecting the prognosis of patients with chronic renal disease (CKD), among whom its morbidity and mortality rates are 10–20 times those in the general population [1–3]. Endothelial dysfunction, an intermediate cardiovascular endpoint [4], plays a significant role in the development of atherosclerosis and vascular lesions, which were associated with CKD in our previous study.

In addition to traditional CVD risk factors (e.g., hypertension and diabetes) and abnormal renal function, the urine albumin-to-creatinine ratio (UACR) was also shown to be an independent risk factor for cardiovascular disease in non-diabetic and diabetic patients in numerous studies [5–7]. However, the mechanisms of the association between albuminuria and CVD are still largely unclear. Little is known about the glomerular filtration rate (GFR)-independent association between endothelial injury, an intermediate step in CVD events, and UACR in CKD patients. We hypothesized that the effect of UACR on the vascular endothelium may be one of the mechanisms leading to CVD in CKD patients. In the present study, we investigated the relationship between UACR and endothelial dysfunction (ED) in CKD patients and the possible mechanism.

Material and Methods

Study design and subjects

This prospective observational study was carried out at the Department of Nephrology, Chinese PLA General Hospital. Between September 2015 and September 2016, consecutive inpatients 18 to 70 years of age with stages 3–5 non-dialysis-dependent CKD were included in this study. To diminish any confounders that may influence patients with endothelial dysfunction, patients were excluded if they: (1) had an established atherosclerotic complication (coronary artery disease, congestive heart failure, or peripheral vascular disease); (2) had a previous diagnosis of diabetes; (3) had a history of chronic dialysis or kidney transplants or had nephrotic syndrome; (4) had a history of use of glucocorticoid or immunosuppressive medication within the last month; and (5) were unwilling to give informed consent. Finally, 151 non-diabetic CKD patients were selected. The etiology of CKD in these patients was chronic glomerulonephritis in 105 and unknown in 46. The research was approved by the Ethics Committee of the General Hospital of the Chinese People's Liberation Army and all subjects signed an informed consent form before participating in the study. A study flowchart is shown in Figure 1.

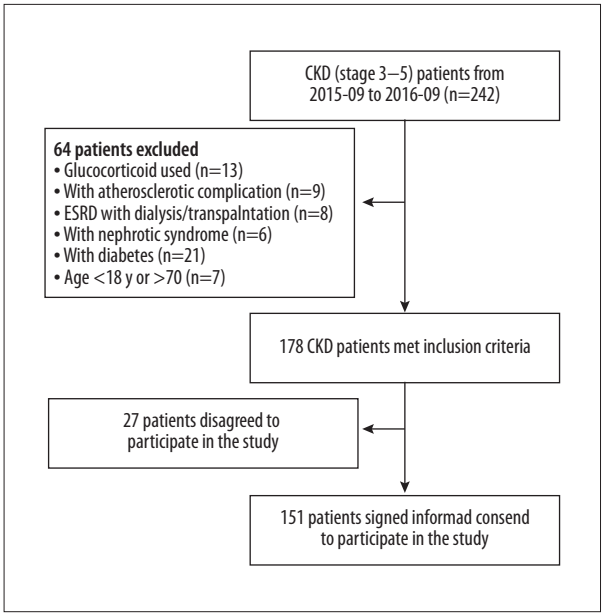

Figure 1. The flow chart of the study.

General data collection

The general characteristics [age, gender, height, weight, systolic blood pressure, and diastolic blood pressure] and laboratory parameters [hemoglobin, white blood cell count, platelet count, serum albumin, serum creatinine, cholesterol, triglycerides, interleukin-2, interleukin-6, superoxide dismutase, and UACR] were determined for all patients.

Body mass index (BMI) and mean arterial pressure (MAP) were calculated as follows: BMI=weight (kg)/[height (m)]^2; MAP=(systolic blood pressure + 2·diastolic blood pressure)/3.

The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used for the estimated glomerular filtration rate (eGFR)[8]. CKD stage was defined in accordance with K/DOQI guidelines [9].

Biomarkers of endothelial function

Specimens of fasting cubital venous blood were collected in the morning. All blood specimens were anticoagulated with 10^9 mmol/L sodium citrate at a ratio of 9: 1 (blood: citrate). Platelet-poor plasma was obtained from blood specimens by centrifugation at 3000×g for 10 min within 1 h of blood collection. The coagulation factor VIII activity was measured using a coagulation assay. Thrombotic von Willebrand factor (vWF) antigen levels and activity were determined using an immunoturbidimetric assay. The instrument (ACL TOP700) and reagents were all purchased from USA Instrumentation Laboratory Company.

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Table 1. Baseline characteristics of the study cohort.

Variables	CKD patients (n=151)	Urine albumin-to-creatinine ratio ≤300 mg/g (n=79)	Urine albumin-to-creatinine ratio >300 mg/g (n=72)	P
Gender, M, n (%)	95 (63%)#	58 (73%)#	37 (51%)*	0.013
Age (year)	43.6±12.9	41.6±12.6	45.9±13.0	0.121
Body mass index (kg/m^2)	24.6±3.7	24.2±3.3	24.9±4.1	0.475
Mean arterial pressure (mmHg)	98.1±11.6	96.6±13.2	99.7±9.2	0.264
Hemoglobin (g/L)	118.5±21.5#	124.2±22.0#	112.3±19.3*	0.003
Platelet count (10^9/l)	212.2±57.3	203.5±51.2	221.8±62.4	0.147
White blood cell count (10^9/l)	7.1±1.9	7.0±1.7	7.3±2.1	0.747
Serum albumin (g/L)	39.9±4.0#	40.9±3.5#	38.7±4.1*	0.004
eGFR (ml/min/1.73 m^2)	30.9±17.7#	36.7±19.6*#	24.4±12.6*	<0.001
CKD3 stage, N (%)	58 (38%)#	43 (54%)*#	15 (21%)*	<0.001
CKD4 stage, N (%)	55 (36%)	23 (29%)	32 (44%)	0.148
CKD5 stage, N (%)	38 (25%)	13 (16%)#	25 (35%)	0.036
Cholesterol (mmol/l)	4.2±0.9	4.0±1.0	4.3±0.8	0.161
Triglycerides (mmol/l)	2.0±0.9	2.0±0.9	2.0±0.9	0.999

Data are expressed as mean ± standard deviation (SD) or median (interquatile range) as appropriate; eGFR – estimated glomerular filtration rate; CKD – chronic kidney disease. * P<0.05, *vs.* CKD group; # P<0.05, *vs.* UACR >300 mg/g group.

Statistical analysis

Data analysis was performed using Statistical Package for Social Science (SPSS) 19 statistical software (Chicago, IL, USA). Results are expressed as the mean ± standard deviation or median (range) for continuous data and as the frequency or percentage for categorical data. We initially compared the baseline characteristics of the study subjects using the t-test, Mann-Whitney U, or chi-squared test, as appropriate. A generalized linear model estimating procedure was used to obtain the adjusted mean levels of procoagulant biomarkers within categories of UACR [10]. Interaction between CKD stage (3 levels: CKD3, CKD4, and CKD5) and UACR (2 levels: UACR ≤300 mg/g and UACR >300 mg/g) was assessed by factorial design analysis of variance (ANOVA). Using multivariable linear regression, we examined the association of eGFR and UACR with markers of endothelial dysfunction. Log transformations were used for biomarkers if they were not normally distributed. The eGFR, UACR, and other baseline characteristics were the independent variables in these analyses, and the hemostatic biomarkers were the dependent variables. P values less than 0.05 were considered statistically significant.

Results

Participant characteristics

According to the definition of microalbuminuria and macroalbuminuria suggested by the American Diabetes Association (2007), we divided the CKD patients into 2 groups: UACR ≤300 mg/g and UACR >300 mg/g. Baseline information is presented in Table 1. The patients in the 2 groups showed no significant differences in body mass index, mean arterial pressure, white blood cell count, cholesterol, or triglycerides. Compared with patients with UACR ≤300 mg/g, patients with UACR >300 mg/g were associated with older age, female sex, and lower hemoglobin, platelet count, serum albumin, eGFR, and superoxide dismutase levels. However, the interleukin-2 and interleukin-6 levels were significantly higher in the UACR >300 mg/g group. The UACR ≤300 mg/g group had a higher proportion of CKD3 patients, whereas the UACR >300 mg/g group had a higher proportion of CKD5 patients.

Procoagulant and inflammatory biomarkers according to UACR categories adjusted for baseline covariates

Regardless of adjustment, a higher UACR was associated with increases in vWF: Ag, vWF activity, factor VIII, interleukin-2, and

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Table 2. Unadjusted and adjusted levels of procoagulant and inflammatory biomarkers.

Variables	Unadjusted		P	Multivariable-adjusted*		P
	Urine albumin-to-creatinine ratio ≤300 mg/g	Urine albumin-to-creatinine ratio >300 mg/g		Urine albumin-to-creatinine ratio ≤300 mg/g	Urine albumin-to-creatinine ratio >300 mg/g	
VWF: Ag (%)	140.2±51.7	181.7±45.7	<0.001	145.1±49.2	177.9±49.7	<0.001
VWF: activity (%)	131.2±49.2	176.9±47.9	<0.001	135.4±49.2	173.6±49.7	<0.001
Factor VIII (%)	120.6±29.7	142.5±23.7	<0.001	124.3±25.2	137.6±25.6	0.030
IL-2 (U/ml)	728.5±201.7	880.2±233.4	0.004	748.3±172.2	859.5±180.0	0.044
Log(IL -6 (pg/ml))	0.53±0.3	0.78±0.3	<0.001	0.57±0.3	0.75±0.3	0.007
SOD (U/ml)	151.7±21.5	135.1±14.7	0.001	148.1±14.7	138.8±15.3	0.036

IL – Interleukin; SOD – Superoxide dismutase. * Adjusted for age, sex, mean arterial pressure, body mass index, hemoglobin, serum albumin, cholesterol, triglycerides, and eGFR.

Table 3. Association of eGFR and UACR with endothelial dysfunction: difference in hemostatic marker per 10 ml/min/1.73 m^2 decrease in eGFR and per 88.5-U increase of UACR.

Cohort and variables	Unadjusted changes (95% CI)*	P	Adjusted changes (95% CI)	P
vWF activity (%)				
UACR, mg/g	8.3 (4.1, 12.5)	<0.001	4.8 (0.9, 9.4)[&]	0.040
eGFR, ml/min per 1.73 m^2	11.0 (6.2, 15.8)	<0.001	6.4 (1.0, 11.8)[#]	0.020
Factor VIII (%)				
UACR, mg/g	6.3 (3.1, 9.5)	<0.001	4.2 (1.0, 7.3)[&]	0.011
eGFR, ml/min per 1.73 m^2	6.6 (3.4, 9.8)	0.017	3.9 (0.7, 7.2)[#]	0.018

* Reported for a 10-U decrease of eGFR or 88.5-U increase of UACR. For UACR, to convert from milligrams per gram to milligrams per millimole, multiply by 0.113; [#] bAdjusted for age, sex, mean arterial pressure, body mass index, hemoglobin, serum albumin, cholesterol, triglycerides, and UACR; [&] Adjusted for age, sex, mean arterial pressure, body mass index, hemoglobin, serum albumin, cholesterol, triglycerides, and eGFR.

log transformed value of interleukin-6 (log [interleukin-6]), as well as a decrease in superoxide dismutase (Table 2).

Association of eGFR and UACR with endothelial dysfunction

When adjusted for demographics, biochemical data, and UACR, for every 10 ml/min/1.73 m^2 decrease in eGFR, the vWF activity and factor VIII were elevated by 6.4% (95% confidence interval [95% CI], 1.0% to 11.8%) and 3.9% (95% CI, 0.7% to 7.2%), respectively. In models adjusted for demographic characteristics, biochemical data, and eGFR, for every 88.5 mg/g (10 mg/mmol) increase in UACR, the vWF activity and factor VIII were elevated by 4.8% (95% CI, 0.9% to 9.4%) and 4.2% (95% CI, 1.0% to 7.3%), respectively (Table 3).

In addition, the interaction between CKD and UACR with vWF activity, vWF: Ag, and factor VIII is presented in Figure 2. There was no significant interaction between these variables (vWF activity: F=0.58, P=0.56; vWF: Ag: F=0.62, P=0.52; factor VIII: F=0.1, P=0.913).

Association of eGFR and UACR with inflammatory biomarkers

Additionally, we analyzed the association of kidney function and UACR with inflammatory cytokines by factorial design ANOVA. The results showed that the main effects of the factors UACR and CKD were both statistically significant: the interleukin-2 and log (interleukin-6) levels were significantly higher, while superoxide dismutase was lower in the UACR >300 mg/g group and in CKD5 patients compared with the UACR

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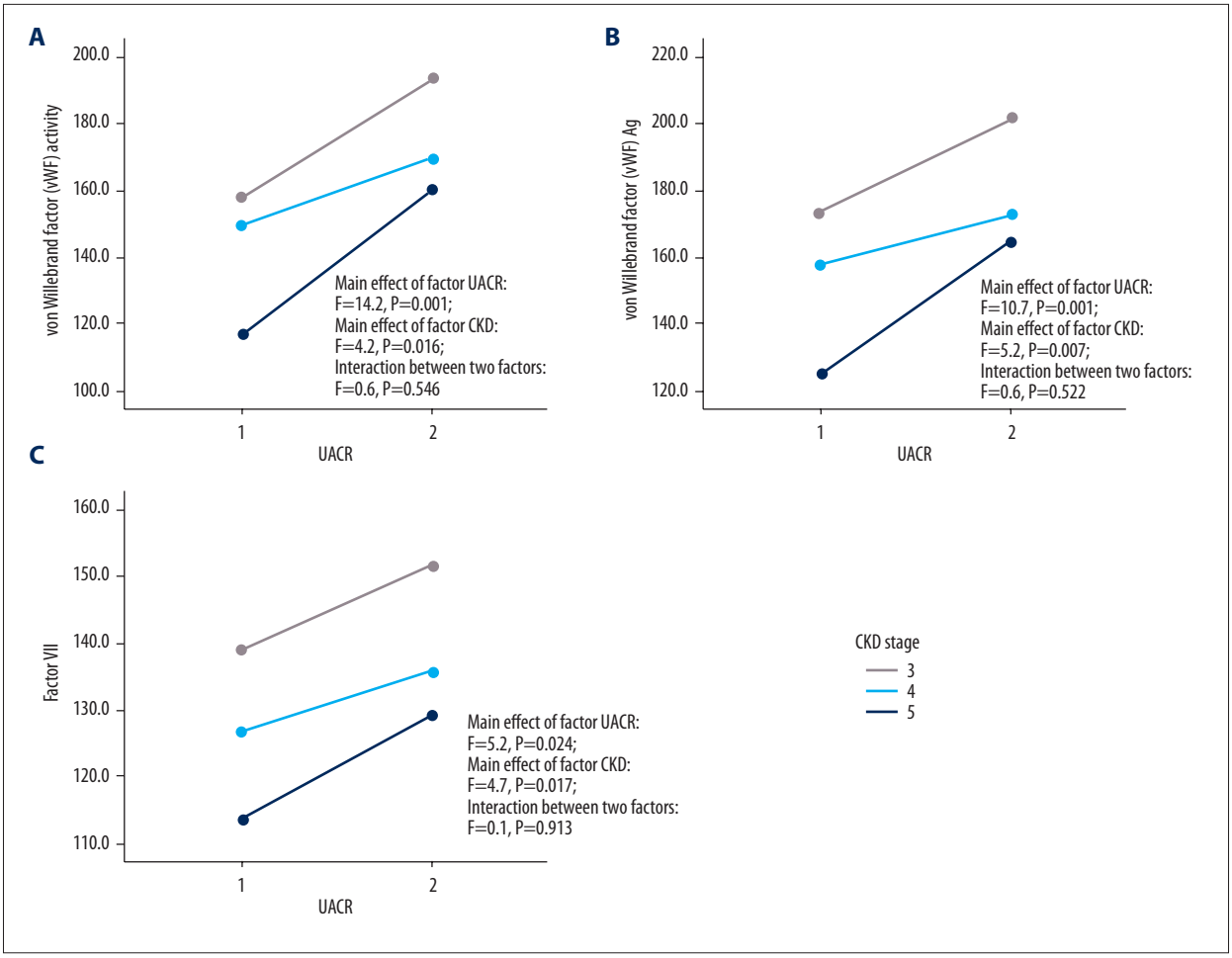

Figure 2. Main effect and interaction between 2 factors: CKD and UACR. (**A**) Effect of CKD and UACR on vWF activity. (**B**) Effect of CKD and UACR on vWF Ag. (**C**) Effect of CKD and UACR on factor VIII.

≤300 mg/g group and CKD3 patients, respectively. There was no significant interaction between any 2 factors (interleukin-2: F=0.26, P=0.77; log (interleukin-6): F=0.29, P=0.75; superoxide dismutase: F=0.25, P=0.78) (Figure 3).

Discussion

Patients with CKD have endothelial dysfunction, which may contribute to increased risk for cardiovascular events. UACR is an independent risk factor for cardiovascular disease. However, the mechanisms underlying the association between albuminuria and CVD are not fully understood. We hypothesized that the effect of UACR on vascular endothelium may be one of the mechanisms leading to CVD in patients with CKD. Thus, we examined the relationship between UACR and biomarkers of endothelial function in patients with CKD.

In addition to the associations between eGFR and hemostatic markers, a strong correlation between UACR and endothelial dysfunction was confirmed in the present study. CKD patients often present with higher levels of traditional risk factors for thromboembolic events, such as hypertension, diabetes, obesity, and dyslipidemia [11], and these factors also affect endothelial function. Before and after adjustment for demographic characteristics and other covariates (e.g., hemoglobin, serum albumin, and blood lipids), the associations between UACR and biomarkers of endothelial dysfunction were attenuated but remained significant. For instance, for every 88.5 mg/g (10 mg/mmol) increase in UACR, the unadjusted vWF activity was elevated by 8.3%, which was attenuated to 4.8% with full adjustment. Moreover, we found no significant interaction between these variables, which indicates that albuminuria does not alter the association between eGFR and endothelial dysfunction.

Extensive basic research has found that inflammation and oxidative stress can induce vascular endothelial cell injury and accelerate atherosclerosis [12,13]. Additionally, a clinical study demonstrated that inflammation and oxidative stress markers

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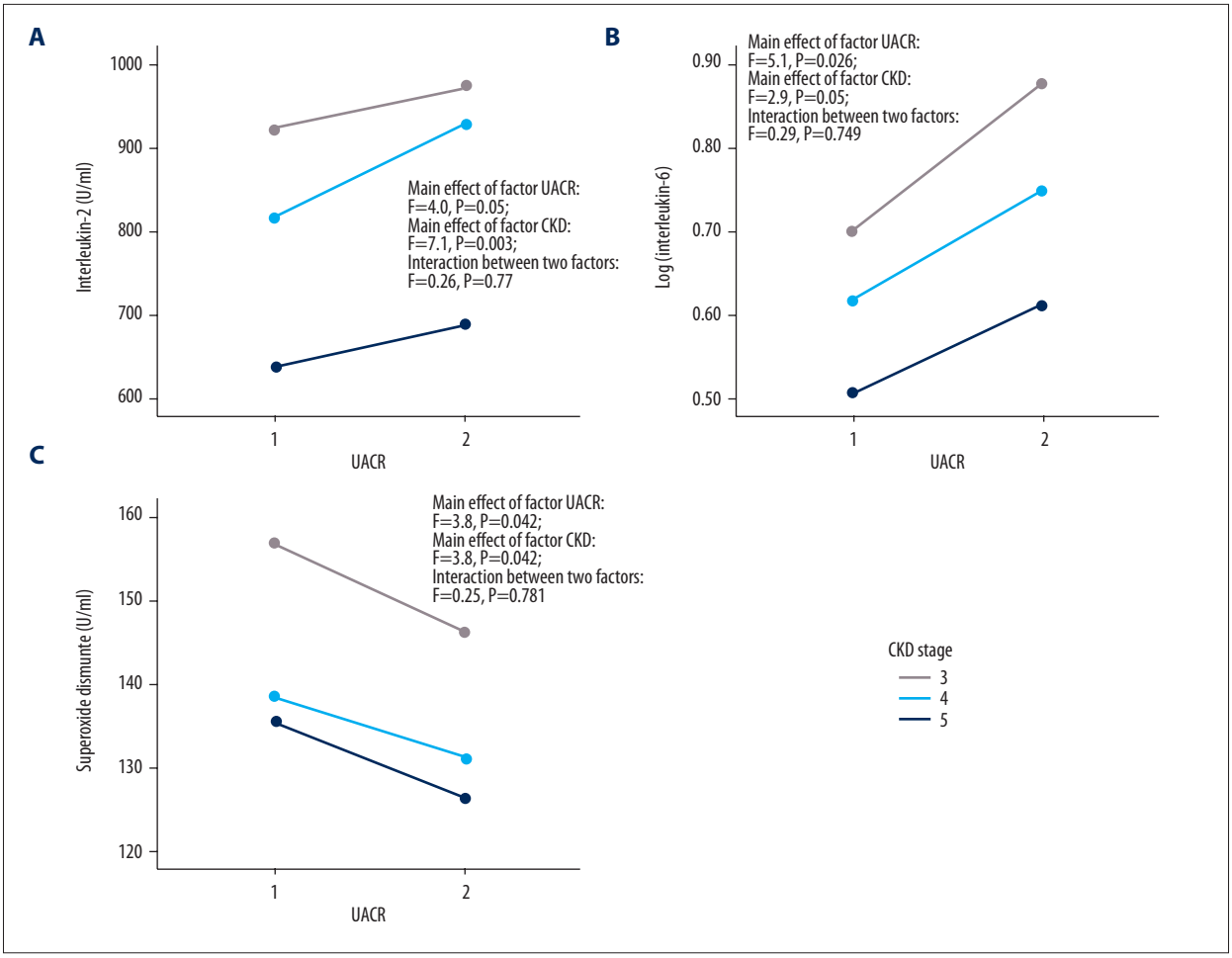

Figure 3. Inflammatory biomarkers associated with level of eGFR and UACR group. (**A**) Effect of CKD and UACR on Interleukin-2. (**B**) Effect of CKD and UACR on log (Interleukin-6). (**C**) Effect of CKD and UACR on superoxide dismutase.

are closely associated with cardiovascular events [14]. In the present study, we found that interleukin-2, interleukin-6, and superoxide dismutase had strong correlations with UACR in CKD patients. We infer that these factors may mediate the association between UACR and endothelial dysfunction. Notably, our findings suggest that inflammation and oxidative stress markers change as UACR increases in CKD patients; however, the present study is a cross-sectional study, which only identifies the association between inflammatory cytokines and UACR but does not demonstrate a causal relationship between inflammatory cytokines, UACR, and endothelial injury. For example, increased inflammation could have caused both UACR and endothelial dysfunction [4,15]. Additionally, albuminuria can alter the levels of inflammatory cytokines and thrombotic cascades [16] and can contribute to CVD.

The present study has certain limitations. First, the vascular endothelium has multiple physiological functions, such as regulating vascular tension, resisting coagulation, and promoting fibrinolysis, in addition to its participation in the inflammatory response. In addition to the serological marker vWF, other indicators, such as reactive hyperemia peripheral arterial tonometry (RH-PAT), should be tested; these indicators can better reflect the physiological functions of the vascular endothelium. However, further investigation was not carried out because such test methods are not yet available at our center. Second, this was a cross-sectional study, and there was no further follow-up after the endpoint of cardiovascular events. We could not establish a relationship between endothelial dysfunction and eventual subsequent thromboembolic events in CKD patients.

Conclusions

This study demonstrates that a higher UACR is GFR-independently associated with elevations in markers of endothelial function and inflammatory factors in CKD patients. These findings suggest that therapies aimed at treating albuminuria may help to alleviate endothelial dysfunction.

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References:

1. Tonelli M, Karumanchi SA, Thadhani R: Epidemiology and mechanisms of uremia-related cardiovascular disease. Circulation, 2016; 133: 518–36
2. Schiffrin EL, Lipman ML, Mann JF: Chronic kidney disease: effects on the cardiovascular system. Circulation, 2007; 116: 85–97
3. Go AS, Chertow GM, Fan D et al: Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med, 2004; 351: 1296–305
4. Hogas SM, Voroneanu L, Serban DN et al: Methods and potential biomarkers for the evaluation of endothelial dysfunction in chronic kidney disease: A critical approach. J Am Soc Hypertens, 2010; 4: 116–27
5. Matsushita K, Coresh J, Sang Y et al: Estimated glomerular filtration rate and albuminuria for prediction of cardiovascular outcomes: A collaborative meta-analysis of individual participant data. Lancet Diabetes Endocrinol, 2015; 3: 514–25
6. Solomon SD, Lin J, Solomon CG et al: Influence of albuminuria on cardiovascular risk in patients with stable coronary artery disease. Circulation, 2007; 116: 2687–93
7. Muntner P, Bowling CB, Gao L et al: Age-specific association of reduced estimated glomerular filtration rate and albuminuria with all-cause mortality. Clin J Am Soc Nephrol, 2011; 6: 2200–7
8. Levey AS, Stevens LA, Schmid CH et al: A new equation to estimate glomerular filtration rate. Ann Intern Med, 2009; 150: 604–12
9. National Kidney Foundation: K/DOQI clinical practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. Am J Kidney Dis, 2002; 39: S1–266
10. Shlipak MG, Fried LF, Crump C et al: Elevations of inflammatory and procoagulant biomarkers in elderly persons with renal insufficiency. Circulation, 2003; 107: 87–92
11. Rucker D, Tonelli M: Cardiovascular risk and management in chronic kidney disease. Nat Rev Nephrol, 2009; 5: 287–96
12. Guzik B, Sagan A, Ludew D et al: Mechanisms of oxidative stress in human aortic aneurysms – association with clinical risk factors for atherosclerosis and disease severity. Int J Cardiol, 2013; 168: 2389–96
13. D'Apolito M, Du X, Pisanelli D et al: Urea-induced ROS cause endothelial dysfunction in chronic renal failure. Atherosclerosis, 2015; 239: 393–400
14. Wannamethee SG, Shaper AG, Lowe GD et al: Renal function and cardiovascular mortality in elderly men: The role of inflammatory, procoagulant, and endothelial biomarkers. Eur Heart J, 2006; 27: 2975–81
15. Hartman J, Frishman WH: Inflammation and atherosclerosis: a review of the role of interleukin-6 in the development of atherosclerosis and the potential for targeted drug therapy. Cardiol Rev, 2014; 22: 147–51
16. Kario K, Matsuo T, Kobayashi H et al: Factor VII hyperactivity and endothelial cell damage are found in elderly hypertensives only when concomitant with microalbuminuria. Arterioscler Thromb Vasc Biol, 1996; 16: 455–61

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