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Alteration and association between serum ACE2/angiotensin(1-7)/Mas axis and oxidative stress in chronic kidney disease

A pilot study

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Abstract

Activation of the renin angiotensin system and renal oxidative stress (OS) are critical contributors in the progression of chronic kidney disease(CKD). Recent studies have confirmed that the angiotensin-converting enzyme 2-angiotensin (1-7)-Mas(ACE2/Ang(1-7)/Mas) axis, the important components of renin angiotensin system, protected kidneys against damage by antagonizing angiotensin II and attenuating OS in rats with several nephropathy models, but its effect needs to be further evaluated in clinic. In this study, we aimed to detected serum ACE2/Ang (1-7)/Mas axis, OS conditions and described its clinical associations in patients with CKD at different stages.

A total of 48 patients with CKD and 6 healthy controls (CT) were enrolled, and serum angiotensin converting enzyme (ACE), ACE2, Ang (1-7), 8-hydroxy-2'-deoxyguanosine (8-OHdG) were determined by ELISA. Serum extracellular glutathione peroxidase(eGSH-Px) activity and renal functions were determined by the biochemical method.

Serum ACE and ACE2 levels in CKD stages 3 to 5 and serum Ang(1-7) levels in CKD stages 4 to 5 without Ang II receptor blockers treatment significantly increased compared to those in the CT group. However, ACE2 was decreased and Ang(1-7) level increased in early CKD stage with Ang II receptor blockers treatment. Higher serum 8-OHdG levels and lower eGSH-Px activity were noted in CKD stages 4 to 5. Serum 8-OHdG level was correlated with serum ACE2, Ang(1-7) expression. Estimated glomerular filtration rate (eGFR) was correlated with serum ACE, ACE2, Ang(1-7), 8-OHdG, Hcy levels and serum eGSH-Px activity. Multiple-regression analysis eGFR was predicted by ACE, Hcy, eGSH-Px, and also can be predicted by ACE2, Ang(1-7), Hcy in CT subgroup.

The ACE2/Ang(1-7)/Mas axis is associated with OS, and both them were associated with eGFR in the progression of CKD. Activation of ACE2/Ang(1-7)/Mas axis may have renoprotective effect and can be a potential therapeutic target in patients with early CKD stages.

Abbreviations: 8-OHdG = 8-hydroxy-2′ -deoxyguanosine, ACE = angiotensin-converting enzyme, ACE2 = angiotensin-converting enzyme 2, ACEIs = ACE inhibitors, Ang II = angiotensin II, Ang(1-7) = angiotensin(1-7), ARB = Ang II receptor blockers, CKD = chronic kidney disease, CT = healthy control, eGSH-Px = extracellular glutathione peroxidase, ESRD = end stage renal disease, OS = oxidative stress, RAS = renin angiotensin system.

Keywords: angiotensin-converting enzyme 2/angiotensin(1-7) Mas axis, chronic kidney disease, ; oxidative stress

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SC and LK contributed equally to this work

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Written informed consent for publication was obtained from all participants.

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1. Introduction

Chronic kidney disease(CKD) is a clinical syndrome that is associated with abnormality in kidney structure, function[1] and the prevalence of CKD reached 14.3% in the general populations.[2] Renal fibrosis is a common pathological manifestation of CKD significantly accelerated due to the activation of the renin angiotensin system (RAS) and oxidative stress(OS). Previous studies proved that the increase in biologically active angiotensin II (AngII), the main effector of RAS, enhanced renal OS and promoted renal inflammation,[3,4] played an important role in kidney damage. Clinical application of angiotensin-converting enzyme (ACE) inhibitors (ACEIs) or AngII receptor blockers(ARBs), the blocker of the RAS, reduced multiple risk factors and delayed the progression of CKD. But it is limited in renal artery stenosis, severe renal insufficiency, hyperkalemia, etc. Therefore, it makes sense to find new blocking targets of RAS to treat CKD.

As the new components of the RAS, ACE2 cleaves the main effector Ang II directly to the biologically active angiotensin (1-7) Ang (1-7), combined with other ways, and accelerated the metabolism of AngII which protected the kidney against injury.[5,6] Clinical data found that the activity of ACE2 in the circulation[7,8] and the level of ACE2 in the urine[9] were both increased in patients with CKD and suggested that ACE2 can be used as a biomarker to predict cardiovascular risk and ACE/ACE2 ratios could reflect the level of RAS activation to some extent.[6] However, there are research reports that ACE2 activity in the circulation decreased in CKD stages 3 to 5, which was detected in human ethylenediamine-tetraacetic acid plasma samples with zinc added.[10] Therefore, it is necessary to clarify the alterations in ACE2 in patients with CKD and eliminate related influencing factors. Many studies have shown that the Ang(1-7), degradation of AngII by ACE2, has a protective effect in experimental model of kidney disease. Infusion of Ang(1-7) reverses glomerulosclerosis by counteracting the AngII effect[11] and prevents renal fibrosis by regulating TGF-β1/Smad signaling[12] in rats with unilateral ureteral obstruction model. There are few correlative studies on Ang(1-7) in clinical trials indicated that the level of Ang(1-7) in CKD may be useful for further research.

The RAS is closely related to OS in CKD, while AngII induced reactive oxygen species(ROS) production in the mitochondria, especially in diabetic nephropathy,[13] causing renal inflammation and deposition of the extracellular matrix.[14] Accumulated evidence suggests that Ang(1-7) administration reduces inflammation, attenuate OS and tubulointerstitial fibrosis in mice with diabetic nephropathy models.[15,16] However, these changes may be not specific in patients with CKD except diabetic nephropathy.

In this study, we aimed to determine whether the ACE2/Ang(1-7)/Mas axis, OS differed and describe clinical associations between patients with CKD.

2. Methods

2.1. Subjects

This was a cross-sectional analysis of patients who met the diagnostic criteria of KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease.[17] The study protocol was approved by The Ethics Committee of Zhejiang Chinese Medical University First Affiliated Hospital (Hangzhou, China). A total of 48 patients hospitalized for CKD in Zhejiang Chinese Medical University Affiliated First Hospital between January 2018 and June 2018 were recruited. The exclusion criteria were renal replacement therapy, acute renal failure, renal artery stenosis, heart failure, respiratory failure, malignant tumor, tuberculosis and pregnant women.

All patients provided informed consent to participate in the sduty and related indexes were subsequently determined. The 48 patients were assigned to 5 groups according to their CKD stages: group 1(estimated glomerular filtration rate [eGFR]≥90 mL/min/1.73m^2), group 2(eGFR > 60–90 mL/min/1.73m^2), group 3 (eGFR > 30–60 mL/min/1.73m^2), group 4(eGFR > 15–30 mL/min/1.73 m^2), group 5(eGFR≤15 mL/min/1.73 m^2) and 6 healthy volunteers served as controls. eGFR were calculated using the CKD-EPI-Cys C[18] except in healthy volunteers (calculated by CKD-EPI).

2.2. Sample collection

Blood samples were obtained from participants using the Z Serum Clot Activator tube(Greiner Bio-One, Kremsmunster, Austria) and centrifuged at 3000 rpm for 10 minutes; then, the serum was frozen at −80°C until measurements were performed.

2.3. Measurements

Clinical laboratory data were measured in Zhejiang Chinese Medical University First Affiliated Hospital with the Accelerator a3600(Abbott, Chicago).

Serum ACE, ACE2, Ang(1-7) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were measured by enzyme-linked immunosorbent assay (ELISA) kits(L180521118, L180521025, L180529107 and L180502292, USCN, Wuhan, China) according to the manufacturer's instructions. Briefly, 50 μL of control, standard or serum sample per well was added, covered with the adhesive strip, incubated with 50 uL of detection antigen liquid at 37°C incubator for 30 min. Moreover, the plates were washed 5 times and incubated with 50 uL of avidin-HRP at 37°C for 30 min. Subsequently, detection A and B to per well were added and incubated in the dark at 37°C for 10 minute. Finally, the reaction was terminated with 50 uL of stop solution and the O.D. was measured at 450 nm with SpectraMax Plus384 Microplate Reader (Molecular Devices, Silicon Valley). The levels were calculated by standard curve that was created through O.D. and concentration.

Extracellular glutathione peroxidase (eGSH-Px)(20180530, Jiancheng, Nanjing, China) activity were determined by the biochemical method. Briefly, added 100 μL serum sample was added in the enzyme tube and non-enzyme tube in 37°C warm bath for 5 minute. Next, the enzymatic reaction was conducted using glutathione as a substrate. After the centrifuge process at 3500 rpm for 10 minute, 1 mL of supernatant was obtained for color reaction. After letting it stand at 25°C for 15 minute, the O.D. was measured at 412 nm with SpectraMax Plus384 Microplate Reader (Molecular Devices, Silicon Valley) and eGSH-Px activity was calculated by O.D.

2.4. Statistical analysis

Data were expressed as mean ± SD. Statistical significance between 2 groups was analyzed by independet *T*-test. Comparison of multiple groups was performed by ANOVA. Correlations were determined using Pearson's correlation coefficients(Spear-

man's rank correlation coefficient for non-normal distribution). Single-regression of the respective independent variables on eGFR was calculated. The correlation between eGFR and multiple independent variables was determined by multiple linear regression. A P-value $< .05$ was considered statistically significant(SPSS17.0, Chicago).

3. Results

3.1. Analysis of Clinical data

The profiles and clinical data of the included subjects are presented in Table 1. Significant differences between patients with CKD stages 1 to 5 were observed in Age, Cr, Cys C, eGFR, Bun, Cl, Glb, Hcy, and Hb.

3.2. Activation of ACE2/Ang(1-7)/Mas axis in advanced CKD (Fig. 1)

The expression of ACE increased in the CKD stages 4–5 group compared to those in the healthy control (CT) group and decreased in the CKD stage 1 group compared to that in the CKD stages 3 to 5 group(Fig. 1A). The RAS activation increased with CKD progression at some degree. Similarly, the levels of ACE2 in the CKD stages 4 to 5 group and Ang(1-7) in the CKD stage 5 group significantly increased compared to those in other groups (Fig. 1B,D). However, the ACE/ACE2 ratio between groups was not significantly different(Fig. 1C). In other words, ACE2/Ang(1-7)/Mas axis was activated in advanced CKD.

3.3. Activation of ACE2/Ang(1-7)/Mas axis in early CKD after ARB treatment

The RAS blocker, ARB, is wildly used in the clinic and delays the progression of CKD. Subgroup analysis found that serum ACE and ACE2 levels in the CKD stages 3 to 5 were higher than those in the CKD stage 1 and CT group in no ARB treatment (NA) subgroups (Fig. 2A,B). However, the serum ACE2 level decreased in CKD stage 1 and 3 with ARB treatment (A) subgroups compared with those in the NA subgroups (Fig. 2B). We also found that the ACE/ACE2 ratio in CKD stages 1 to 3 A subgroups increased but was not statistically significant ($P > .05$, Fig. 2C). Differently, Ang(1-7) level increased in CKD stages 4 to 5 NA subgroups and CKD stage 1 A subgroup(Fig. 2D). Combining the changes in ACE2 and Ang(1-7), the increase in Ang(1-7) level, as a negative feedback regulation, caused a decrease in ACE2 level. Accordingly, our results suggested that

Table 1

Clinical data.

Characteristics	I(n = 11)	II(n = 11)	III(n = 12)	IV(n = 9)	V(n = 5)	P	CT(n = 6)
Age	41.45 ± 16.27	55.73 ± 12.95	55.67 ± 13.41	57.00 ± 11.43	62.20 ± 13.57	.03	33.80 ± 4.12
Male/female	4/7	9/2	6/6	2/7	1/4	-	3/3
ARB/NARB	3/8	5/6	6/6	0/9	0/5	-	-
BMI(kg/m^2)	23.21 ± 3.88	23.93 ± 2.72	25.44 ± 4.56	23.43 ± 3.75	23.11 ± 2.30	.74	23.12 ± 3.04
HR (beats/min)	82.55 ± 9.79	89.18 ± 9.50	84.75 ± 10.37	77.00 ± 14.71	84.20 ± 9.65	.19	78.33 ± 7.76
SBP (mm Hg)	136.27 ± 27.02	127.00 ± 27.73	134.50 ± 16.61	138.67 ± 30.93	142.20 ± 20.95	.79	122.00 ± 6.96
DBP (mm Hg)	83.36 ± 17.63	76.09 ± 14.72	83.92 ± 10.66	82.22 ± 19.41	77.20 ± 17.31	.73	75.83 ± 7.36
Glu (mmol/L)	5.31 ± 2.56	4.62 ± 0.70	4.56 ± 0.73	4.91 ± 1.17	5.05 ± 1.18	.75	4.85 ± 0.46
Uric acid (umol/L)	355.91 ± 88.74	471.38 ± 148.01	438.35 ± 108.98	412.97 ± 102.62	465.00 ± 83.24	.16	382.67 ± 90.14
Cr (mg/dL)	0.70 ± 0.08	0.85 ± 4.27	1.53 ± 0.42	2.61 ± 0.45	4.45 ± 1.56	.00	0.68 ± 0.19
Cys C (mg/L)	0.80 ± 0.18	1.13 ± 0.12	1.70 ± 0.31	2.72 ± 0.57	3.89 ± 0.69	.00	-
eGFR(ml/min/1.73m^2)	108.29 ± 13.7	78.98 ± 7.43	41.48 ± 8.43	22.29 ± 3.98	11.08 ± 1.67	.00	119.25 ± 12.2
Bun (mmol/L)	5.14 ± 2.04	6.98 ± 4.27	9.60 ± 3.78	15.06 ± 4.04	18.26 ± 7.48	.00	4.27 ± 0.97
K (mmol/L)	3.88 ± 0.42	4.17 ± 0.53	4.23 ± 0.52	4.07 ± 0.63	4.54 ± 0.56	.21	–
Na (mmol/L)	143.11 ± 1.73	142.30 ± 3.68	143.83 ± 2.29	142.29 ± 2.28	144.08 ± 1.84	.46	–
Cl (mmol/L)	105.82 ± 2.99	105.86 ± 3.46	109.08 ± 2.33	107.62 ± 2.15	112.64 ± 1.76	.00	–
TG (mmol/L)	1.88 ± 1.43	1.90 ± 1.06	1.88 ± 0.88	2.58 ± 1.63	1.64 ± 0.57	.60	1.43 ± 0.63
TC (mmol/L)	6.11 ± 2.27	7.92 ± 3.99	6.22 ± 2.25	6.96 ± 2.35	4.34 ± 0.83	.17	4.19 ± 0.7
HDL (mmol/L)	1.66 ± 0.5	2.06 ± 0.85	1.65 ± 0.54	1.76 ± 0.49	1.20 ± 0.19	.12	1.33 ± 0.08
LDL (mmol/L)	3.64 ± 1.99	5.05 ± 2.95	3.87 ± 1.61	4.19 ± 1.61	2.39 ± 0.67	.19	2.17 ± 0.58
Apo A (g/L)	1.62 ± 0.21	1.58 ± 0.28	1.44 ± 0.31	1.40 ± 0.18	1.30 ± 0.16	.08	-
Apo B (g/L)	1.13 ± 0.55	1.51 ± 0.71	1.30 ± 0.51	1.33 ± 0.39	0.89 ± 0.16	.26	-
Alb (g/L)	30.53 ± 10.48	25.4 ± 10.24	26.45 ± 8.31	28.43 ± 8.36	35.62 ± 6.65	.27	44.52 ± 2.47
Glb (g/L)	25.65 ± 5.03	24.58 ± 3.31	24.37 ± 4.84	25.74 ± 3.48	34.64 ± 2.95	.00	28.48 ± 4.49
Tb (umol/l)	12.43 ± 7.83	8.64 ± 1.87	8.91 ± 4.89	7.03 ± 1.87	8.36 ± 0.8	.13	11.30 ± 3.45
AST (U/L)	25.36 ± 20.86	30.64 ± 18.24	19.67 ± 5.09	22.67 ± 6.71	16.00 ± 5.15	.27	-
ALT (U/L)	22.00 ± 27.15	25.82 ± 14.51	18.58 ± 13.99	19.78 ± 4.89	11.40 ± 3.78	.58	12.00 ± 5.29
Hcy (umol/L)	9.40 ± 1.97	11.53 ± 4.94	16.69 ± 7.19	29.03 ± 11.24	43.88 ± 53.03	.00	-
Free fatty acid (umol/L)	412.36 ± 262.94	356.89 ± 117.99	333.83 ± 117.97	283.33 ± 106.66	327.60 ± 122.61	.52	-
Hb (g/L)	125.91 ± 19.18	132.36 ± 19.58	116.08 ± 21.01	98.44 ± 22.71	78.6 ± 17.42	.00	141.17 ± 18.24

I = CKD stage 1, II = CKD stage II, III = CKD stage III, IV = CKD stage 1 V; V = CKD stage V, CT = control, ARB = angiotensin receptor blocker treatment, NARB = no angiotensin receptor blocker treatment, BMI = body mass index, HR = heart rate, SBP = systolic blood pressure, DBP = diastolic blood pressure, Cr = creatinine, Cys C = Cystatin C, BUN = blood urea nitrogen, K = potassium, NA = sodium, Cl = chloride, TG = triglyceride, TC = total cholesterol, HDL = high-density lipoprotein, LDL = low-density lipoprotein, Apo A = apolipoprotein A, Apo B = apolipoprotein B, Alb = Albumin, Glb = Globulin, AST = aspartate aminotransferase, Tb = Total bilirubin, ALT = alanine aminotransferase, Hcy = homocysteine, Hb = hemoglobin.

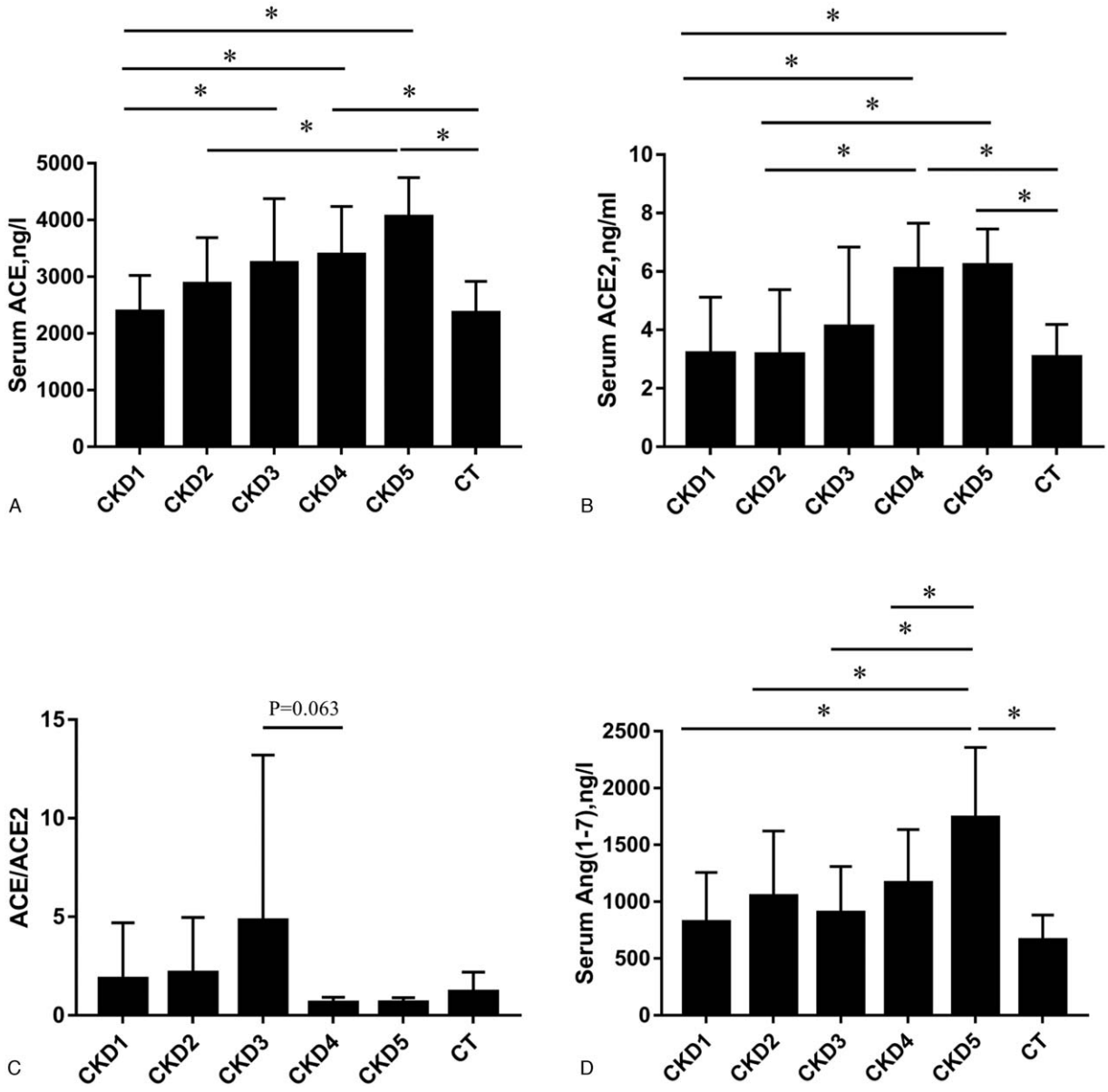

Figure 1. Activation of ACE2/Ang(1-7)/Mas axis in advanced chronic kidney disease. A. Serum ACE level significantly increased in the CKD stages 4-5 compared to that in the CT group and decreased in CKD stage 1 compared to that in the CKD stages 3-5. B. The CKD stages 4-5 group had higher ACE2 content than other groups. C. There was no statistical significantly in ACE/ACE2 ratio between the groups. D. The content of Ang(1-7) in the CKD stage 5 was higher than those in other groups. ACE:angiotensin converting enzyme; ACE2:angiotensin converting enzyme 2; Ang(1-7): angiotensin(1-7);CT:control; *:$P<.05$.

ACE2/Ang(1-7)/Mas axis was activated in advanced CKD and could be activated early by intervention.

3.4. Enhanced OS in advanced CKD

The body has physiologic defense mechanisms against OS, but imbalance in oxidant generation results in renal injury in patients with CKD. We examined the OS state of CKD at different stages and found that the level of 8-OHdG, marker of leukocyte DNA oxidative damage, increased in CKD stages 4–5 (Fig. 3A), but the activity of eGSH-Px, enzymatic antioxidant, decreased in CKD stages 4 to 5 (Fig. 3B). Then, we further divide the participants into subgroups and the results showed that oxidation products increased and antioxidant enzyme activity decreased with the progression of CKD (Fig. 3C,D).

3.5. The expression of ACE2/Ang(1-7)/Mas axis had difference in various pathological diagnoses

The primary pathological diagnosis of kidney disease were minimal change disease (MCD, n=5), IgA nephropathy (IgAN, n=7), membranous nephropathy (MN, n=10), mesangial proliferative glomerulonephritis (MPG, n=6), focal segmental glomerulosclerosis(FSGS, n=3), diabetic kidney disease(DKD, n=2), crescentic glomerulonephritis(n=1) and unknown(n=14). As shown in Figure 4, the levels of ACE2 decreased in the IgAN group (Fig. 4B). Ang(1-7) level decreased in the MCD group and IgAN groups(Fig. 4C). We also found that Ang(1-7) level relatively increased in multiple groups(Fig. 4C). Therefore, the expression of the ACE2/Ang(1-7)/Mas axis differs in various pathological diagnoses. There were also significant differences

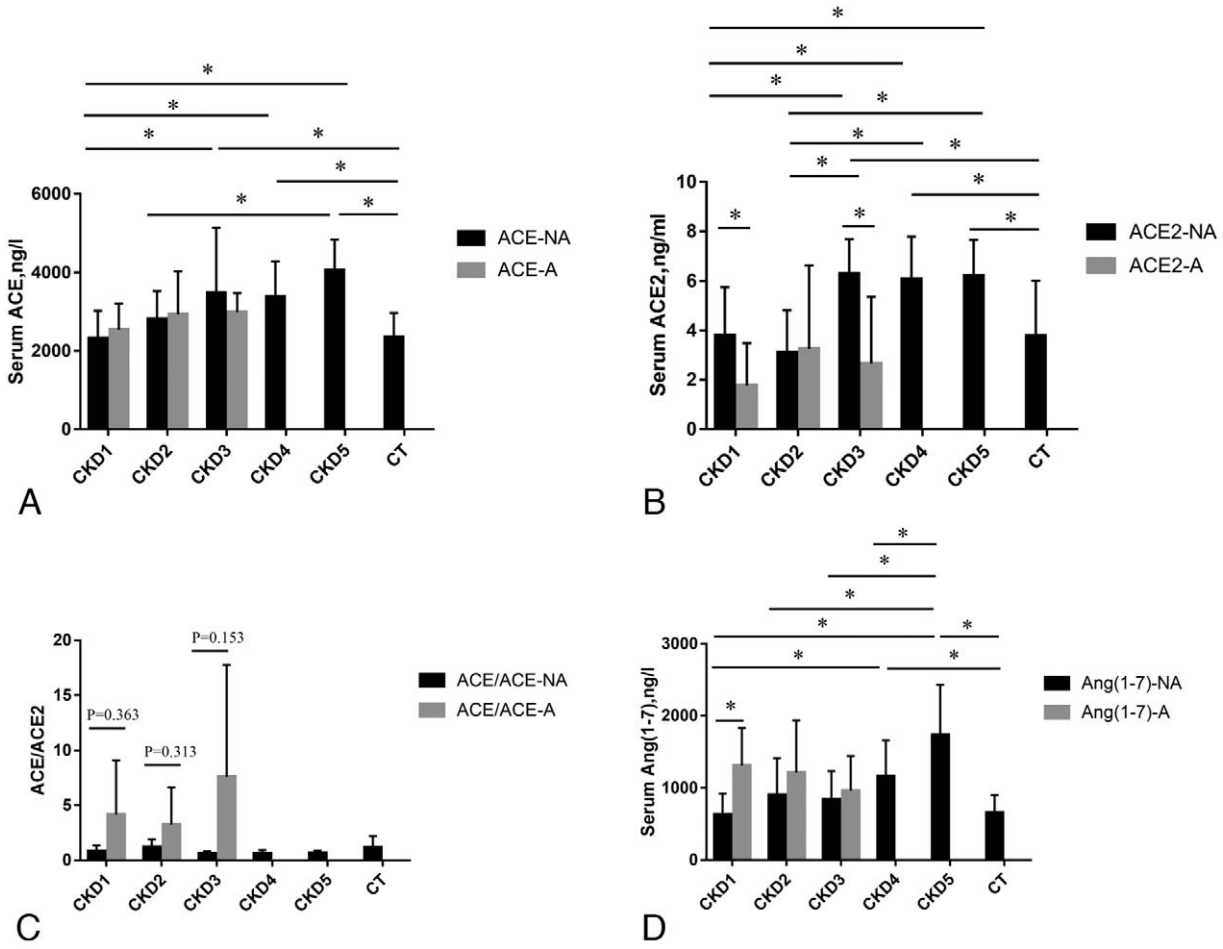

Figure 2. Activation of ACE2/Ang(1-7)/Mas axis in early chronic kidney disease after ARB treatment. A. Serum ACE level significantly increased in CKD stages 3-5 NA subgroups compared to that in the CT group and decreased in the CKD stage 1 NA subgroup compared to those in the CKD stages 3-5 NA subgroups. The comparison between the CKD stages 1-3 subgroups had no difference. B. ACE2 level was higher in CKD stages 3-5 NA groups than those in other groups. CKD stages 1,3 NA subgroups higher than those in the A subgroups. C. There was no statistically significant difference in ACE/ACE2 ratio between the groups, including the subgroups. D. The Ang(1-7) level in the CKD stage 5 group was higher than those in other groups. CKD stages 4-5 groups higher than those in CT group. CKD stage 1 NA group lower than those in the CKD stage 1 A group and stages 4-5 group. NA: no ARB treatment; A: ARB treatment.

between MCD and IgAN in eGSH-Px activity(Fig. 4E). But the levels of ACE and 8-OHdG were not statistically different between groups(Fig. 4A, D).

3.6. Correlation between ACE2/Ang(1-7)/Mas axis and OS

It is known that RAS is closely related to OS in CKD and AngII-induced ROS production.[13] As shown in Table 2, we further found that 8-OHdG and Hcy, markers of nucleic acid damage and protein oxidation, were significantly correlated with serum ACE2 expression except in the A subgroups. Ang(1-7) correlated with 8-OHdG and Hcy in the A subgroup. However, there was no correlation between ACE2/Ang(1-7)/Mas axis and eGSH-Px in all subjects.

3.7. Correlation analysis of eGFR with the respective independent variables

As shown in Table 3, eGFR had a negative correlation with ACE, ACE2, Ang(1-7), 8-OHdG and Hcy. We divided those samples into subgroups and found that the above indicators were still negatively correlated with eGFR in the NA subgroups. eGSH-Px activity was significantly correlated with eGFR in all groups.

3.8. Single-Regression of eGFR with the respective independent variables

The results showed that eGFR had a negative correlation with ACE (Fig. 5A), ACE2 (Fig. 5B), Ang(1-7) (Fig. 5C), 8-OHdG (Fig. 5D), Hcy (Fig. 5E) and a positive correlation with eGSH-Px (Fig. 5F). Then, we repeated the single-regression analysis of eGFR with above indicators after dividing participants into NA and A subgroups. eGFR was negatively correlated with ACE (Fig. 6A), ACE2 (Fig. 6B), Ang(1-7) (Fig. 6C), 8-OHdG (Fig. 6D), Hcy(Fig. 6E) in the NA subgroups and positive correlated with eGSH-Px activity (Fig. 6F,G) in all groups.

3.9. Multiple-regression analysis by the Stepwise method for eGFR

As described in Table 4, the indicators, singly correlated with eGFR significantly, were selected as explanatory variables in the multiple-regression analysis. The results showed that ACE2, Ang (1-7), 8-OHdG were eliminated and eGFR was predicted by ACE, Hcy, eGSH-Px. However, the results were different in the subgroup analysis. eGFR in NA subgroups was predicted by

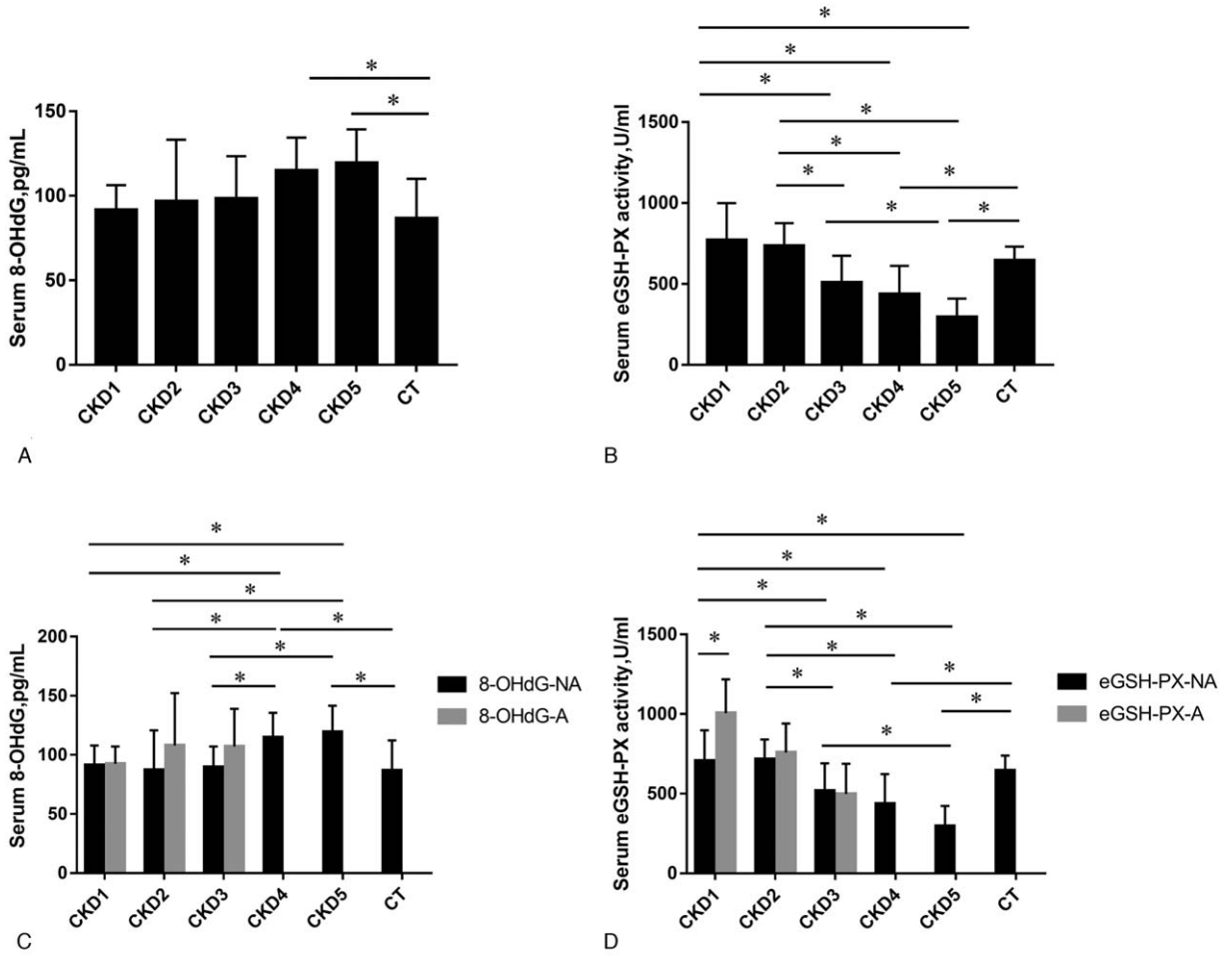

Figure 3. Enhanced OS in advanced chronic kidney disease. A,C Serum 8-OHdG level increased in CKD stages 4-5 compared to those in CT group. Serum 8-OHdG content in CKD stages 4-5 higher than those to CKD stages 1-3 NA subgroups and CT group. There was no statistical significance in CKD stages 1-3 group and subgroup. B,D. The activity of eGSH-Px decreased in CKD stages 3-5 compared with those in CKD stages 1-2. Moreover, serum eGSH-Px activity increased in CKD stage 1 A subgroup. 8-OHdG: 8-hydroxy-2'-deoxyguanosine; eGSH-Px: extracellular glutathione peroxidase.

ACE2, Ang(1-7), Hcy, and eGFR in A subgroups was independently predicted by eGSH-Px.

4. Discussion

CKD is an important health problem worldwide that causes enormous social, economic and family burdens. Blocking or inhibiting the classical RAS axis, ACEI/ARB, has become the common protocol of treating CKD treatment.[19] However, the inevitable side effects and limitations in treatment with ACEI/ARB resulted in the lack of available medicine in some situations. It's important to excavate potential targets to supplement the defects of classical axis. Therefore, we are devoted to researching ACE2/Ang(1-7)/Mas axis and the underlying mechanistic pathways in order to provide new ideas for the treatment of CKD.

ACE, a key enzyme of the classical axis, converts Ang I to Ang II which promotes water-sodium retention, vasoconstriction, induction of ROS, apoptosis and stimulation of extracellular matrix synthesis.[20] Usual daily doses of an ACEI decreased plasma Ang II levels by inhibiting the ACE activity. Differently, only administrated ARB does not suppress ACE activity.[21] Many studies have proven that other RAS components, like ACE2/Ang(1-7)/Mas axis, regulated renal inflammation, fibrosis, changed as CKD progresses and also in response to the treatment of ACEI/ARB.[5,6,22] It has been demonstrated that circulation ACE2 activity[8] and urinary ACE2 levels[9] increased in patients with CKD. However, circulation ACE2 activity decreased in CKD stages 3 to 5 which measured in human ethylenediaminetetraacetic acid -plasma with zinc added.[10] The balance between the 2 enzymes, ACE/ACE2 ratio, was driving the regulation of the renal RAS[23] and intrarenal ACE/ACE2 ratio plays a vital role in the progression of hypertensive nephrosclerosis.[24] In this study, we found that serum ACE, ACE2 levels were low in CT and progressively increased with deterioration of renal function. After the patients who received ARB treatment were included into subgroup in CKD stages 1 to 3, the change of serum ACE levels was not obvious, but serum ACE2 levels was decreased in A subgroup in CKD stages 1,3. Similarly, the serum ACE, ACE2 levels in patients without ARB treatment increased with the progression of CKD. The ACE/ACE2 ratio increased in the CKD stage 3 group and CKD stages 1 to 3 NA groups but had no statistical significance compared to those in other groups. These results suggested that the classical ACE/AngII/AT_1R axis was activated with eGFR decreasing gradually and higher ACE2 level

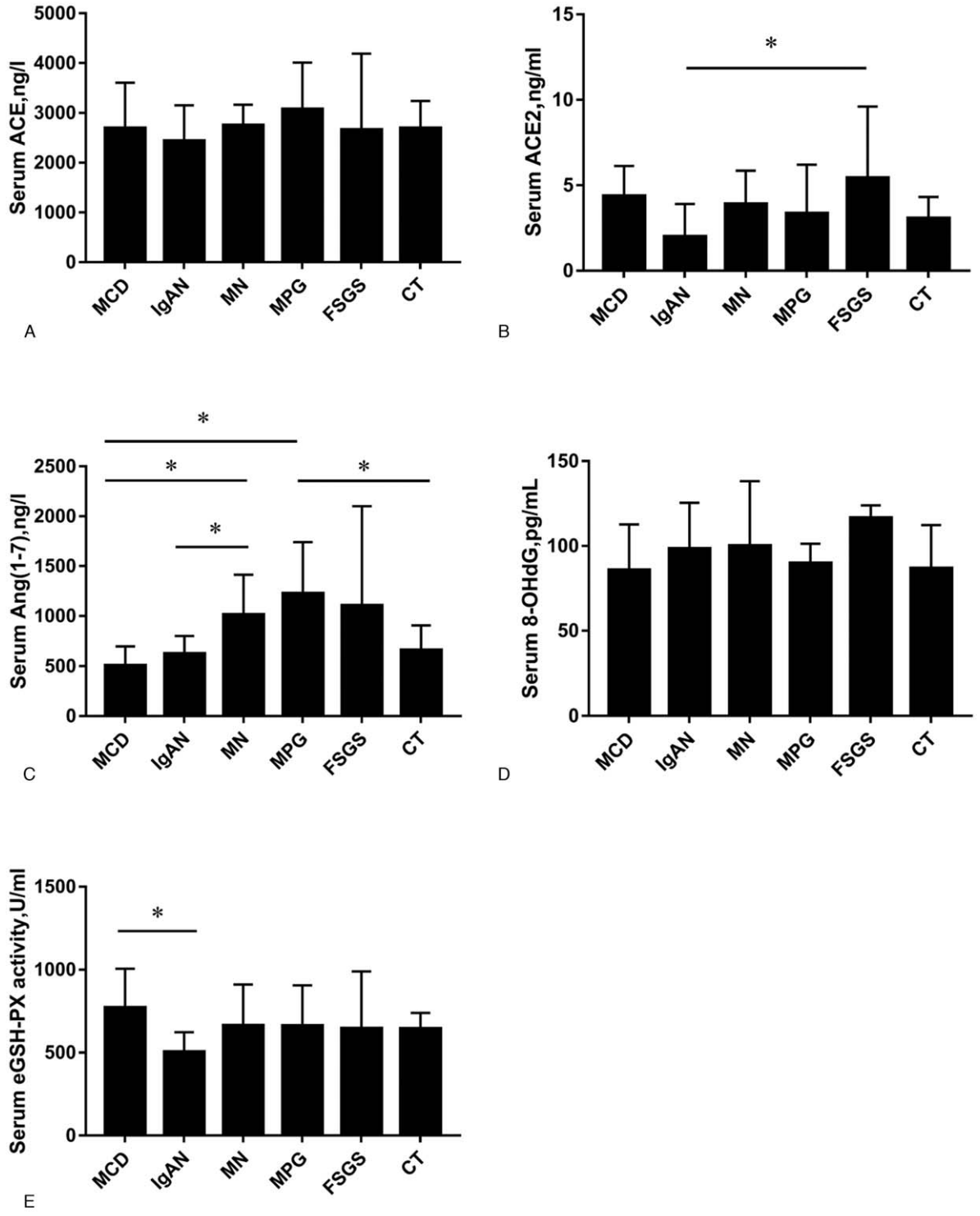

Figure 4. The expression of ACE2/Ang(1-7)/Mas axis had difference in various pathological diagnoses. A,D. There was no statistical significance in the expression of ACE and 8-OHdX between different pathological diagnoses. B. Serum ACE2 level in IgAN lower than that in FSGS. C. Serum Ang(1-7) level was highest in MPG and higher in MN than those in MCD and IgAN. D. Serum eGSH-Px activity in MCD was higher than that in IgAN. MCD: minimal change disease; IgAN: IgA nephropathy; MN: membranous nephropathy; MPG: mesangial proliferative glomerulonephritis; FSGS: focal segmental glomerulosclerosis.

seemed to reflect renal dysfunction as it was associated with eGFR levels. ACE2, as a large molecular weight protein, cannot pass through the glomerular filtration membrane, so the opinion that serum ACE2 increased due to decreased filtration function was unconvincing. Anguiano[8] pointed out that circulating ACE2 activity was associated with cardiovascular disease(CVD) in patients with CKD. Accordingly, we considered that serum ACE2 increased with the progression of CKD might be associated

Table 2

Correlation between ACE2/Ang(1-7)/Mas axis and OS.

Variables	N		8-OHdG	Hcy	eGSH-Px
ACE2	41	Pearson	0.333	0.322	−0.189
		P	.034	0.043	0.235
Ang(1-7)	48	Spearman	0.109	0.075	−0.051
		P	.46	0.616	0.732
ACE2-NA	28	Pearson	0.405	0.381	−0.298
		P	.033	0.045	0.123
Ang(1-7)-NA	34	Spearman	0.139	0.300	−0.330
		P	.433	0.085	0.056
ACE2-A	13	Pearson	0.233	−0.211	0.192
		P	.443	0.510	0.529
Ang(1-7)-A	14	Spearman	0.002	−0.706	0.464
		P	.994	0.001	0.095

ACE = angiotensin-converting enzyme, ACE2 = angiotensin-converting enzyme 2, Ang(1-7) = angiotensin(1-7).

with high incidence of CVD in end stage renal disease (ESRD) patients.

AngII is the main biological component of the classical axis and plays a central mediator in many pathophysiological processes such as hypertension, OS, renal inflammation and fibrosis.[25] However, Ang(1-7) is now recognized as a central role in the RAS because it exerts a series of opposite actions on effector peptide AngII.[26] Studies have shown that Ang (1-7) protects the kidney against damage in multiple kidney disease models.[11,12,15,16,27–30] Genetic deletion of MAS, the Ang(1-7) receptor, led to microalbuminuria, glomerular hyperfiltration and renal fibrosis.[27] Ang(1-7) administration ameliorated renal injury in diabetic[15,16,28] and chronic intermittent hypoxia rats[29] in which beneficial effects were associated with reduction of inflammation, OS and fibrosis. In high fat diet-fed mice, infusion of Ang(1-7) reduced renal injury caused by abnormal lipid metabolism through the LDLr-SREBP2-SCAP pathway.[30] Although the protective effects of Ang(1-7) in experimental nephropathy models, the clinical studies on Ang(1-7) are insufficient. Research showed that Ang(1-7) increased in CKD stages 3–4 after ARB treatment[31] and ESRD.[32] In this study, we observed a negative association between serum Ang(1-7) and eGFR. The highest serum Ang(1-7) level was found in CKD stage 5. Then, subgroup analysis suggested that the serum Ang(1-7) levels was increased significantly in the A subgroup in CKD stage 1 and also in CKD stages 4–5 compared to the CT group. Taken together, the combined analysis of the decrease in ACE2 level with increase in Ang(1-7) level in A subgroup in CKD stage 1 suggested that these changes between ACE2 and Ang(1-7) may be caused by a negative feedback and intervention in ACE2/Ang(1-7)/Mas axis in early CKD stages could be more meaningful to some extent. In contrast, higher serum ACE2, Ang(1-7) levels with lower eGFR could provide a protective and compensatory mechanism which was designed to antagonize the associated damage induced by the classical axis.[33] We speculate that, the higher Ang(1-7) levels with ARB treatment, activation of ACE2/Ang(1-7)/Mas axis may have renoprotective effect in patients with CKD.

OS plays an important role in the progression of CKD. Excessive OS can be considered as a major factor in hypertension, renal ischemia, glomerular damage, inflammation and endothelial dysfunction.[34,35] We choose 2 indicators, 8-OHdG and GSH-Px, to reflect the pro-/antioxidant balance in patients with CKD. 8-OHdG is the most frequently used OS biomarkers among more than 100 oxidative DNA-modifications,[36] which increased in patients with ESRD and regarded as an independent predictor of all-cause mortality in dialysis patients.[37] GSH-Px is 1 of primary enzymatic antioxidants and play an important role

Table 3

Correlation analysis of eGFR with the respective independent variables.

Variables	Pearson/Spearman	*P*	N	Subgroup	Pearson/Spearman	*P*	N
ACE	−0.562	.000	48	ACE-NA	−0.603	.000	34
				ACE-A	−0.385	.175	14
ACE2	−0.502	.001	41	ACE2-NA	−0.542	.003	28
				ACE2-A	−0.322	.284	13
ACE/ACE2	0.237	.136	41	ACE/ACE2-NA	0.179	.361	28
				ACE/ACE2-A	0.250	.409	13
Ang(1-7)	−0.384	.007	48	Ang(1-7)-NA	−0.619	.000	34
				Ang(1-7)-A	0.178	.543	14
8-OHdG	−0.371	.009	48	8-OHdG-NA	−0.408	.017	34
				8-OHdG-A	−0.332	.246	14
Hcy	−0.663	.000	46	Hcy-NA	−0.665	.000	34
				Hcy-A	−0.231	.418	13
eGSH-Px	0.570	.000	48	eGSH-Px-NA	0.558	.001	34
				eGSH-Px-A	0.622	.018	14

8-OHdG = 8-hydroxy-2'-deoxyguanosine, ACE = angiotensin-converting enzyme, ACE2 = angiotensin-converting enzyme 2, Ang(1-7) = angiotensin(1-7).

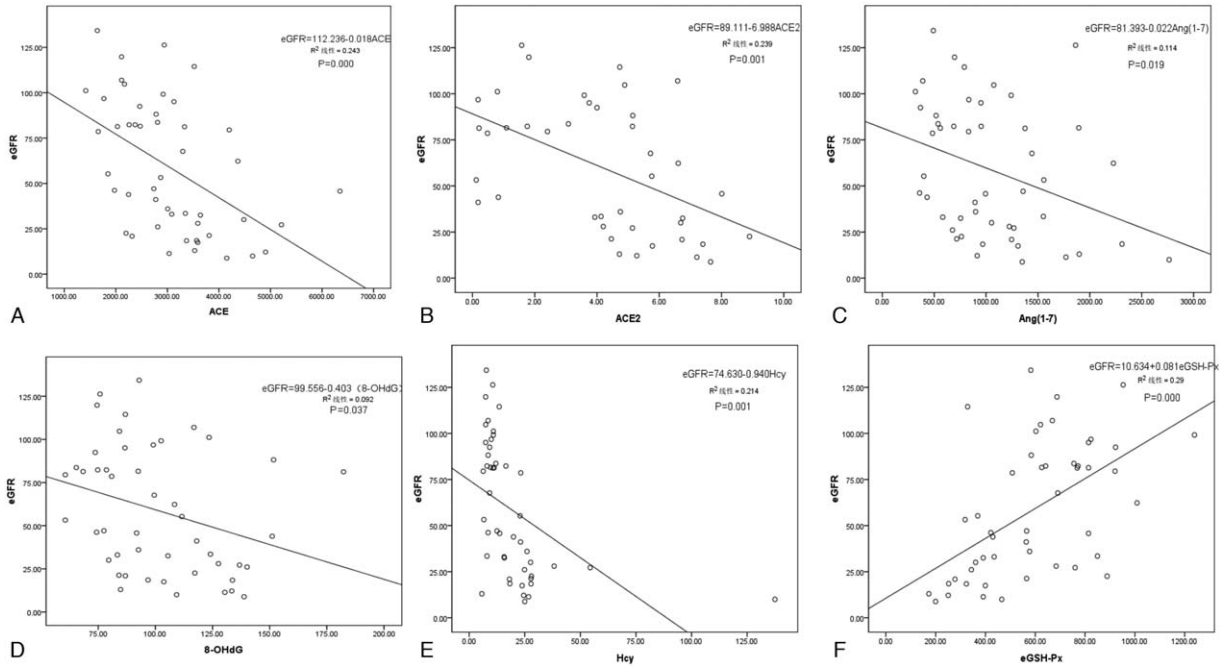

Figure 5. Single-Regression of eGFR with the respective independent variables. eGFR was correlated with ACE(A), ACE2(B), Ang(1-7)(C), 8-OHdG(D), Hcy(E), eGSH-Px(F).

in ROS metabolism. The activity of GSH-Px gradually decreased during CKD progression.[38] Similarly, results showed increased 8-OHdG levels and decreased eGSH-Px activity in the progression of CKD were associated with eGFR. Thus, we conducted a correlation analysis between ACE2/Ang(1-7)/Mas axis and OS, and found that ACE2 positively correlated with 8-OHdG and Hcy. Then, Hcy, a common biomarker of protein oxidation in CKD, correlated with Ang(1-7) in A subgroup. Accumulating evidences suggested that AngII stimulated ROS production by inducing NADPH-oxidase, but few about ACE2/Ang(1-7)/Mas axis. We proved that the new axis correlated with OS and specific mechanisms needed to be further explored. But eGFR negatively

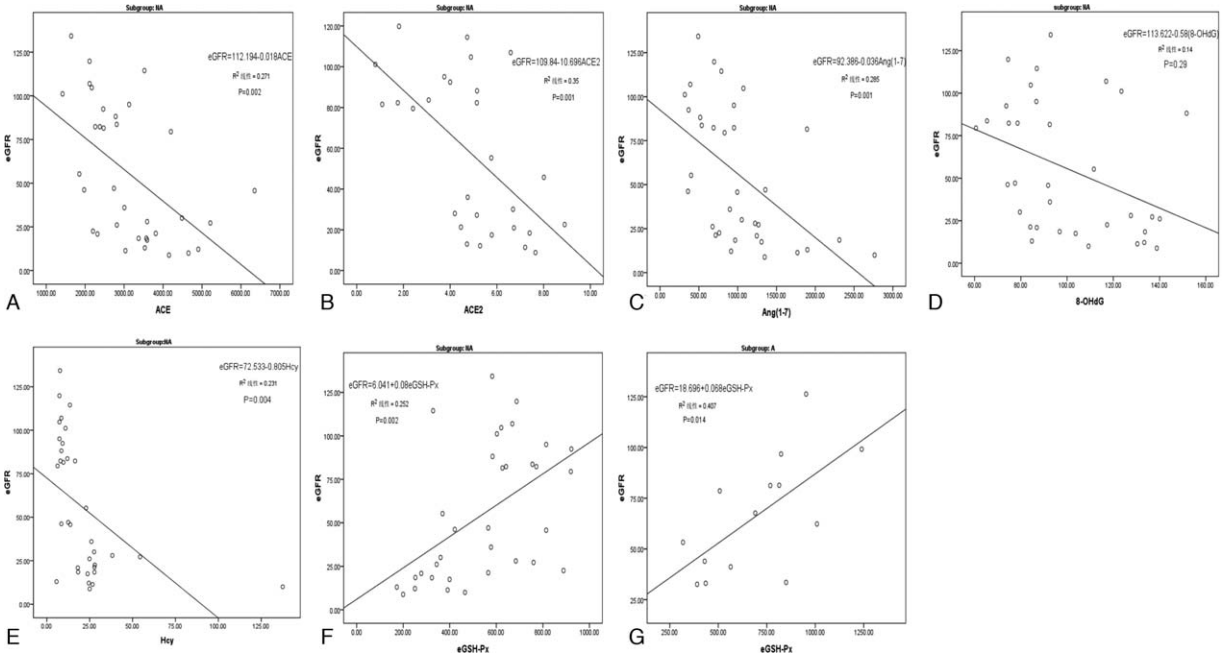

Figure 6. Single-Regression of eGFR with the respective independent variables in subgroups. eGFR was correlated with ACE(A), ACE2(B), Ang(1-7)(C), 8-OHdG (D),Hcy(E), eGSH-Px (F) in the NA subgroup and eGSH-Px (G) in the A subgroup.

Table 4

Multiple-regression analysis by the stepwise method for eGFR.

Variables	Beta	*P*	R^2	*N*	Subgroup	Beta	*P*	R^2	*N*
ACE	−0.010	.017	0.618	48	ACE-NA	−0.074	.567	0.719	34
					ACE-A	−0.328	.098	0.471	14
ACE2	−0.182	.131	0.618	41	ACE2-NA	−6.428	.006	0.719	28
					ACE2-A	−0.353	.130	0.471	13
Ang(1-7)	−0.159	.150	0.618	48	Ang(1-7)-NA	−0.033	.003	0.719	34
					Ang(1-7)-A	−0.055	.839	0.471	14
8-OHdG	−0.098	.417	0.618	48	8-OHdG-NA	−0.021	.875	0.719	34
					8-OHdG-A	−0.409	.072	0.471	14
Hcy	−1.451	.001	0.618	47	Hcy-NA	−1.527	.001	0.719	34
					Hcy-A	−0.002	.995	0.471	13
eGSH-Px	0.062	.000	0.618	48	eGSH-Px-NA	0.204	.095	0.719	34
					eGSH-Px-A	0.077	.014	0.471	14
Intercept	77.074	.000	0.618	-	Intercept -NA	148.154	.000	0.719	-
					Intercept -A	13.865	.000	0.471	-

R^2: coefficient of determination. By multiple-regression analysis, ACE, Hcy, eGSH-Px were found as potential predictors of eGFR. In the multiple linear regression after subgroup analysis, ACE2, Ang(1-7), Hcy were found as potential predictors of eGFR in the NA subgroup and only eGSH-Px in A subgroup.
8-OHdG = 8-hydroxy-2'-deoxyguanosine, ACE = angiotensin-converting enzyme, ACE2 = angiotensin-converting enzyme 2, Ang(1-7) = angiotensin(1-7).

correlated with middle molecular substance(Ang(1-7) and 8-OHdG) suggested that substances increased might be associated with renal function damage.

With the generalization of percutaneous renal biopsy, pathological diagnosis had a guiding role in the selection of clinical treatment options and judgment of prognosis. The nonspecific pathological and physiological mechanisms in different pathological types of kidney disease lead us to study the influence of these types on ACE2/Ang(1-7)/Mas axis and OS. Recent studies indicated decreased ACE2 expression in the kidneys of patients with diabetic nephropathy and IgAN.[39,40] Our results showed that serum ACE2, Ang(1-7) levels and eGSH-Px activity decreased in IgAN compared with those in FSGS, MN and MCD, but had no difference compared with those in the CT group. Ang(1-7) levels also decreased in MCD compared with those in MN and MPG. These changes suggested that different pathological types of kidney disease had various reflections on ACE2/Ang(1-7)/Mas axis and the specific mechanism need further research to explore. but insufficient number of participants so that without knowledge of GFR in each group. It should be noted that insufficient number of participants results in unequal GFR in each group, and actual result may be deviated

Deterioration of renal function causes changes in multiple regulatory systems in physiological and pathological mechanisms. Significant correlations of eGFR with ACE, ACE2, Ang(1-7), 8-OHdG, Hcy and eGSH-Px in patients with CKD were observed in our study. Multiple regression analysis showed that eGFR was predicted by ACE, Hcy and eGSH-Px. Further analysis in subgroups showed that eGFR was predicted by ACE2, Ang(1-7) and Hcy in NA subgroups and independently predicted by eGSH-Px in A subgroups. Results suggested that ACE2/Ang(1-7)/Mas axis greatly influenced eGFR and regulation of the axis, like ARB treatment, was a potential therapeutic target.

The present study has certain limitations. The number of recruited patients and non-dialysis patients with CKD stage 5 was relatively small so that the limited level of evidence obtained. Furthermore, we only detected these indicators in the serum rather than in the kidney tissue.

5. Conclusion

ACE2/Ang(1-7)/Mas axis is associated with OS in patients with CKD and the specific mechanism needs further study. The expression of the axis and OS are both associated with eGFR and increased in CKD progression. These data suggest that ACE2/Ang(1-7)/Mas axis is a potential therapeutic target in early CKD stages and it is of great significance to conduct a prospective clinical trial to establish the clinical significance in the correlation of eGFR and ACE2/Ang(1-7)/Mas axis.

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Author contributions

Zhang BB, Lu KD, and Shi CQ conceptualized the study and its objective. Zhang BB and Lu KD designed the study, extracted and analyzed the data statistically, and contributed in the interpretation of the results. Shi CQ wrote the manuscript. Xia H and Zhang PP revised the manuscript critically and contributed substantially to the content of the article. All the authors read and approved the final manuscript.
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