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Angiotensin-converting enzyme 2 and transmembrane protease serine 2 in female and male patients with end-stage kidney disease

Samsul Arefin[1] | **Leah Hernandez**[1] | **Liam J. Ward**[1,2] | **Angelina Schwarz**[1] | **GOING-FWD Collaborators** | **Peter Barany**[1] | **Peter Stenvinkel**[1] | **Karolina Kublickiene**[1]

[1]Division of Renal Medicine, Department of Clinical Science, Intervention & Technology, Karolinska Institutet, Stockholm, Sweden

[2]Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping, Sweden

Correspondence
Karolina Kublickiene, Division of Renal Medicine, Department of Clinical Science, Intervention & Technology, Karolinska Institutet, Karolinska University Hospital-Flemingsberg campus, 14186 Stockholm, Sweden.
Email: karolina.kublickiene@ki.se

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Abstract

Background: Individuals with chronic kidney disease are affected by acute respiratory syndrome coronavirus 2 (SARS-CoV-2) due to multiple comorbidities and altered immune system. The first step of the infection process is the binding of SARS-CoV-2 with angiotensin-converting enzyme 2 (ACE2) receptor, followed by its priming by transmembrane protease serine 2 (TMPRSS2). We hypothesized that circulating soluble ACE2 levels, as well as the expressions of ACE2 and TMPRSS2 in the microvasculature, are increased in patients with end-stage kidney disease (ESKD).

Methods: A total of 210 participants were enrolled, representing 80 ESKD patients and 73 non-CKD controls for soluble ACE2, and 31 ESKD and 26 non-CKD controls for vasculature and fat tissue bioassays. We have assessed ACE2 expression in blood using ELISA and in tissue using immunofluorescence.

Results: Soluble ACE2 levels were higher in ESKD patients compared to controls; however, there is no sex difference observed. In ESKD and controls, soluble ACE2 positively correlated with Interleukin 6 (IL-6) and C-reactive protein (CRP), respectively. Similarly, ACE2 tissue expression in the vasculature was higher in ESKD patients; moreover, this higher ACE2 expression was observed only in male ESKD patients. In addition, TMPRSS2 expression was observed in vessels from males and females but showed no sex difference. The expression of ACE2 receptor was higher in ESKD patients on ACE-inhibitor/angiotensin blocker treatment.

Conclusion: ESKD is associated with increased ACE2 levels in the circulation and pronounced in male vasculature; however, further studies are warranted to assess possible sex differences on specific treatment regime(s) for different comorbidities present in ESKD.

†See Acknowledgements for GOING-FWD Collaborators.

KEYWORDS
ACE2, ESKD, TMPRSS2

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) remains a worldwide threat to health. Patients with chronic kidney disease (CKD) have several comorbidities, including hypertension, diabetes mellitus (DM) and cardiovascular disease (CVD), that are established risk factors for poor outcomes in COVID-19. Wu et al. reported that patients on haemodialysis with COVID-19 are at a higher risk of death than hospitalized controls without kidney failure (14% vs. 4%, respectively).[1] Immunosuppressed patients with end-stage kidney disease (ESKD) that have undergone kidney transplantation are at high risk for poor outcomes following COVID-19.[2] Moreover, meta-analysis data have shown that men are associated with increased risk for COVID-19.[3]

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) enters cells via binding to angiotensin-converting enzyme 2 (ACE2), followed by its priming by transmembrane protease serine 2 (TMPRSS2).[4] ACE2, a homolog of ACE, has an important role in regulating blood pressure, fluid volume and sodium retention.[5] There are two forms of ACE2, the full-length ACE2 transmembrane protein with an ectodomain, and a soluble form of ACE2 found in peripheral blood.[6] The membrane-bound ACE2 undergoes proteolysis facilitated by the protease "a disintegrin and metalloproteinase-17" (ADAM-17), also known as tumour necrosis factor-converting enzyme (TACE).[7] Although the physiological role of ACE2 in most tissues has not been exemplified, it is thought to be a crucial regulator of cardiac function.[8] ACE2 expression has been found in the heart, kidney, lung, testis, epithelial cells, arterial and venous endothelial cells, arterial smooth muscle cells,[9] small intestine, thyroid, adipose tissue, brain, bone marrow and spleen.[10] ACE2 is known to facilitate the conversion of angiotensin II (Ang II) into angiotensin 1-7 (Ang 1-7), and Ang 1 to Ang 1-9 thus offsetting the effects of ACE-Ang II- type 1 (AT1) receptor axis.[7] The opposing action of ACE2 against Ang II results in vasodilatation, decreased inflammation and fibrosis and induces natriuresis.[11] Aside from its role in the regulation of the renin-angiotensin system (RAS), the ACE2 protein serves as the membrane receptor for the spike protein of the SARS-COV-2 virus.[12] Thus, ACE2 receptors expressed in tissues constitute an important entry mechanism for SARS-COV2 in its subsequent infection of the cells in the human host. Although current recommendations support the continuation of angiotensin-converting enzyme inhibitors (ACEi)/angiotensin receptor blockers (ARBs) during SARS-CoV-2 infection,[13] it is not yet established if patients on ACE-i/ARBs are more susceptible to COVID-19 and have worse outcomes via upregulation of ACE2.[14]

Resistance arteries are vessels with lumen diameters measuring <400 μm that constitute the major site of vascular resistance.[15] Resistance vessels were selected for this study as they are involved in the pathophysiology of hypertension in both animal and human studies.[16] The vascular networks of hypertensive patients are manifested by both decreased lumen size and increased vessel stiffness that results in increased peripheral resistance.[17] The ability of vessels to adapt to changes in blood pressure depends on vascular distensibility and compliance.[16] Current guidelines recommend that hypertension in CKD should be managed by antihypertensive medications that includes ACEi and ARBs,[18] which block Ang II and formation of AT1R mediated actions and shift the RAS system balance, and increases the levels of Ang 1-7.[19] Thus, ACE2 plays an important role in the bidirectional nature of CKD and hypertension. Reports of COVID-19 show worse outcome for males; therefore, it is important to determine if there is sex bias in the expression of ACE2 among patients with ESKD.[20]

We hypothesized that patients with ESKD have increased circulating soluble ACE2 levels as well as increased expressions of ACE2 and TMPRSS2 in the microvasculature compared to controls and that sex differences exist. Therefore, we aimed to investigate whether there are differences in the circulating soluble ACE2 levels alongside expression of ACE2 and TMPRSS2 receptors in the resistance artery and subcutaneous adipose tissue in ESKD patients versus non-CKD controls. The levels of soluble ACE2 mainly reflect constitutive shedding, on the other hand, the vascular expression of ACE2 refers to the question of expression levels influence the risk of secondary viral establishment in the vasculature and other organs.

2 | METHODS

2.1 | Study participants

The investigation was undertaken with the approval of the Ethical Committee at Karolinska University Hospital, Huddinge, with the informed consent of each patient and in accordance with the principles outlined in the Declaration of Helsinki. Two sets of population groups

were included in this study. For soluble ACE2 investigation, a subset of prevalent haemodialysis patients ($n = 80$), with available serum samples, were taken from the MIMICK-1 (Mapping of Inflammation Markers in Chronic Kidney Disease) cohort[21] and available non-CKD control serum samples ($n = 73$) were taken from the PRIMA-control cohort. The control cohort was recruited from an age- and sex-matched population, respective to the entire MIMICK-1 cohort, randomly selected from the Stockholm Region, Sweden, by the Statistics Bureau of Sweden—a government agency. No other exclusion criteria other than unwillingness to participate in the study were applied to the selection of the control participants.

For the receptor expression investigations, ESKD patients undergoing living-donor kidney transplantation (including patients in haemodialysis or peritoneal dialysis) with an eGFR < 15 ml/min and the non-CKD controls (subcutaneous fat biopsies from kidney donors or subjects undergoing planned hernia and gallbladder operation or bariatric surgery) with a median eGFR > 100 ml/min/1.73 m^2 were included. We did not include any mild/moderate CKD patients in the control groups. For the baseline characteristics, the concentrations of serum creatinine, albumin, calcium, phosphate, 25(OH)-vitamin D, triglycerides, cholesterol, high-density lipoprotein (HDL) cholesterol, high-sensitivity C-reactive protein (hsCRP), glucose, glycated haemoglobin A1c (HbA1c) were measured at the Department of Laboratory Medicine, Karolinska University Hospital, Huddinge, Sweden. Plasma interleukin IL-6, IL-10, and tumour necrosis factor (TNF) were analysed by commercial kits available for an Immulite automatic analyser (Siemens Medical Solutions, CA, USA) and run in accordance with the manufacturer instructions. Vascular cell adhesion protein 1 (VCAM-1) was analysed using the enzyme-linked immunosorbent assay (ELISA) technique.

2.2 | Soluble ACE2 assay

Serum samples stored at −80°C were obtained from Karolinska Institutet, CLINTEC, Division of Renal Medicine biobank. Soluble ACE2 protein concentration was detected using a commercially available ELISA kit (Lot# L200526530, Cloud Clone Corp., USA) and run according to the manufacturer's instructions. Samples were run in duplicates with an intra-assay coefficient of variation of 9%.

2.3 | Immunohistochemistry

Resistance arteries, with a size of about <400 μm, were isolated from subcutaneous fat. Freshly isolated artery segments and adipose tissue were frozen on dry ice and preserved at −80°C. Before the experiments, transverse 10 μm cryosections were cut using a Cryostat HM 500 OM (MICROM International GmbH, Germany). Sections were mounted on glass slides (Thermo Fisher Scientific, Germany) and stored at −20°C until staining. The experiments were performed in two-day cycles. Before immunohistochemical staining, the sections were thawed and post-fixed with 4% formaldehyde. Upon staining, all tissue sections were first blocked with 10% goat serum (Sigma-Aldrich, USA) for 30 min. The diluted primary antibody ACE2 (1:200; Nordic BioSite, cat no: CSB-PA866317LA01HU-50, Sweden), TMPRSS2 (1:400; cat no HPA035787, Sigma Aldrich) or CD31 (1:100, cat no: 553370, BD Biosciences) with PBS and 5% goat serum was added on the slide and incubated overnight in 4°C. For the negative controls, the primary antibody was replaced with 5% normal goat serum. The secondary Alexa Fluor 594 goat anti-rabbit antibody (1:600; Thermo Fisher Scientific) was added for 1 h, in the dark at room temperature. The slides were then dipped with DAPI (1:50,000) for 1 min. Finally, coverslips were added to slides using Aqua-Mount mounting media (Thermo Fisher Scientific) and were examined and photographed under fluorescence microscopy (Zeiss, Japan) with x20 and x40 objectives. For the validation of the antibodies, positive control tissues (human placenta and kidney) were stained (The human protein Atlas). The placenta and the kidney were obtained from non-CKD participants. To exclude antigen-independent staining, negative control for which the primary antibody was omitted was examined. In addition, antibody concentrations were optimized before investigations to determine the optimal concentration for final staining experiments with tissues used in the manuscript. The quantification of IHC staining was performed digitally using ImageJ software (National Institute of Health, USA) where the area of positive staining measured was expressed as a percentage of total tissue area. The analysis of the IHC images was performed blinded.

2.4 | Statistical analysis

Shapiro-Wilk normality tests were performed for all data variables included in this study. Continuous data are expressed as either median (interquartile range) or mean (standard deviation) dependent on data distribution, either not normal or normal distribution, respectively. Statistical analyses were selected in accordance with the data distribution. Categorical data are expressed as the frequency with percentage. Comparisons between clinical and biochemical markers, as well as *in vivo* and staining studies, comparing ESKD to control participants were

assessed using non-parametric Mann-Whitney U test or parametric Student's *t* test. Categorical data were compared using chi-squared test. Sex-divided statistics were also performed, and when significant presented, otherwise displayed as a group. Correlation analyses were performed using non-parametric Spearman's rank correlation method for continuous variables. Multivariable linear regression analyses were performed to assess the strength of the relationship between the dependent variable and several predictor variables. For not-normally distributed variables, data were log-transformed (log10) prior inclusion to the models. Statistical significance was set at $p < 0.05$. Statistical analyses were carried out using SPSS (v.26.0, IBM, USA) and GraphPad Prism 6.0 (GraphPad Software Inc., CA, USA). Reporting of the study conforms to broad EQUATOR guidelines.[22]

3 | RESULTS

3.1 | Study population for soluble ACE2

A total of 80 prevalent haemodialysis patients with ESKD and 73 non-CKD control participants were included for the measurement of soluble ACE2 concentrations. Baseline characteristics of the study populations for soluble ACE2 are shown in Table 1. As expected, ESKD patients more often had CVD and diabetes mellitus (DM) and were more often on medications for hypertension and lipid control. As controls were selected randomly from the general population without CKD, only a small number of controls also had CVD and DM. The biochemical parameters showed a typical profile of ESKD patients with significantly higher levels of triglycerides, S-creatinine, VCAM-1, IL-6, fibrinogen, and hsCRP ($p < 0.0001$), while BMI ($p < 0.028$) and albumin ($p < 0.001$) were significantly lower in ESKD compared to controls. Soluble ACE2 was significantly ($p < 0.0002$) higher in ESKD (3.8 ng/ml, IQR 2.4–5.5 ng/ml) compared to controls (2.7 ng/ml, IQR 2.1–3.7 ng/ml) (Figure 1A). There were no significant differences in the sex-divided analysis of soluble ACE2 concentration in ESKD and controls (Figure 1B). We identified that soluble ACE2 related to the inflammatory response, reflected by correlations with hsCRP, IL-6 and TNF. In ESKD, soluble ACE2 was positively correlated with IL-6 (rho 0.257, $p = 0.021$), whilst negatively correlated with cholesterol (*rho* −0.248, $p = 0.029$), while in controls soluble ACE2 showed positive correlation with hsCRP (*rho* −0.254, $p = 0.03$) (Table 2). We have adjusted the soluble ACE2 levels in the ESKD group with age, sex, BMI, CVD, DM, and ACEi/ARB treatment in three different models and all three models showed a stronger association to IL-6 ($p = 0.003$) and cholesterol ($p = 0.003$) (Table 3). In addition, in model 3 when we have adjusted for age, sex, BMI, CVD, DM, and ACEi/ARB treatment soluble ACE2 showed a positive association with TNF ($p = 0.05$), which was not significant in the earlier two models (Table 3) or when performed univariate correlation analysis (Table 2). In the non-CKD control group, after adjusting the age, sex, BMI, CVD, DM, and ACEi/ARB treatment, soluble ACE2 showed a positive association with IL-6 ($p = 0.005$; Table 4). Nevertheless, the association with CRP was lost that showed a positive correlation in the earlier univariate correlation analysis (Table 2).

Soluble ACE2 levels were not significantly different in ESKD patients for those with and without CVD, DM, comorbidities (CVD + DM), or those in the ACEi/ARB treated and non-treated group (Figure 2). Moreover, sex-divided analysis of soluble ACE2 levels in ESKD patients treated and non-treated with ACEi/ARB, with and without CVD, DM and combined CVD + DM comorbidities showed no significant differences (Figure S1).

3.2 | Study population for immunohistochemistry

A total of 57 participants were enrolled for the staining study, representing 31 ESKD and 26 non-CKD controls. However, the arteries (ESKD, $n = 23$, and controls, $n = 15$) and adipose tissue (ESKD, $n = 11$, and controls, $n = 12$) samples used for staining were not automatically obtained from the same participants. The samples were obtained depending on the available tissues received from the surgery. Age, demographic, biochemical, and clinical characteristics for all participants are shown in Table 5.

3.3 | ACE2 and TMPRSS2 localization

Immunofluorescence was used to determine the presence of ACE2 and TMPRSS2 in isolated subcutaneous resistance arteries from ESKD patients and controls. We have confirmed the expression of ACE2 on the human placenta and kidney and TMPRSS2 on the placenta as a positive control (Figure S2). The positive signal in the kidney was prominent in the glomeruli of the cortex region. To exclude possible auto-fluorescence or antigen-independent staining negative controls were examined and there was no expression on negative controls observed (Figure S2). The expression of ACE2 was observed on both endothelium and vascular smooth muscle cells (VSMC) in resistance arteries from ESKD patients and controls (Figure 3C). The expression of ACE2 was higher in ESKD patients compared with controls (Figure 3B). Sex-divided

TABLE 1 Clinical characteristics of patients with ESKD and controls for soluble ACE2

Clinical parameters	Control ($n = 73$)	ESKD ($n = 80$)	p value
Age, years	61 (55–70)	66 (51–76)	0.360
Males, *n* (%)	49 (67%)	43 (54%)	0.092
Females, *n* (%)	24 (33%)	37 (46%)	0.092
Systolic BP, mmHg	138 (127–154)	[a]	
Diastolic BP, mmHg	84 (77–94)	[a]	
DM, *n* (%)	4 (5%)	20 (25%)	<0.001
CVD, *n* (%)	7 (9%)	50 (63%)	<0.001
ACEi/ARB, *n* (%)	10 (14%)	17 (21%)	0.221
Beta blockers, *n* (%)	14 (19%)	31 (39%)	0.008
Statins, *n* (%)	10 (14%)	18 (23%)	0.160
BMI, kg/m^2	26.16 ± 4.7, $n = 72$	24.31 ± 5.3, $n = 79$	0.026
Cholesterol, mg/dl	5.1 (4.6–5.7), $n = 64$	4.4 (3.7–5.2), $n = 78$	<0.001
Triglycerides, mg/dl	1.1 (0.75–1.8), $n = 64$	1.7 (1.1–2.2), $n = 79$	0.003
Glucose (mg/dl)	5.2 (4.9–5.5), $n = 62$	—	
HbA1c (%)	4.7 (4–5.5.0), $n = 63$	—	
Serum creatinine, mmol/L	80.1 ± 15.7; $n = 65$	764.2 ± 210	<0.001
eGFR, ml/min/1.73m^2	81.1 (74.7–97.7)	5.4 (4.7–8.5)	<0.001
Albumin, g/L	39.1 ± 2.5; $n = 65$	35.0 ± 3.7	<0.001
IL-6, pg/ml	1.9 (1.0–3.5), $n = 26$	7.3 (4.1–14.0)	<0.001
VCAM-1, ng/ml	689 (572–822), $n = 57$	1832 (1419–2221), $n = 79$	<0.001
IL-10, ng/ml	—	1.2 (0.9–1.8)	
BNP, ng/L	—	10.1 (3.4–28.2), $n = 77$	
TNF, pg/ml	—	13.1 (10.7–15.9)	
Fibrinogen, g/L	2.9 ± 0.56, $n = 63$	3.9 ± 0.9, $n = 64$	<0.001
hsCRP, mg/L	1.3 (0.6–2.9), $n = 63$	6.3 (2.4–17.5), $n = 77$	<0.001

Note: Data are expressed as mean ± standard deviation for normally distributed variables and median ± quartile range (Q1–Q3) for not normally distributed variables. Statistical comparisons are performed by parametric *t* test for normally distributed variables and non-parametric Mann-Whitney U test for variables not-normally distributed. Nominal data expressed as frequency (%) and statistical comparison by chi-squared test.

Abbreviations: BP, blood pressure; DM, diabetes mellitus; CVD, cardiovascular disease; ACEi/ARB, angiotensin-converting enzyme inhibitor/angiotensin-receptor blocker; BMI, body mass index; HBA1c, glycated haemoglobin; eGFR, estimated glomerular filtration rate; IL, interleukin; VCAM-1, vascular cell adhesion molecule-1; BNP, brain natriuretic peptide; TNF, tumour necrosis factor; hsCRP, high-sensitivity C-reactive protein; ESKD, end-stage kidney disease.

[a]About BP data in ESKD patients: a single BP-measurement in haemodialysis patients provide no/little reliable information. BP varies considerable during a haemodialysis session and depends on the session and the day BP was measured. To measure BP accurately in haemodialysis patients someone needed to perform 24 h ambulatory blood pressure; a possibility that was not feasible for this cohort of patients included in the manuscript.[55]

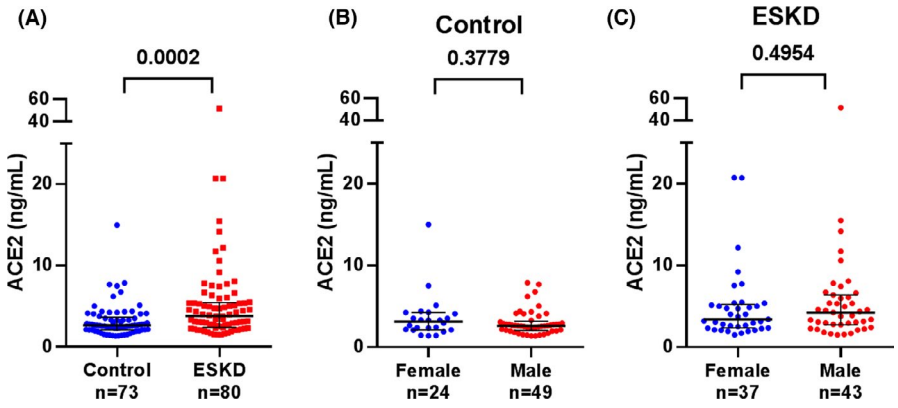

FIGURE 1 Serum concentration of soluble angiotensin-converting enzyme 2 (ACE2) in (A) controls versus patients with end-stage kidney disease (ESKD), (B, C) sex divided differences into controls and ESKD subjects. Results are expressed as the median and interquartile range (IQR). Significance $p < 0.05$

TABLE 2 Correlation of soluble ACE2 with clinical and other biochemical parameters in controls and ESKD patients

	Control ($n = 73$)		ESKD ($n = 80$)	
Variable	***rho***	***p* value**	***rho***	***p* value**
Age	0.010	0.933	0.076	0.502
BMI	0.028	0.812	0.032, $n = 79$	0.777
Albumin	−0.160, $n = 63$	0.211	0.126	0.264
hsCRP	0.254	0.030	0.121, $n = 77$	0.296
Ferritin	—	—	0.037, $n = 68$	0.763
Fibrinogen	0.067, $n = 63$	0.601	0.163, $n = 64$	0.197
IL-10	—	—	−0.058	0.612
VCAM-1	0.047, $n = 57$	0.728	−0.198, $n = 79$	0.080
IL-6	0.309, $n = 26$	0.125	0.257	0.021
TNF	—	—	0.204	0.070
s-Ca	−0.195, $n = 62$	0.129	−0.103, $n = 79$	0.364
s-PO_4	0.056, $n = 62$	0.668	0.036, $n = 79$	0.752
Cholesterol	−0.118, $n = 62$	0.360	−0.248, $n = 78$	0.029
Creatinine	−0.117, $n = 63$	0.363	0.201	0.074
Haemoglobin	0.007, $n = 63$	0.955	−0.174	0.122

Note: Statistical analyses were performed by Spearman correlation (rho) analysis.

Abbreviations: BMI, body mass index; ESKD, end-stage kidney disease; hsCRP, high-sensitivity C-reactive protein; IL, interleukin; s-Ca, serum calcium; s-PO4, serum phosphate; TNF, tumour necrosis factor.

statistics show that this significance remains in males only (Figure 4B). In the non-CKD controls, females had higher expression of ACE2 compared with males (Figure 4B). Patients with ESKD who were treated with ACEi/ARBs showed higher expression of ACE2 compared with those not on such treatment (Figure 5B). Subcutaneous adipose tissue staining shows positive ACE2 staining on both ESKD and control groups, however, there was no statistical difference in the expression among the groups (Figure S3). We have investigated the expression of TMPRSS2 on the artery and adipose tissue from ESKD patients and controls. Although, both artery and adipose tissue expressed TMPRSS2 we observed no differences in the expression (Figure 6). We did not find any sex difference for the expression of TMPRSS2 (data not shown). In addition, TMPRSS2 was expressed both on endothelium and VSMC in the resistance artery (Figure S4).

4 | DISCUSSION

Patients with ESKD are expected to be at higher risk of severe COVID-19 since they are more susceptible to infectious complications and have a higher prevalence of CVD due to early vascular ageing than the general population.[23] It is also of importance to understand if sex-specific prerequisites exist towards susceptibility to infection by the SARS-CoV-2 virus. ACE2 is the functional receptor for SARS-CoV-2, and in this study, we report on the detection of ACE2 in the circulation, as well as in resistance

TABLE 3 Soluble ACE2 multivariable linear regression analysis in ESKD participants

	Estimate	Standard error	*p*-value
Model 1			
IL-6	0.355	0.082	**0.003**
CRP	0.174	0.67	0.149
TNF	0.201	0.273	0.09
Cholesterol	−0.344	0.325	**0.004**
Model 2			
IL-6	0.438	0.084	**0.0001**
CRP	0.213	0.068	0.085
TNF	0.204	0.272	0.08
Cholesterol	−0.341	0.332	**0.005**
Model 3			
IL-6	0.385	0.001	**0.001**
CRP	0.217	0.068	0.079
TNF	0.227	0.273	**0.05**
Cholesterol	−0.337	0.332	**0.006**

Note: Bold signifies statistical significance $p < 0.05$

Model 1: Adjusted for age, sex and BMI.

Model 2: Model 1+ comorbidities (DM + CVD).

Model 3: Model 1+ comorbidities + ACEi/ARB treatment.

Abbreviations: hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; TNF, tumour necrosis factor.

TABLE 4 Soluble ACE2 multivariable linear regression analysis in non-CKD control participants

	Estimate	Standard error	*p*-value
Model 1			
IL-6	0.710	0.140	**0.011**
CRP	0.225	0.055	0.087
Model 2			
IL-6	0.897	0.172	**0.010**
CRP	0.217	0.057	0.107
Model 3			
IL-6	1.03	0.178	**0.005**
CRP	0.231	0.61	0.109

Note: Bold signifies statistical significance $p < 0.05$.

Model 1: Adjusted for age, sex and BMI.

Model 2: Model 1+ comorbidities (DM + CVD).

Model 3: Model 1+ comorbidities + ACEi/ARB treatment.

Abbreviations: hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6.

artery and adipose tissues of ESKD patients and non-CKD controls. Indeed, ACE2 levels were significantly increased both in the circulation and resistance artery tissues of ESKD patients compared to controls. However, no sex differences were observed in soluble ACE2 levels in ESKD, but the expression of ACE2 was higher in resistance artery from male patients with ESKD. In ESKD and controls soluble ACE2 has shown a positive association with inflammatory marker IL-6 and hsCRP, respectively. The ACE2 and TMPRSS2 receptors were present both in the endothelium and vascular smooth muscle cells from resistance arteries. There was no difference in the expression levels of TMPRSS2 in isolated arteries and fat tissue between ESKD patients and controls.

Our observation of elevated soluble ACE2 in the peripheral circulation of ESKD patients concurs with another study that reported higher levels of soluble ACE2 in serum of patients with advanced CKD stage 3/5 patients compared to controls.[24] Changes in soluble ACE2 levels have been implicated in the pathophysiology of CVD, with higher levels being associated with myocardial infarction, stroke, heart failure, and diabetes in a large multinational population study.[25] Soluble ACE2, shed from epithelial cells by ADAM-17, retains catalytic activity.[26] Studies have shown that a higher soluble ACE2 enzymatic activity is associated with a higher risk for atherosclerosis in CKD stage 3/5 patients.[27] Concurrently, elevated soluble ACE2 observed in ESKD patients may indicate elevated ADAM-17 levels and/or activity, as previously suggested in diabetic patients,[28] with ADAM-17 activity correlating with worsening renal function.[29] As ablation of ADAM-17 expression reduces ACE2 shedding[30] it may confer a protective mechanism.[30] However, as the role of soluble ACE2 remains unclear, and the shedding of ACE2 may mechanistically regulate transmembrane ACE2 activity,[4] further studies need to explore the relation between transmembrane ACE2 activity, ADAM-17 and soluble ACE2 levels.

In adults, high soluble ACE2 levels associate with classical Framingham risk factors such as BMI, diabetes, hypertension, LDL cholesterol, and smoking.[25] In kidney transplant patients soluble ACE2 correlates with age, liver function and glycosylated haemoglobin.[31] We report that soluble ACE2 levels were positively correlated with IL-6 and negatively with cholesterol levels. Multivariable linear regression revealed pronounced independent interplay of IL-6, TNF and cholesterol with soluble ACE2 adjusted for age, sex, BMI, CVD, DM, and ACEi/ARB treatment. As ESKD is characterized by an inflammatory phenotype, increased levels of soluble ACE2 may suggest participation in resolving the inflammatory response.[32] In the context of COVID-19, our findings of higher soluble ACE2 correlating with inflammation suggest that ESKD patients are more susceptible to an exacerbated inflammatory response with cytokine storm and worse outcomes. IL-6 is an inflammatory

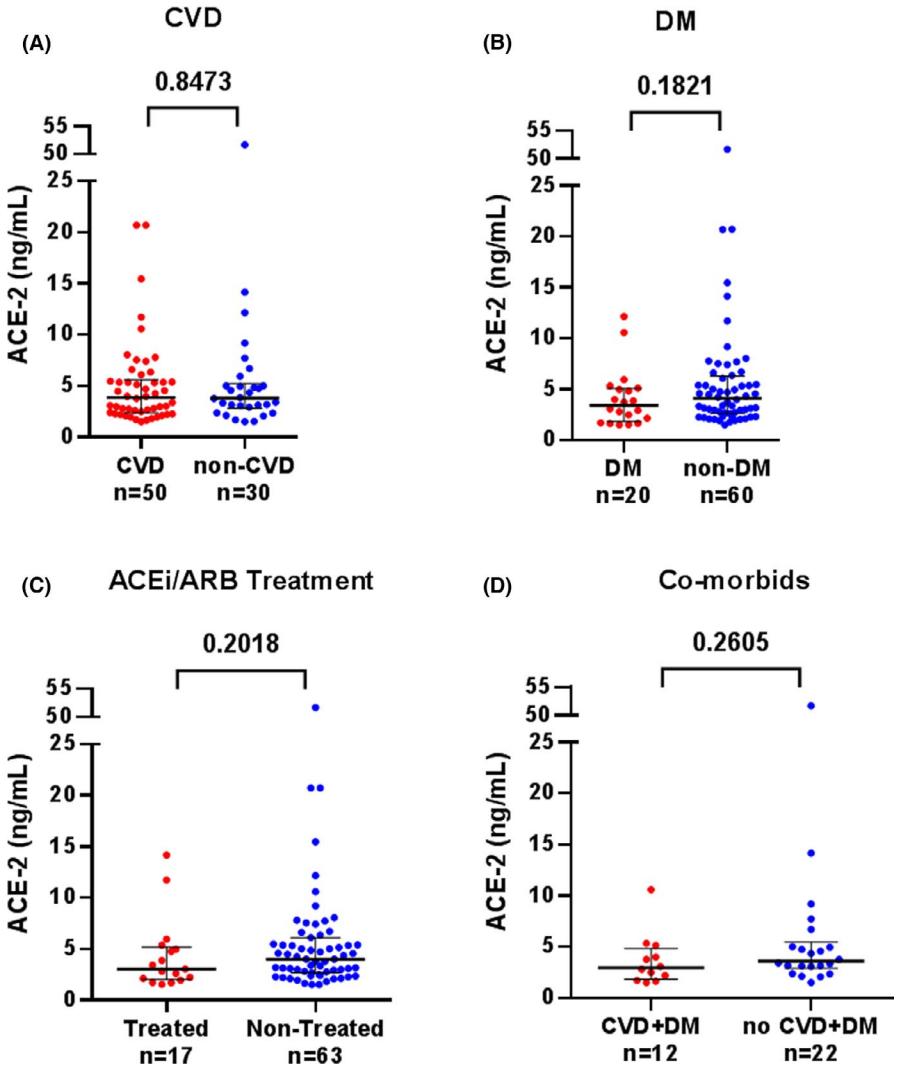

FIGURE 2 Soluble ACE2 levels in patients with end-stage kidney disease (ESKD) according to treatment and presence of comorbidities cardiovascular disease (CVD), diabetes mellitus (DM) and combined CVD + DM. Results are expressed as the median and interquartile range (IQR). Significance $p < 0.05$

regulator of the senescence-associated secretory phenotype. As ESKD can be seen as a clinical model of senescence and early vascular ageing, increased IL-6 and soluble ACE2 levels may reflect senescence status.[33] The negative correlation between soluble ACE2 and cholesterol levels observed may be a reflection of the "reverse epidemiology phenomenon"[34] and independent of age, sex, BMI, CVD, DM, and ACEi/ARB treatment.

We find no differences in the levels of soluble ACE2 between males and females in either ESKD patients or controls. In contrast, previous studies have reported that males have elevated soluble ACE2 levels in both the general population[35] and in heart failure.[36] The menopausal status of the women in the current study may be an explanation. As postmenopausal women have higher soluble ACE2 levels than premenopausal women,[35] hormonal dysregulation, as well as reduced power, may explain the lack of sex differences in soluble ACE2.

Our finding of ACE2 expression on both the endothelium and VSMC in resistance artery from both ESKD patients and controls concur with previous studies.[37,38] The ESC Working Group for Atherosclerosis and Vascular Biology and the ESC Council of Basic Cardiovascular emphasized the link between ACE2, cardiovascular physiology, and susceptibility to COVID-19 infection.[39] On the other hand, whether endothelial cells and VSMCs can be infected by SARS-CoV-2 and/or can support substantial viral replication is controversial as different researchers addressed different findings and opinions. A lack of evidence for replicative infection by SARS-CoV-2 in human endothelial cells through ACE2 was recently reported.[40] Nevertheless, the higher ACE2 expression in resistance arteries from patients with ESKD may be indicative of a role in vascular calcification.[41] The observation of elevated vascular ACE2 in ESKD patients indicates that they are at risk of SARS-CoV-2 infection and/or greater severity of COVID-19. In contrast to the findings in arteries, we find no differences in adipose ACE2 expression between ESKD patients and controls. Although a recent study highlights an association between high visceral adiposity and severity of COVID-19, it can be postulated that adipose ACE2 may trigger the cytokine storm of COVID-19.[42]

TABLE 5 Clinical characteristics of patients with ESKD and controls measured for immunohistochemistry

Clinical parameters	Control ($n = 26$)	ESKD ($n = 31$)	p value
Age (years)	48.6 ± 10.6	50.0 ± 16	0.684
Males, *n* (%)	9 (34%)	20 (64%)	**<0.001**
Females, *n* (%)	17 (66%)	11 (36%)	**<0.001**
Systolic BP, mmHg	140.1 ± 15.2	143.6 ± 20.4	0.661
Diastolic BP, mmHg	81.2 ± 10.4	88.1 ± 12.9	0.128
ACEi/ARB, *n* (%)	2 (8%)	17 (54%)	**<0.001**
Beta-blockers, *n* (%)	2 (8%)	19 (61%)	**<0.001**
Statins, *n* (%)	1 (4%)	12 (38%)	**<0.001**
BMI, kg/m^2	25 (23–28), $n = 18$	23.7 (21.4–26.5), $n = 29$	0.279
Cholesterol, mg/dl	5.1 ± 1.0, $n = 20$	4.3 ± 1.1, $n = 30$	**0.014**
HDL, mmol/L	1.8 ± 0.55, $n = 17$	1.52 ± 0.61, $n = 30$	0.162
Triglycerides, mg/dl	0.9 ± 0.45, $n = 19$	1.4 ± 0.58, $n = 30$	**0.007**
HbA1c, %	35.2 ± 1.8, $n = 9$	37.8 ± 10.4, $n = 31$	0.891
Serum creatinine, mmol/L	75 (67–81), $n = 19$	666 (542–794), $n = 30$	**<0.001**
eGFR, ml/min/1.73 m^2	100 (85–108)	6 (5–8)	**<0.001**
hsCRP, mg/L	0.47 (0.33–2.9), $n = 20$	0.83 (0.21–2.5), $n = 30$	0.897
Albumin, g/L	38.1 ± 2.6, $n = 20$	33.7 ± 3.8, $n = 30$	**<0.001**
Calcium, mmol/L	2.28 ± 0.07, $n = 20$	2.26 ± 0.18, $n = 31$	0.879
Phosphate, mmol/L	1.0 ± 0.25, $n = 20$	1.8 ± 0.53, $n = 31$	**<0.001**
25-OH D-vitamin, nmol/L	54.1 ± 20.2, $n = 11$	54.6 ± 25.0, $n = 29$	0.952

Note: Data are expressed as mean ± standard deviation for normally distributed variables and median ± quartile range (Q1–Q3) for not normally distributed variables. Statistical comparisons are performed by parametric *t* test for normally distributed variables and non-parametric Mann-Whitney U test for variables not-normally distributed. Nominal data expressed as frequency (%) and statistical comparison by chi-squared test.

Abbreviations: ACEi/ARB, angiotensin converting enzyme inhibitor/angiotensin-receptor blocker; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein.

Although no sex differences were found in soluble ACE2 levels, we report that female controls had a significantly higher arterial expression of ACE2 than male controls. Whereas male patients with ESKD had a significantly higher arterial expression of ACE2 compared to control males, no difference was observed between female ESKD patients and controls. The observation that females have a higher basal ACE2 expression than males in the general population may be explained the stimulatory effects of oestrogens on the expression of ACE2.[43] In addition, the *ACE2* gene is overexpressed in females, as the gene is located on the X chromosome in sites commonly escaping X chromosome inactivation.[44] Thus, since females may have twice the capacity to produce two types of ACE2, the SARS-CoV-2 virus have to unlock two forms of ACE2 while males only have a single form to unlock.[45] As estrogen has been described to be protective during SARS by activation of the immune response and suppressing SARS-CoV replication,[46] there may be similar interactions with COVID-19/SARS-CoV-2. These results may have relevance for COVID-19 pathology: a higher ACE2 tissue expression in healthy females vs males (which is in line with animal experiments) and, as ACE2 is more 'inducible' in males, a stronger up-regulation may occur under pathological circumstances.[47] These findings highlight the need for investigations into the role of sex hormones in the regulation of ACE2 and protection during COVID-19.

The expression of TMPRSS2 in resistance arteries and adipose tissue did not differ between ESKD patients and controls. The expression of TMPRSS2 expression in microvascular endothelial cells has been considered so low that it is hard to detect except during active angiogenic/tubulogenic responses.[48] However, we find detectable levels of TMPRSS2 expression in the endothelium of resistance

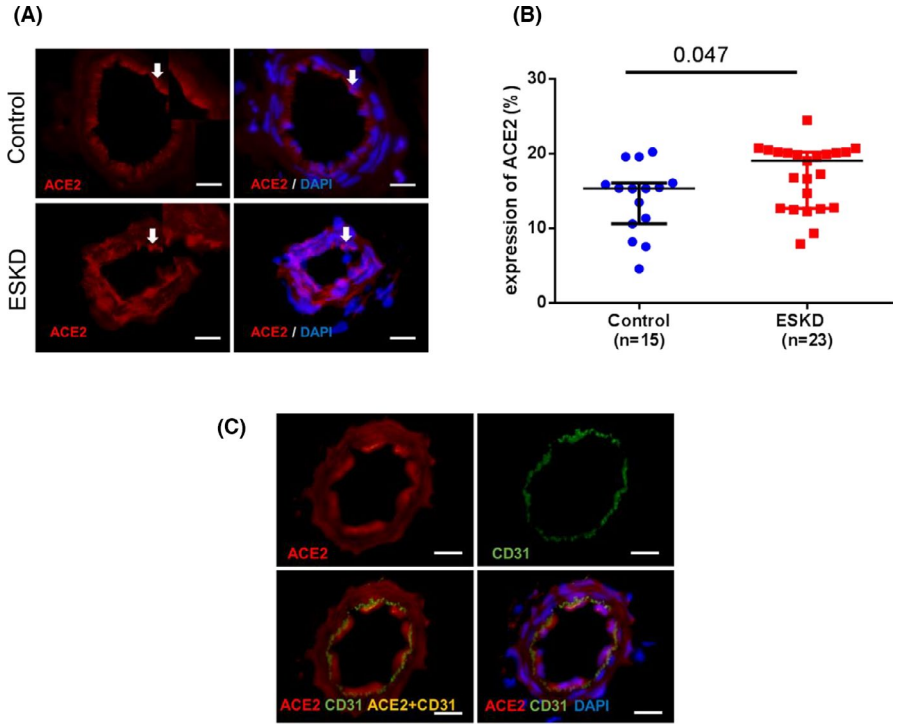

FIGURE 3 Angiotensin-converting enzyme 2 (ACE2) expression in resistance artery sections from patients with end-stage kidney disease (ESKD) and controls. (A) Representative immunofluorescence staining of ACE2, (B) Statistical analysis of ACE2 expression between controls ($n = 15$) and ESKD ($n = 23$), (C) Representative immunofluorescence staining of ACE2 expression and CD31 endothelial cell marker expression in resistance artery from patients with ESKD. ACE2 (red), CD31 (green) and nuclear DAPI staining (blue). Co-localization of ACE2 and CD31 expression is seen in yellow. Bar = 100 μm. Significance $p < 0.05$; ESKD, end-stage kidney disease

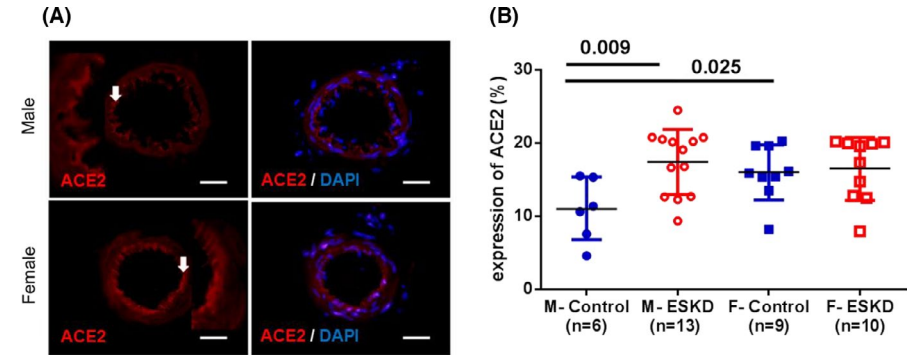

FIGURE 4 Sex-divided analysis of angiotensin-converting enzyme 2 (ACE2) expression in resistance artery sections from patients with end-stage kidney disease (ESKD) and controls. (A) Representative immunofluorescence staining of ACE2 (red) in male and female controls, Bar = 100μm, (B) Sex divided statistical analysis of ACE2 expression in male vs female controls ($n = 6/9$, respectively), and male versus female ESKD patients ($n = 13/10$, respectively). M, male; F, female; Significance $p < 0.05$; ESKD, end-stage kidney disease

arteries both in controls and ESKD patients. Nevertheless, TMPRSS2 expression alone is not predictive of its functional TMPRSS2, which is regulated by nitrosylation.[49] Therefore, it is conceivable that nitric oxide (NO) synthase activity and the subsequent production of NO may influence viral infection of endothelium by altering the activity of TMPRSS2.

In this study, we find no differences in soluble ACE2 levels between ESKD patients treated or not treated with ACEi/ARBs. Our findings are in accordance with the observation that levels of soluble ACE2 are not influenced by ACEi/ARBs.[36] In contrast to soluble levels, resistance arteries showed higher ACE2 expression in the ACEi/ARBs treated group compared to the non-treated group. Increased ACE2 expressions in multiple tissues have been observed in patients on ACEi/ARB.[50] In the case of COVID-19 infection, ACEi/ARBs seem to be a double-edged sword as upregulation of ACE2 can increase the risk for SARS-CoV-2 infection whilst at the same time they reduce the pro-inflammatory, vasoconstrictor and pro-fibrotic effects of Ang II.[51] To this date, guidelines have recommended that withdrawal of ACEi/ARBs is not recommended in high-risk individuals.[52] It is important to note that ESKD patients and their associated co-morbidities are usually under a multidrug regimen. Aside from ACEi/ARBs, other clinically approved drugs given to ESKD patients such as statins, GLP-1 agonists, thiazide diuretics, beta-blockers, and calcium channel blockers have been reported to affect the levels of ACE2 expression of different animals or human tissues or plasma.[53,54] Further

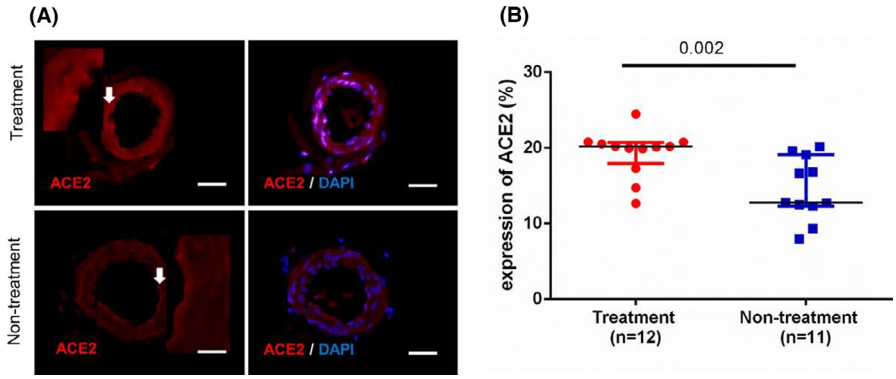

FIGURE 5 Angiotensin-converting enzyme 2 (ACE2) expression in resistance artery sections from patients with end-stage kidney disease (ESKD) with or without ACE-inhibitor/angiotensin receptor blocker (ARB) treatment. (A) Representative immunofluorescence staining of ACE2 (red) in ACE-inhibitor/ARB treated and non-treated arteries from ESKD patients, Bar = 100μm, (B) Statistical analysis of ACE2 expression in ACE-inhibitor/ARB treated ($n = 12$) and non-treated ($n = 11$) ESKD; Significance $p < 0.05$

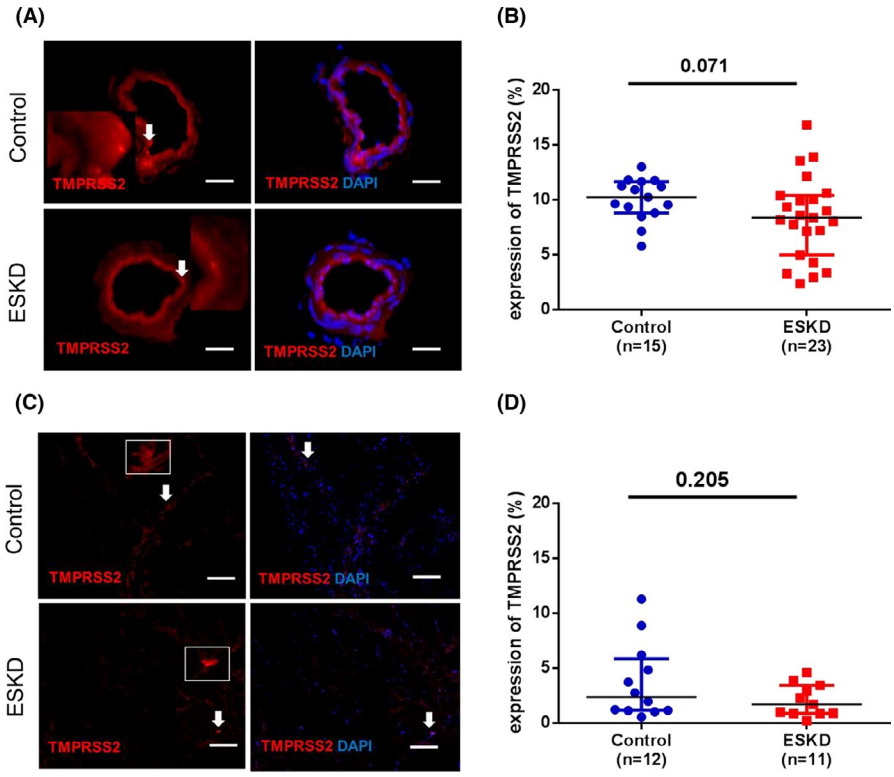

FIGURE 6 Transmembrane protease serine 2 (TMPRSS2) expression in resistance artery sections (A + B), and adipose tissue (C + D) from patients with end-stage kidney disease (ESKD) and controls. (A**)** Representative immunofluorescence staining of TMPRSS2 in arteries, (B) Statistical analysis of TMPRSS2 expression between controls ($n = 15$) and ESKD ($n = 23$) in resistance arteries, (C) Representative immunofluorescence staining of TMPRSS2 in adipose tissue, (D) Statistical analysis of TMPRSS2 expression between control ($n = 12$) and ESKD ($n = 11$) in adipose tissue. ACE2 (red) and nuclear staining with DAPI (blue). Bar = 100 μm. Significance $p < 0.05$; ESKD, end-stage kidney disease

studies however, are needed to determine the effects of these medications (whether as single or in combination therapy) in increasing ACE2 in the ESKD population and relation to SARS-CoV-2 infection. Some strengths and weaknesses of this study are worth addressing. The use of unique human material is an apparent strength of this study. Regarding the higher tissue expression of ACE2 in ESKD patients, ACEi/ARB may have a role in the greater tissue expression of ACE2 in ESKD patients, however, as we have limited access to the study materials further investigations are warranted to elaborate on this issue. In addition, as the numbers of males and females were low in ACEi/ARB treatment vs non-treatment group in ESKD, we pooled both males and females that deserve further exploration with larger groups.

In conclusion, our findings suggest that ACE2 in the circulation and vasculature are differently expressed in ESKD and controls. A better understanding of the sex predisposition of ACE2 could provide further insight on possible mechanisms of COVID-19 pathology in high-risk patients that could establish a personalized treatment approach.

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GOING-FWD Consortium Co-Principal Investigators: Louise Pilote—McGill University Health Center and McGill University, Canada; Colleen M. Norris—University of Alberta, Canada; Valeria Raparelli—University of Ferrara, Italy. Site Principal Investigators: Alexandra Kautzky-Willer—Medical University of Vienna, Austria; Karolina Kublickiene—Karolinska Institutet, Sweden; Maria Trinidad Herrero—Universidad de Murcia, Spain.

CONFLICT OF INTEREST

PS serves on the Scientific Advisory Boards of Baxter, Astra Zeneca and REATA. All other authors declare no competing interests.

AUTHOR CONTRIBUTION

KK, PS contributed to the conceptualization of the project and together with SA, LH, LJW for study design. SA and LH contributed to performing experiments. SA, LH, LJW and KK analysed the data. SA, LH, LJW and KK drafted the manuscript. PB, AS, PS and KK critically reviewed the manuscript. All authors read and approved the final manuscript.

ORCID

Samsul Arefin https://orcid.org/0000-0002-8050-9100

REFERENCES

1. Wu J, Li J, Zhu G, et al. Clinical features of maintenance hemodialysis patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *Clin J Am Soc Nephrol*. 2020;15(8):1139-1145.
2. Ulu S, Gungor O, Gok Oguz E, Hasbal NB, Turgut D, Arici M. COVID-19: a novel menace for the practice of nephrology and how to manage it with minor devastation? *Ren Fail*. 2020;42(1):710-725.
3. Figliozzi S, Masci PG, Ahmadi N, et al. Predictors of adverse prognosis in COVID-19: a systematic review and meta-analysis. *Eur J Clin Invest*. 2020;50(10):e13362.
4. Zores F, Rebeaud ME. COVID and the renin-angiotensin system: are hypertension or its treatments deleterious? *Front Cardiovasc Med*. 2020;7:71.
5. Patel VB, Zhong JC, Grant MB, Oudit GY. Role of the ACE2/angiotensin 1–7 axis of the renin-angiotensin system in heart failure. *Circ Res*. 2016;118(8):1313-1326.
6. Batlle D, Wysocki J, Satchell K. Soluble angiotensin-converting enzyme 2: a potential approach for coronavirus infection therapy? *Clin Sci (Lond)*. 2020;134(5):543-545.
7. Anguiano L, Riera M, Pascual J, Soler MJ. Circulating ACE2 in cardiovascular and kidney diseases. *Curr Med Chem*. 2017;24(30):3231-3241.
8. Crackower MA, Sarao R, Oudit GY, et al. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature*. 2002;417(6891):822-828.
9. Cheng H, Wang Y, Wang GQ. Organ-protective effect of angiotensin-converting enzyme 2 and its effect on the prognosis of COVID-19. *J Med Virol*. 2020;92(7):726-730.
10. Li MY, Li L, Zhang Y, Wang XS. Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. *Infect Dis Poverty*. 2020;9(1):45.
11. Mizuiri S, Ohashi Y. ACE and ACE2 in kidney disease. *World J Nephrol*. 2015;4(1):74-82.
12. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med*. 2020;46(4):586-590.
13. Chung MK, Karnik S, Saef J, et al. SARS-CoV-2 and ACE2: the biology and clinical data settling the ARB and ACEI controversy. *EBioMedicine*. 2020;58:102907.
14. Rossi GP, Sanga V, Barton M. Potential harmful effects of discontinuing ACE-inhibitors and ARBs in COVID-19 patients. *Elife*. 2020;9.
15. Christensen KL, Mulvany MJ. Location of resistance arteries. *J Vasc Res*. 2001;38(1):1-12.
16. Intengan HD, Schiffrin EL. Structure and mechanical properties of resistance arteries in hypertension: role of adhesion molecules and extracellular matrix determinants. *Hypertension*. 2000;36(3):312-318.
17. Schiffrin EL. Vascular remodeling in hypertension: mechanisms and treatment. *Hypertension*. 2012;59(2):367-374.
18. Judd E, Calhoun DA. Management of hypertension in CKD: beyond the guidelines. *Adv Chronic Kidney Dis*. 2015;22(2):116-122.
19. Gurwitz D. Angiotensin receptor blockers as tentative SARS-CoV-2 therapeutics. *Drug Dev Res*. 2020;81(5):537-540.
20. Peckham H, de Gruijter NM, Raine C, et al. Male sex identified by global COVID-19 meta-analysis as a risk factor for death and ITU admission. *Nat Commun*. 2020;11(1):6317.
21. Snaedal S, Qureshi AR, Lund SH, et al. Dialysis modality and nutritional status are associated with variability of inflammatory markers. *Nephrol Dial Transplant*. 2016;31(8):1320-1327.
22. Simera I, Moher D, Hoey J, Schulz KF, Altman DG. A catalogue of reporting guidelines for health research. *Eur J Clin Invest*. 2010;40(1):35-53.
23. Council E-E, Group EW. Chronic kidney disease is a key risk factor for severe COVID-19: a call to action by the ERA-EDTA. *Nephrol Dial Transplant*. 2021;36(1):87-94.
24. Shi C, Lu K, Xia H, Zhang P, Zhang B. Alteration and association between serum ACE2/ angiotensin(1–7)/Mas axis and oxidative stress in chronic kidney disease: a pilot study. *Medicine (Baltimore)*. 2020;99(31):e21492.
25. Narula S, Yusuf S, Chong M, et al. Plasma ACE2 and risk of death or cardiometabolic diseases: a case-cohort analysis. *Lancet*. 2020;396(10256):968-976.
26. Wu J, Deng W, Li S, Yang X. Advances in research on ACE2 as a receptor for 2019-nCoV. *Cell Mol Life Sci*. 2021;78(2):531-544.

27. Anguiano L, Riera M, Pascual J, et al. Circulating angiotensin converting enzyme 2 activity as a biomarker of silent atherosclerosis in patients with chronic kidney disease. *Atherosclerosis*. 2016;253:135-143.
28. Soro-Paavonen A, Gordin D, Forsblom C, et al. Circulating ACE2 activity is increased in patients with type 1 diabetes and vascular complications. *J Hypertens*. 2012;30(2):375-383.
29. Palau V, Riera M, Duran X, et al. Circulating ADAMs are associated with renal and cardiovascular outcomes in chronic kidney disease patients. *Nephrol Dial Transplant*. 2020;35(1):130-138.
30. Lambert DW, Yarski M, Warner FJ, et al. Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). *J Biol Chem*. 2005;280(34):30113-30119.
31. Soler MJ, Riera M, Crespo M, et al. Circulating angiotensin-converting enzyme 2 activity in kidney transplantation: a longitudinal pilot study. *Nephron Clin Pract*. 2012;121(3–4):c144-c150.
32. Iwasaki M, Saito J, Zhao H, Sakamoto A, Hirota K, Ma D. Inflammation triggered by SARS-CoV-2 and ACE2 augment drives multiple organ failure of severe COVID-19: molecular mechanisms and implications. *Inflammation*. 2021;44(1):13-34.
33. Kooman JP, Dekker MJ, Usvyat LA, et al. Inflammation and premature aging in advanced chronic kidney disease. *Am J Physiol Renal Physiol*. 2017;313(4):F938-F950.
34. Reiss AB, Voloshyna I, De Leon J, Miyawaki N, Mattana J. Cholesterol Metabolism in CKD. *Am J Kidney Dis*. 2015;66(6):1071-1082.
35. Kornilov SA, Lucas I, Jade K, Dai CL, Lovejoy JC, Magis AT. Plasma levels of soluble ACE2are associated with sex, metabolic syndrome, and its biomarkers in a large cohort, pointing to a possible mechanism for increased severity in COVID-19. *Crit Care*. 2020;24(1):452.
36. Sama IE, Ravera A, Santema BT, et al. Circulating plasma concentrations of angiotensin-converting enzyme 2 in men and women with heart failure and effects of renin-angiotensin-aldosterone inhibitors. *Eur Heart J*. 2020;41(19):1810-1817.
37. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol*. 2004;203(2):631-637.
38. Hikmet F, Méar L, Edvinsson Å, Micke P, Uhlén M, Lindskog C. The protein expression profile of ACE2 in human tissues. *Mol Syst Biol*. 2020;16(7):e9610.
39. Evans PC, Rainger GE, Mason JC, et al. Endothelial dysfunction in COVID-19: a position paper of the ESC working group for atherosclerosis and vascular biology, and the ESC council of basic cardiovascular science. *Cardiovasc Res*. 2020;116(14):2177-2184.
40. McCracken IR, Saginc G, He L, et al. Lack of evidence of angiotensin-converting enzyme 2 expression and replicative infection by SARS-CoV-2 in human endothelial cells. *Circulation*. 2021;143(8):865-868.
41. Zhang Q, Sun L, Jin L. Association between angiotensin-converting enzyme 2 and coronary artery calcification in patients on maintenance hemodialysis therapy. *Ther Apher Dial*. 2015;19(5):466-470.
42. Favre G, Legueult K, Pradier C, et al. Visceral fat is associated to the severity of COVID-19. *Metabolism*. 2021;115:154440.
43. Bukowska A, Spiller L, Wolke C, et al. Protective regulation of the ACE2/ACE gene expression by estrogen in human atrial tissue from elderly men. *Exp Biol Med (Maywood)*. 2017;242(14):1412-1423.
44. Gagliardi MC, Tieri P, Ortona E, Ruggieri A. ACE2 expression and sex disparity in COVID-19. *Cell Death Discov*. 2020;6:37.
45. Conti P, Younes A. Coronavirus COV-19/SARS-CoV-2 affects women less than men: clinical response to viral infection. *J Biol Regul Homeost Agents*. 2020;34(2):339-343.
46. Channappanavar R, Fett C, Mack M, Ten Eyck PP, Meyerholz DK, Perlman S. Sex-based differences in susceptibility to severe acute respiratory syndrome coronavirus infection. *J Immunol*. 2017;198(10):4046-4053.
47. Pedersen KB, Chodavarapu H, Porretta C, Robinson LK, Lazartigues E. Dynamics of ADAM17-mediated shedding of ACE2 applied to pancreatic islets of male db/db mice. *Endocrinology*. 2015;156(12):4411-4425.
48. Aimes RT, Zijlstra A, Hooper JD, et al. Endothelial cell serine proteases expressed during vascular morphogenesis and angiogenesis. *Thromb Haemost*. 2003;89(3):561-572.
49. Stamler JS, Lamas S, Nitrosylation FFC. the prototypic redox-based signaling mechanism. *Cell*. 2001;106(6):675-683.
50. Wysocki J, Lores E, Ye M, Soler MJ, Batlle D. Kidney and lung ACE2 expression after an ACE inhibitor or an Ang II receptor blocker: implications for COVID-19. *J Am Soc Nephrol*. 2020;31(9):1941-1943.
51. Guo J, Huang Z, Lin L, Lv J. Coronavirus disease 2019 (COVID-19) and cardiovascular disease: a viewpoint on the potential influence of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers on onset and severity of severe acute respiratory syndrome coronavirus 2 infection. *J Am Heart Assoc*. 2020;9(7):e016219.
52. Vaduganathan M, Vardeny O, Michel T, McMurray JJV, Pfeffer MA, Solomon SD. Renin-angiotensin-aldosterone system inhibitors in patients with Covid-19. *N Engl J Med*. 2020;382(17):1653-1659.
53. Akhtar S, Benter IF, Danjuma MI, Doi SAR, Hasan SS, Habib AM. Pharmacotherapy in COVID-19 patients: a review of ACE2-raising drugs and their clinical safety. *J Drug Target*. 2020;28(7-8):683-699.
54. Dambha-Miller H, Albasri A, Hodgson S, et al. Currently prescribed drugs in the UK that could upregulate or downregulate ACE2 in COVID-19 disease: a systematic review. *BMJ Open*. 2020;10(9):e040644.
55. Agarwal R, Peixoto AJ, Santos SF, Zoccali C. Pre- and post-dialysis blood pressures are imprecise estimates of interdialytic ambulatory blood pressure. *Clin J Am Soc Nephrol*. 2006;1(3):389-398.

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