

## Altered kidney function induced by SARS-CoV-2 infection and acute kidney damage markers predict survival outcomes of COVID-19 patients: a prospective pilot study

Mustafa Zafer Temiz<sup>a</sup> , Ibrahim Hacibey<sup>a</sup> , Ramazan Omer Yazar<sup>a</sup> , Mehmet Salih Sevd<sup>b</sup> ,  
Suat Hayri Kucuk<sup>c</sup> , Gizem Alkurt<sup>d</sup> , Levent Doganay<sup>d</sup> , Gizem Dinler Doganay<sup>e</sup> ,  
Muhammet Murat Dincer<sup>a</sup> , Emrah Yuruk<sup>f</sup> , Kerem Erkalp<sup>b</sup>  and Ahmet Yaser Muslumanoglu<sup>a</sup> 

<sup>a</sup>Department of Urology, Bagcilar Training and Research Hospital, Istanbul, Turkey; <sup>b</sup>Department of Anesthesiology and Reanimation, Bagcilar Training and Research Hospital, Istanbul, Turkey; <sup>c</sup>Department of Biochemistry, Bagcilar Training and Research Hospital, Istanbul, Turkey; <sup>d</sup>Genomic Laboratory (GLAB), Umraniye Training and Research Hospital, Elmalikent, Istanbul, Turkey; <sup>e</sup>Department of Molecular Biology and Genetics, Istanbul Technical University, Istanbul, Turkey; <sup>f</sup>Department of Urology, BHT Clinic Istanbul Tema Hospital, Istanbul, Turkey

### ABSTRACT

**Background:** Literature with regard to coronavirus disease 2019 (COVID-19) associated morbidities and the risk factors for death are still emerging. In this study, we investigated the presence of kidney damage markers and their predictive value for survival among hospitalized subjects with COVID-19.

**Methods:** Forty-seven participants was included and grouped as: 'COVID-19 patients before treatment', 'COVID-19 patients after treatment', 'COVID-19 patients under treatment in intensive care unit (ICU)', and 'controls'. Kidney function tests and several kidney injury biomarkers were compared between the groups. Cumulative rates of death from COVID-19 were determined using the Kaplan–Meier method. The associations between covariates including kidney injury markers and death from COVID-19 were examined, as well.

**Results:** Serum creatinine and cystatin C levels, urine Kidney Injury Molecule-1 (KIM-1)/creatinine ratio, and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), CKD-EPI cystatin C, and CKD-EPI creatinine–cystatin C levels demonstrated significant difference among the groups. The most significant difference was noted between the groups 'COVID-19 patients before treatment' and 'COVID-19 patients under treatment in ICU'. Advancing age, proteinuria, elevated serum cystatin C, and urine KIM-1/creatinine ratio were all significant univariate correlates of death ( $p < 0.05$ , for all). However, only elevated urine KIM-1/creatinine ratio retained significance in an age, sex, and comorbidities adjusted multivariable Cox regression (OR 6.11; 95% CI: 1.22–30.53;  $p = 0.02$ ), whereas serum cystatin C showing only a statistically non-significant trend (OR 1.42; 95% CI: 0.00–2.52;  $p = 0.09$ ).

**Conclusions:** Our findings clearly demonstrated the acute kidney injury related to COVID-19. Moreover, urine KIM-1/creatinine ratio was associated with COVID-19 specific death.

### ARTICLE HISTORY

Received 13 September 2021  
Revised 10 January 2022  
Accepted 10 January 2022

### KEYWORDS

COVID-19; kidney; acute kidney injury; survival analysis

## Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, coronavirus disease 2019 (COVID-19) affects the respiratory tract as the primary target in most of the cases and has been known as the latest pandemic of the modern world [1,2]. The clinical manifestation of the disease ranges from asymptomatic course to severe pneumonia [2]. The central role in the

pathogenesis has been attributed to angiotensin-converting enzyme 2 (ACE-2) as it enables viral entry into the target cells [3]. Being a member of the respiratory system virus family, settling down of the SARS-CoV-2 on the highly ACE-2 expressing respiratory tract is not surprising [3,4]. However, various tissues, including the kidney proximal tubule cells, outside the lung have ACE-2 expression and are potential target for the virus [3,5].

**CONTACT** Mustafa Zafer Temiz  [dr\\_mustafazafertemiz@hotmail.com](mailto:dr_mustafazafertemiz@hotmail.com)  Department of Urology, Bagcilar Training and Research Hospital, Merkez Mh, Dr. Sadik Ahmet Caddesi, 34100 Bagcilar, Istanbul, Turkey

 Supplemental data for this article can be accessed [here](#).

© 2022 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

In the present study, we primarily aimed to investigate whether there is a kidney damage during the course of COVID-19 through a prospective analytical study with a pilot design. The secondary aim was identifying the predictive value of several kidney injury biomarkers for estimating the survival from COVID-19.

## Materials and methods

### Study design and setting

A prospective, pilot study was conducted at a tertiary referral center declared as one of the pandemic hospitals in Istanbul/Turkey by Turkish Ministry of Health (TMOH). The Institutional Review Board approved the study (approval no.: 2020.05.1.06.038). The principles outlined in the Declaration of Helsinki were followed and informed consent from participants and/or their relatives for patients in intensive care unit (ICU) was obtained, as well. Approval of TMOH has also been obtained for the study. A total of 75 patients with confirmed and probable COVID-19 diagnose and 11 healthy controls were prospectively enrolled between April and May 2020.

### Participants

All the participants were selected from urology and ICU departments of our hospital. Patients aged 18 and over who were highly suspicious for COVID-19 with specific computerized tomography (CT) findings [6] before treatment, who completed treatment for laboratory confirmed COVID-19 or who were under treatment in ICU for laboratory confirmed COVID-19. Controls were selected among healthy volunteers from hospital staff without any clinical or laboratory findings consistent with COVID-19. The presence of end-stage renal disease, previous kidney surgery history, documented acute urinary tract infection, and current urinary stone disease were the exclusion criteria. Patients with solitary kidney and urogenital malformation or pregnant and lactating women were also not included in the study.

Diagnosis of the COVID-19 was confirmed on the nasopharyngeal and oropharyngeal swab samples with real-time reverse-transcription polymerase chain reaction (RT-PCR) in our genomic laboratory. We performed the RT-PCR according to manufacturer's instructions (Coyote Bioscience Co., Ltd., Beijing, China). Suspected COVID-19 cases were determined using latest updated version of our national COVID-19 guidelines [7]. Suspected cases with specific thorax CT findings [6] were termed as highly suspected COVID-19 cases. We again used latest updated version of our national

COVID-19 guidelines to determine the treatment algorithms, as well. Blood and urine samples were collected before starting to treatment in highly suspected COVID-19 cases and immediately after finishing the five days treatment course in COVID-19 cases. Samples were collected at fifth day of treatment for COVID-19 patients in ICU. Blood and urine samples were collected from the controls, as well. After obtaining of the RT-PCR tests results, the diagnosis of COVID-19 was confirmed for all of the highly suspected cases.

### Outcomes

Outcomes of interest were: transmission of the SARS-CoV-2 into the urine, incidence of acute kidney injury (AKI) in COVID-19 patients, effects of SARS-CoV-2 on kidney function and kidney injury biomarkers. Proteinuria and hematuria were also evaluated. The survival analysis and determination of the potential predictor parameters for specific mortality of COVID-19 were secondary outcomes of interest.

### Data source/measurement, variables, and covariates

The demographic characteristics, laboratory data, and medications were extracted from our prospectively noted and/or past medical records. AKI was defined as an increase in serum creatinine by 0.3 mg/dL within 48 h or a 50% increase in serum creatinine from baseline within seven days according to the KDIGO criteria [8]. Venous blood samples were collected from the participants to test serum creatinine and cystatin C levels using a photometric test on an AU5800 clinical chemistry analyzer (Beckman Coulter Inc., Brea, CA) and nephelometric test on Immage 800 Rate Nephelometer (Beckman Coulter Inc., Brea, CA), respectively. Then, we estimated glomerular filtration rates (eGFRs) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations from serum creatinine and cystatin C levels as described previously [9,10]. Urine samples were collected as single voided morning urine samples and/or single morning urine samples from urethral catheter. Spot urine Kidney Injury Molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) levels were determined by enzyme-linked immunosorbent assay (ELISA) using specific Human KIM-1 ELISA kit (SinoGeneClon Biotech, Hangzhou, China) and specific Human NGAL ELISA kit (Thermo Fisher Scientific, Rockford, IL), respectively. Urine creatinine levels were determined using a photometric test on an AU5800 clinical chemistry analyzer (Beckman Coulter Inc., Brea,

CA). Spot urine protein and red blood cells (RBCs) estimations were performed by FUS-200/H-800 automated urinalysis system with flow cell digital imaging technology (Dirui Industrial Co., Ltd., Changchun, China).

At beginning, the participants divided the two main groups as COVID-19 patients and controls, and then, divided to four final groups as 'COVID-19 patients before treatment', 'COVID-19 patients after treatment', 'COVID-19 patients under treatment in ICU', and 'controls'. The parameters age, body mass index (BMI), comorbidity, serum creatinine and cystatin C levels, CKD-EPI, CKD-EPI cystatin C and CKD-EPI creatinine–cystatin C eGFR levels, urine KIM-1 and NGAL levels, urine KIM-1/creatinine and NGAL/creatinine ratios were statistically compared between the groups. Urine pH levels and presence of proteinuria and microhematuria were also compared.

### Data analysis

Due to the lack of associated data in the literature, a minimum number of participants for sample size could not be calculated. Instead of this, a pilot study has been designed. Statistical analysis was performed with SPSS Version 22.0 statistic software package (IBM SPSS Inc., Chicago, IL). Data distributions and test of normality were evaluated with Shapiro–Wilk's test. Descriptive statistic methods (mean  $\pm$  standard deviation and median  $\pm$  interquartile range) were used to evaluate data. We compared the normally distributed and not normally distributed parametric data between the main groups using independent *t*-test and Mann–Whitney's *U* test, respectively. In the analysis of the final groups, we used the one-way ANOVA and Kruskal–Wallis tests, respectively. For post hoc analysis of the one-way ANOVA and Kruskal–Wallis tests, we used Tukey's and Mann–Whitney's *U* tests, respectively. Chi-square test was also used in the comparison of the nonparametric categorical variables. Cumulative rates of death from COVID-19 were determined using the Kaplan–Meier method. The associations between covariates including kidney disease indicators and death from COVID-19 were examined using Cox proportional hazard regression analysis.

Based on our comparative statistical results, several covariates were selected for Kaplan–Meier survival and Cox regression analyses. The compared parameters exhibiting *p* values  $\leq 0.1$  were considered as covariates. These covariates were age, comorbidities, serum creatinine and cystatin C levels, urine KM-1/creatinine and

NGAL/creatinine levels, and CKD-EPI, CKD-EPI cystatin C and CKD-EPI creatinine–cystatin C eGFR levels.

In the multivariable model, age, sex, and comorbidities adjusted analysis was performed. Among the variables with statistically significant roles in univariable analysis, we included only specific kidney biomarkers, serum cystatin C level and urine KIM-1/creatinine ratio, to the multivariable analysis as covariates. Differences were considered as significant at two-sided *p* < 0.05 and 95% confidence interval.

## Results

### Demographics and baseline characteristics

A total of 47 participants (26 [55.3%] male, 21 [44.7%] female) who met the inclusion criteria were included to the study. Among them, 36 (78.3%) were diagnosed with COVID-19 and remaining were the controls. The mean age of the study cohort was  $55.77 \pm 17.47$  years. Twenty-six participants (55.3%) had comorbidities while 15 had multiple comorbidities. The presence of a comorbidity was similar among the patient and control groups. Demographics of COVID-19 patients and controls are provided in Table 1. The median duration from onset of symptoms to hospitalization was 3.5 (2–6) days among the COVID-19 patients. The median duration from onset symptoms to ICU admission and from hospitalization to ICU admission was four (3–8) and three (1–4) days, respectively. The most common symptoms at onset of the disease were fever (*n* = 30, 81.1%), fatigue and myalgia (*n* = 21, 56.7%), and cough (*n* = 17, 46%). The less common presenting symptoms were diarrhea (*n* = 11, 29.8%), headache and dizziness (*n* = 11, 29.8%), and shortness of breath (*n* = 10, 27%). Specific thoracic CT findings were noted in all of the patients with COVID-19. All of the patients, except one, in the COVID-19 patients before treatment group were treated in the specific COVID-19 clinic and discharged uneventfully after a median of 4.5 (4–9) days of hospital stay. One patient died (7.69%) from COVID-19 on his 5th day of treatment. The median duration of the hospital stay was five (5–20) and 13.5 (7–20) days for the COVID-19 patients after treatment group and COVID-19 patients treated in ICU group, respectively. Eleven patients (91.7%) died from COVID-19 in the COVID-19 patients treated in ICU group and one patient was discharged after 15 days treatment in ICU. The intubation rate was 58.3% in the COVID-19 patients treated in ICU. The median time from admission to ICU to intubation and duration of intubation period were 5 (1–9) days and 12 (2–16) days.

**Table 1.** Demographics, kidney function parameters, kidney damage markers, and urine analyses results of COVID-19 patients and controls.

	COVID-19 patients (n = 36)	Controls (n = 11)	p
Age (years) (mean ± SD)	59.78 ± 17.11	42.64 ± 11.51	0.03*
BMI (mean ± SD)	27.20 ± 2.68	27.83 ± 3.10	0.51*
Sex (n, %)			0.30**
Male	18 (50%)	8 (72.7%)	
Female	18 (50%)	3 (27.3%)	
Comorbidity (n, %)			0.50**
Yes	21 (58.3%)	5 (45.5%)	
(Some participants had multiple)			
None	15 (41.7%)	6 (54.5%)	
DM	12 (33.3%)	4 (36.4%)	0.73**
Hypertension	7 (19.4%)	1 (9.1%)	
CAD	1 (2.8%)	0 (0.0%)	
Others	1 (2.8%)	0 (0.0%)	
Multiple comorbidity (n, %)			
Yes	13 (36.1%)	2 (18.2%)	0.46**
SARS-CoV-2 positive urine samples (n)	1	0	
Serum Cre (mg/dL) (median ± IQR)	0.75 ± 0.39	0.76 ± 0.22	0.33#
Serum Cyst C (mg/L) (median ± IQR)	0.96 ± 0.59	0.70 ± 0.17	0.003#
CKD-EPI (mean ± SD)	86.66 ± 28.02	114.72 ± 9.40	0.002*
CKD-EPI Cyst C (mean ± SD)	86.66 ± 49.01	136.33 ± 25.35	0.002*
CKD-EPI Cre-Cyst C (mean ± SD)	99.57 ± 46.63	136.39 ± 24.99	0.01*
Urine KIM-1 (ng/mL) (mean ± SD)	12.95 ± 5.82	10.42 ± 4.02	0.18*
Urine KIM-1/urine Cre (ng/mg) (median ± IQR)	15.97 ± 54.24	9.31 ± 15.48	0.46#
Urine NGAL (ng/mL) (median ± IQR)	61.26 ± 75.35	21.73 ± 154.60	0.11#
Urine NGAL/urine Cre (ng/mg) (median ± IQR)	39.22 ± 126.66	12.48 ± 169.50	0.09#
Urine pH (median ± IQR)	6.0 ± 0.9	6.0 ± 0.0	0.17#
Microhematuria (n, %)			
Yes	16 (44.4%)	1 (9.1%)	0.03**
Proteinuria (n, %)			
Yes	17 (47.2%)	0 (0.0%)	0.004**

COVID-19: coronavirus disease 2019; BMI: body mass index; DM: diabetes mellitus; CAD: coronary artery disease; Cre: creatinine; Cyst C: cystatin C; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; KIM-1: kidney injury molecule 1; NGAL: neutrophil gelatinase-associated lipocalin; SD: standard deviation; IQR: interquartile range.

\*Independent *t*-test.

\*\*Chi-square test.

#Mann-Whitney's *U* test.

### Urine RT-PCR test, AKI, and kidney function and injury parameters

In only one patient (2.78%), SARS-CoV-2 was isolated from the urine sample. The incidence of AKI could not be evaluated in the COVID-19 patients after treatment group, because of the lack of the baseline serum creatinine levels. Overall AKI incidence was 16% in the COVID-19 patients. Specific AKI incidences in the COVID-19 patients before treatment and COVID-19 patients in treated in ICU groups were 15.38% (2/13 patients) and 16.66% (2/12 patients), respectively. Serum cystatin C levels were detected significantly higher in patients with COVID-19. Moreover, kidney function parameters CKD-EPI, CKD-EPI cystatin C, and CKD-EPI creatinine–cystatin C eGFR levels were significantly lower in the COVID-19 patients compared to the controls (Table 1). In the urine analysis, micro-hematuria and proteinuria were detected more frequently in patients with COVID-19 ( $p = 0.03$  and  $p = 0.004$ , respectively) (Table 1).

In the comparative analysis of the final groups, mean age was significantly higher in the COVID-19 patients under treatment in ICU (Table 2 and

Supplementary Table 1). Serum creatinine and cystatin C levels, urine KIM-1/creatinine ratio, and CKD-EPI, CKD-EPI cystatin C and CKD-EPI creatinine–cystatin C eGFR levels exhibited significant difference among groups (Table 2). The *post hoc* analyses indicated that this difference was related to the COVID-19 patients before treatment and COVID-19 patients under treatment in ICU groups (Supplementary Table 2) as the kidney function was much more altered and the kidney injury was more deeper in these patients (Supplementary Tables 1 and 2). Additionally, presence of comorbidities and proteinuria were more prevalent in the COVID-19 patients under treatment in ICU group (Supplementary Table 3). In the COVID-19 patients before treatment group, median serum creatinine level significantly decreased and mean CKD-EPI eGFR level significantly increased during the course of the treatment. On the other hand, nevertheless, these parameters got worse in COVID-19 patients under treatment in ICU group (Supplementary Table 4).

The mean age, median serum cystatin C level, and median urine KIM-1/creatinine and urine NGAL/

**Table 2.** Demographics, kidney function parameters, kidney damage markers, and urine analyses results of the participants in the groups.

	COVID-19 patients before treatment (n = 13)	COVID-19 patients after treatment (n = 11)	COVID-19 patients under treatment in ICU (n = 12)	Controls (n = 11)	p
Age (years) (mean ± SD)	56.46 ± 15.95	51.00 ± 14.91	71.42 ± 14.62	42.64 ± 11.51	<0.001*
BMI (mean ± SD)	28.35 ± 2.25	27.19 ± 3.50	25.96 ± 1.71	27.83 ± 3.10	0.16*
SARS-CoV-2 positive urine samples (n)	2	0	0	0	
Serum Cre (mg/dL) (median ± IQR)	0.93 ± 0.34	0.67 ± 0.23	0.83 ± 0.38	0.76 ± 0.22	<0.001#
Serum Cyst C (mg/L) (median ± IQR)	1.04 ± 0.69	0.80 ± 0.33	1.23 ± 0.84	0.70 ± 0.17	<0.001#
CKD-EPI (mean ± SD)	74.53 ± 27.59	111.45 ± 10.83	77.08 ± 25.94	114.72 ± 9.40	<0.001*
CKD-EPI Cyst C (mean ± SD)	79.21 ± 47.81	121.85 ± 44.00	62.47 ± 37.76	136.33 ± 25.35	<0.001*
CKD-EPI Cre-Cyst C (mean ± SD)	85.57 ± 39.75	141.40 ± 38.69	75.27 ± 37.70	136.39 ± 24.99	<0.001*
Urine KIM-1 (ng/mL) (mean ± SD)	11.09 ± 6.56	12.90 ± 0.11	15.00 ± 4.27	10.42 ± 4.02	0.08*
Urine KIM-1/urine Cre (ng/mg) (median ± IQR)	4.23 ± 5.93	6.09 ± 29.32	69.58 ± 27.10	9.31 ± 15.48	<0.001#
Urine NGAL (ng/mL) (median ± IQR)	47.58 ± 58.64	66.21 ± 83.54	81.05 ± 93.80	21.73 ± 154.60	0.44#
Urine NGAL/urine Cre (ng/mg) (median ± IQR)	27.89 ± 54.34	25.95 ± 108.63	244.28 ± 350.27	12.48 ± 169.50	0.007#
Urine pH (median ± IQR)	6.0 ± 1.0	6.0 ± 0.5	5.5 ± 0.5	6.0 ± 0.0	0.17#

COVID-19: coronavirus disease 2019; ICU: intensive care unit; BMI: body mass index; Cre: creatinine; Cyst C: cystatin C; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; KIM-1: kidney injury molecule 1; NGAL: neutrophil gelatinase-associated lipocalin; SD: standard deviation; IQR: interquartile range.

\*One-way ANOVA test.

#Kruskal–Wallis test.

creatinine levels were significantly higher among patients who were died because of COVID-19 compared to survivors of COVID-19. The mean CKD-EPI level was significantly lower for cases of COVID-19 deaths, as well (Supplementary Table 5). Among patients who were died of COVID-19, the rate of comorbidities and proteinuria were also significantly higher (Supplementary Table 6).

### Association of kidney function and injury parameters with COVID-19 specific mortality

Kaplan–Meier's analysis revealed a significantly higher COVID-19 specific death rates for elderly patients. Moreover, patients with altered kidney function and abnormal kidney injury biomarkers, including elevated serum cystatin C level, decreased CKD-EPI, CKD-EPI cystatin C and CKD-EPI creatinine–cystatin C eGFR levels, and elevated urine KIM-1/creatinine and urine NGAL/creatinine levels exhibited higher COVID-19 specific mortality (Figure 1). Univariate Cox regression analysis showed that age above 65 years was associated with COVID-19 specific mortality. In addition, the kidney function and injury biomarkers including elevated serum cystatin C level, elevated urine KIM-1/creatinine, CKD-EPI eGFR levels, and proteinuria were also associated with COVID-19 specific mortality (Table 3). After adjusting for age, sex, and comorbidities, the parameter urine KIM-1/creatinine ratio was associated with COVID-19 specific mortality in the multivariable model. However, serum cystatin C was not associated with COVID-19 specific mortality (Table 4).

**Table 3.** Univariable Cox regression analysis of association between abnormal kidney function and kidney damage with the COVID-19 specific death in patients with COVID-19.

Age >65 years	3.08	1.001–9.501	0.04
Any comorbidity	2.93	0.85–10.72	0.07
Cyst C elevated	5.95	1.62–21.79	0.002
Urine KIM-1/Cre elevated	6.57	1.43–30.02	0.004
Urine NGAL/Cre elevated	29.04	0.09–90.06	0.06
CKDEPI elevated	3.81	1.04–13.90	0.02
CKDEPI Cyst C elevated	3.99	1.09–14.63	0.02
CKDEPI Cre-Cyst C elevated	3.38	1.04–11.06	0.03
Proteinuria any degree	3.38	1.03–11.04	0.03

Cyst C: cystatin C; Cre: creatinine; KIM-1: kidney injury molecule 1; NGAL: neutrophil gelatinase-associated lipocalin; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration.

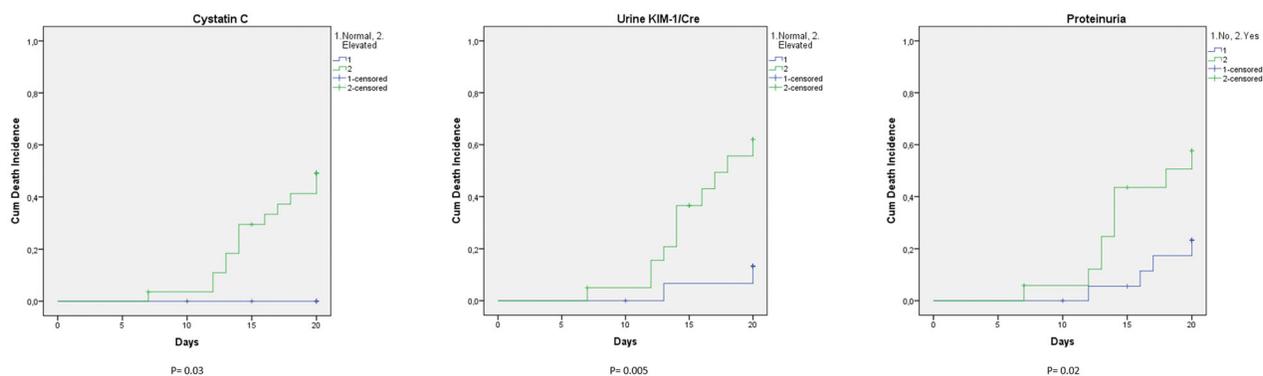
**Table 4.** Age, sex, and comorbidities adjusted multivariable Cox regression analysis of association between kidney damage markers with the COVID-19 specific death in patients with COVID-19.

	HRs	95% CI	p Value
Cyt C elevated	1.42	0.00–2.52	0.09
Urine KIM-1/Cre elevated	6.11	1.22–30.53	0.02

Cyst C: cystatin C; Cre: creatinine; KIM-1: kidney injury molecule 1.

## Discussion

It is now known that involvement of the kidneys during the course of COVID-19 is not uncommon. Typical CT scan findings of the COVID-19 involvement have been described as reduced density in the kidneys, which is compatible with inflammation and edema [11]. Proteinuria and hematuria may be documented in about 40% of the COVID-19 patients upon hospital admission [12]. Increased blood urea nitrogen and serum creatinine levels were reported in 14% and 10%



**Figure 1.** Cumulative incidence for death of patients with coronavirus disease 2019 subgrouped by kidney damage indicators.

of the hospitalized COVID-19 patients, respectively. Of these patients, some may experience gradual worsening of kidney function despite lack of any sign of AKI upon admission to the hospital [12]. According to the recent literature, AKI incidence in hospitalized COVID-19 patients ranges from 8% to 22% [13].

Apart from the alveolar cells in the lungs, many other tissues including the heart, the gut and the kidneys have also considerable ACE2 expression levels [14]. The National Center for Biotechnology Information's (NCBI) gene database reports that, in the human body, the kidney is the fourth highly ACE2 expressing organ following the small intestine, duodenum and gall bladder [15]. ACE2 expression level is almost 100-fold higher in the kidney than that in the respiratory organs [15]. In the kidney, the brush border of proximal tubular cells is the main source of the ACE2 expression followed by the podocytes to a lesser extent. Currently, it is still under debate whether ACE2 expressing kidney is affected by SARS-CoV-2 infection. However, several reports have already demonstrated the co-occurrence of AKI with COVID-19 [11,13,16,17] supporting that SARS-CoV-2 may have a tropism for the kidney. In this regard, a recent study by Diao et al. [18] is quite remarkable. The study showed that one of the specific targets for SARS-CoV-2 is the kidney tissue. The authors found specific SARS-CoV-2 nucleocapsid protein in the kidney specimens and isolated viral antigens accumulated in kidney tubules by postmortem tissue analysis. They concluded that SARS-CoV-2 directly infect human kidney tubules to induce acute tubular damage. In their opinion, beside direct cytotoxicity, it also initiates macrophage and complements mediated tubular pathogenesis secondary to accumulated viral antigens. In a similar study, Pan et al. [19] also concluded that the cytopathic effects of SARS-CoV-2 on proximal straight tubule cells and podocytes might cause AKI in patients with COVID-19. Recent evidence provides that AKI is associated with increased morbidity and mortality in

COVID-19 patients. It is considered as a marker of disease severity and a negative prognostic factor for survival, as well [13,17,20,21]. Although the possible mechanism of kidney damage by SARS-Cov-2 and COVID-19 associated AKI is well described in the literature, concrete evidence for acute kidney damage in COVID-19 patients is still not efficient. Additionally, available data on COVID-19 associated AKI are reporting incidence based on case series, retrospective studies and a few prospective studies. All of those studies determined the AKI based on the serum creatinine levels without using accepted AKI biomarkers [12].

In this prospective pilot study, we investigated the incidence of AKI prospectively in the COVID-19 patients. Moreover, we examined the AKI biomarkers to obtain a concrete evidence in terms of COVID-19 associated acute kidney damage. This is the first study investigating the kidney damage markers in COVID-19 patients. We found that COVID-19 patients under treatment in ICU exhibited extremely higher levels of serum cystatin C, and urine KIM-1/creatinine and urine NGAL/creatinine ratios. Our results clearly described the COVID-19 associated acute kidney damage using molecular kidney damage markers for the first time in the literature. Lowered CKD-EPI, CKD-EPI cystatin C, and CKD-EPI creatinine-cystatin C eGFR levels were determined in COVID-19 patients under treatment in ICU, as well. We observed that the overall incidence of AKI as 16% in hospitalized patients with COVID-19. The incidences were 15.38% and 16.66% in the COVID-19 patients after treatment group and COVID-19 patients under treatment in ICU group, respectively. In brief, impairment in the kidney function was significantly higher in the as-yet-untreated COVID-19 patients and COVID-19 patients under treatment in ICU. The incidences of micro-hematuria and proteinuria were also higher in those patients. These findings support that COVID-19 affects kidney functions adversely and proportionally with severity of the disease. Besides, we showed the considerable

recovery in kidney function with the treatment of COVID-19.

Currently, it has been reported that kidney disease on admission and AKI during hospitalization were associated with an increased risk of in-hospital death for COVID-19 patients [17]. In this regard, we used kidney function parameters and kidney damage markers to predict COVID-19 specific mortality in survival analysis and regression models. According to our results, indicators of altered kidney function and kidney damage were significantly higher in died COVID-19 patients compared with the survived ones. Our univariable Cox regression model indicated that kidney function parameters and damage markers could predict the survival outcomes of COVID-19 patients. After adjusting for age, gender and comorbidity, we found that only urine KIM-1/creatinine ratio was associated with COVID-19 specific death, but serum cystatin C level did not. This finding of the current study is consistent with Liu et al.'s [22]. Liu et al. [22] investigated the serum creatine and cystatin C in estimating glomerular filtration rate of critically ill COVID-19 patients and found that reduced eGFRcreatinine ( $<60$  mL/min/ $1.73$  m<sup>2</sup>) was associated with death (HR = 1.939, 95% CI 1.078–3.489,  $p = 0.027$ ), but eGFRcystatin C did not.

The present study has several limitations. First, our cohort has small number of patients. However, we intended to obtain our pilot findings about the topic. Second, our control group was consisted of our healthy hospital workers with relatively younger age. Due to compeller pandemic period, it was difficult to find aged healthy individuals. Additionally, we had no specific control patients for COVID-19 cases in ICU. Third, even we performed adjusted analysis, other parameters and covariates than our investigated might have played a role on survival analysis. Due to the our small cohort, we could include small covariates in multivariable analysis. In other respects, our study has some strengths. One of them is serial monitoring of the serum creatinine levels with available accurate baseline values. Second one is the use of molecular kidney damage markers, which could indicate functional alteration in kidney prior the elevation of serum creatinine levels. This provided us to clearly describe the acute kidney damage by COVID-19 for the first time in the literature. Classification of the COVID-19 patients as before treatment, after treatment and under treatment in ICU might have provided findings that are more accurate. Finally, this is the first study investigating the predictive role of kidney damage markers in determining the survival outcomes of the patients with COVID-19.

Our findings clearly described the acute kidney damage by COVID-19. Moreover, kidney damage was

associated with COVID-19 specific death. In this regard, considering kidney function and damage markers must not be ignored in the COVID-19 patients, and serial monitoring of them should be considered. Impairment in the kidney function and/or emerging kidney damage should alert clinicians during the management of COVID-19 patients. In clinical practice, serum cystatin C level and urine KIM-1/creatinine ratio can be used to predict the survival of COVID-19 patients.

## Acknowledgements

The authors thank administration and staffs of the TUSEB. The authors express our special thanks of gratitude to our clinic nurses for their substantial contribution to the study while they exhibited heroic efforts during the fight against COVID-19. The authors thank our allied health personnel for their infinite pains, as well.

## Disclosure statement

The authors report no involvement in the research by the funder that could have influenced the outcome of this work. MZ Temiz, I Hacibey, RO Yazar, MS Sevdi, SH Kucuk, L Doganay, MM Dincer, K Erkalp, and AY Muslumanoglu were granted by Health Institutes of Turkey (TUSEB).

## Funding

This work supported by the Health Institutes of Turkey (TUSEB) with a Grant number of 2020-CV-01-8708/8969.

## ORCID

Mustafa Zafer Temiz  <http://orcid.org/0000-0002-5736-5495>  
 Ibrahim Hacibey  <http://orcid.org/0000-0002-2212-5504>  
 Ramazan Omer Yazar  <http://orcid.org/0000-0002-5343-5345>  
 Mehmet Salih Sevdi  <http://orcid.org/0000-0001-7484-7501>  
 Suat Hayri Kucuk  <http://orcid.org/0000-0003-0267-1302>  
 Gizem Alkurt  <http://orcid.org/0000-0003-2768-4695>  
 Levent Doganay  <http://orcid.org/0000-0002-2263-6689>  
 Gizem Dinler Doganay  <http://orcid.org/0000-0002-3586-1287>  
 Muhammet Murat Dincer  <http://orcid.org/0000-0001-7110-4155>  
 Emrah Yuruk  <http://orcid.org/0000-0002-2343-8828>  
 Kerem Erkalp  <http://orcid.org/0000-0002-4025-7092>  
 Ahmet Yaser Muslumanoglu  <http://orcid.org/0000-0002-8691-0886>

## Data availability statement

Due to the nature of this research, participants of this study did not agree for their data to be shared publicly, so supporting data are not available.

## References

- [1] Jiang S, Shi Z, Shu Y, et al. A distinct name is needed for the new coronavirus. *Lancet*. 2020;395(10228):949.
- [2] Kakodkar P, Kaka N, Baig MN. A comprehensive literature review on the clinical presentation, and management of the pandemic coronavirus disease 2019 (COVID-19). *Cureus*. 2020;12(4):e7560.
- [3] Xu H, Zhong L, Deng J, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *Int J Oral Sci*. 2020;12(1):8.
- [4] Nichols WG, Peck Campbell AJ, Boeckh M. Respiratory viruses other than influenza virus: impact and therapeutic advances. *Clin Microbiol Rev*. 2008;21(2):274–290.
- [5] Xiao F, Zimpelmann J, Agaybi S, et al. Characterization of angiotensin-converting enzyme 2 ectodomain shedding from mouse proximal tubular cells. *PLOS One*. 2014;9(1):e85958.
- [6] Ai T, Yang Z, Hou H, et al. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. *Radiology*. 2020; 296(2):E32–E40.
- [7] Turkish Ministry of Health GdoPH. T.C. Sağlık Bakanlığı, Halk Sağlığı genel Müdürlüğü, COVID-19 (2019-nCoV HASTALIGI) Rehberi (in Turkish); 2020. p. 4.
- [8] Howitt SH, Grant SW, Caiado C, et al. The KDIGO acute kidney injury guidelines for cardiac surgery patients in critical care: a validation study. *BMC Nephrol*. 2018; 19(1):149.
- [9] Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med*. 2012;367(1):20–29.
- [10] Inker LA, Eneanya ND, Coresh J, et al. New creatinine- and cystatin C-based equations to estimate GFR without race. *N Engl J Med*. 2021;385(19):1737–1749.
- [11] Qian JY, Wang B, Liu BC. Acute kidney injury in the 2019 novel coronavirus disease. *Kidney Dis*. 2020;323: 1–6.
- [12] Cheng Y, Wang W, Wu L, et al. SARS-CoV-2-related kidney injury: current concern and challenges. *SN Compr Clin Med*. 2020;1–10. DOI:10.1007/s42399-020-00529-0
- [13] Zahid U, Ramachandran P, Spitalewitz S, et al. Acute kidney injury in COVID-19 patients: an inner city hospital experience and policy implications. *Am J Nephrol*. 2020;51(10):786–796.
- [14] Ye M, Wysocki J, William J, et al. Glomerular localization and expression of angiotensin-converting enzyme 2 and angiotensin-converting enzyme: implications for albuminuria in diabetes. *J Am Soc Nephrol*. 2006; 17(11):3067–3075.
- [15] Gene Database. The National Center for Biotechnology Information; 2022.
- [16] Rudnick MR, Hilburg R. Acute kidney injury in COVID-19: another challenge for nephrology. *Am J Nephrol*. 2020;51(10):761–763.
- [17] Cheng Y, Luo R, Wang K, et al. Kidney disease is associated with in-hospital death of patients with COVID-19. *Kidney Int*. 2020;97(5):829–838.
- [18] Diao B, Wang C, Wang R, et al. Human kidney is a target for novel severe acute respiratory syndrome coronavirus 2 infection. *Nat Commun*. 2021;12(1):2506.
- [19] Pan XW, Xu D, Zhang H, et al. Identification of a potential mechanism of acute kidney injury during the COVID-19 outbreak: a study based on single-cell transcriptome analysis. *Intensive Care Med*. 2020;46(6): 1114–1116.
- [20] Li Q, Guan X, Wu P, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med*. 2020;382(13):1199–1207.
- [21] Hu Z, Song C, Xu C, et al. Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China. *Sci China Life Sci*. 2020;63(5):706–711.
- [22] Liu Y, Xia P, Cao W, et al. Divergence between serum creatine and cystatin C in estimating glomerular filtration rate of critically ill COVID-19 patients. *Ren Fail*. 2021;43(1):1104–1114.