Original Article
http://mjiri.iums.ac.ir
Medical Journal of the Islamic Republic of Iran (MJIRI)

Med J Islam Repub Iran. 2022 (15 Nov);36.135. https://doi.org/10.47176/mjiri.36.135

Soluble ACE2 as a Risk or Prognostic Factor in COVID-19 Patients: A Cross-sectional Study

Parsa Mohammadi[1], Hesam Aldin Varpaei[2], Arash Seifi[3], Sepideh Zahak Miandoab[4], Saba Beiranvand[5], Sahar Mobaraki[5], Mostafa Mohammadi[6]*, Alireza Abdollahi[7]

Received: 19 Jan 2022 Published: 15 Nov 2022

Abstract

Background: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel severe acute respiratory syndrome coronavirus. The first known receptor for this virus in the human body is angiotensin-converting enzyme 2 (ACE2), the same receptor for the SARS virus.

Methods: A total of 38 hospitalized adult (18 years) patients with laboratory or clinically confirmed coronavirus disease 2019 (COVID-19) were identified in the infectious disease ward of Tehran Imam Khomeini hospital complex in this single-center cross-sectional study. A blood sample was taken at the time of hospitalization and a second one was taken 48 hours later. Blood samples are kept frozen at -80 degrees Celsius. After the complete collection of samples, the ACE2 level of the samples was measured using a serum sACE2 detection ELISA kit. The data were analyzed using SPSS v26. P value of 0.05 was considered statistically significant. An analysis of covariance was performed to examine the mean differences in day 7 serum ACE2 concentration among the 2 groups after adjusting for the baseline serum ACE2 concentration. The 1-way multivariate analysis of variance was used to determine whether there were any differences between independent groups (mechanical ventilation yes/no) on serum ACE2 levels at 3 different times.

Results: The mean age of patients was 64.13 ± 16.49 years, 21 patients (55.3%) were men, 16 patients (42%) were polymerase chain reaction test positive, and 15 patients (39.5%) died. A total of 35 individuals (92.1%) had chest computed tomography images that indicated lung involvement. A comparison of the 2 groups of patients who died and were discharged revealed that serum ACE2 at the first (p=0.033) and third (7th day) measurements were statistically different (p=0.026). Patients had a mean of serum ACE2. The results indicated that the day 7 serum ACE2 concentration did significantly differ between the 2 groups after controlling for the baseline serum ACE2 concentration (p=0.023). The model explained about 73.61% of the variance in the 7-day serum ACE2 concentration. Specifically, after adjusting for the baseline concentration, survived patients had the lowest level of serum ACE2 concentration (1 ± 0.65) on the 7th day compared with the deceased patient group (2.83 ± 1.12).

Conclusion: Soluble ACE2 in the serum of COVID-19 patients who died, later on, was significantly higher than the discharged patients when the samples were taken seven days after admission. It is suggested that serum soluble ACE2 level could be used as a prognostic factor for COVID-19 patients' outcomes and also their need for mechanical ventilation.

Keywords: SARS-CoV-2, Angiotensin-converting enzyme 2, Polymerase chain reaction, Mechanical ventilation, Prognosis, Mortality

Conflicts of Interest: *None declared*
Funding: *None*

Cite this article as: Mohammadi P, Varpaei HA, Seifi A, Zahak Miandoab S, Beiranvand S, Mobaraki S, Mohammadi M, Abdollahi A. Soluble ACE2 as a Risk or Prognostic Factor in COVID-19 Patients: A Cross-sectional Study. *Med J Islam Repub Iran.* 2022 (15 Nov);36:135. https://doi.org/10.47176/mjiri.36.135

Corresponding author: *Dr Mostafa Mohammadi, mohammady_mm@tums.ac.ir*

1. Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. College of Nursing, Michigan State University, MI, USA
3. Department of Infectious Diseases, Tehran University of Medical Sciences, Imam Khomeini Hospital Complex, Tehran, Iran
4. Department of Infectious Diseases, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
5. Department of Nursing, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran
6. Tehran University of Medical Sciences, General ICU of Imam Khomeini Hospital Complex, Tehran, Iran
7. Imam Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran

↑What is "already known" in this topic:

- There have been numerous suggestions for therapeutic options regarding the new coronavirus, among which a significant number are based on the interaction between ACE2 and the viral spike protein.
- For instance, hydroxychloroquine alters a portion of the ACE2 molecule and prevents the binding of spike protein from the virus.
- Blocking the ACE2 receptor and using soluble ACE2 to bind to and neutralize the viral spike protein are further examples of how the mentioned interaction could lead to therapeutical strategies.

→What this article adds:

- Although serum soluble ACE 2 on day 7 of patient admission can be a prognostic factor, its levels on the first and third days of patient admission are not significantly different in patients.
- An increase in serum soluble ACE-2 on days 3 and 7 after hospitalization (compared with the first day) can be a predictor of the need for mechanical ventilation.

Introduction

The severe acute respiratory syndrome c oronavirus 2 (SARS-CoV-2) virus, the causative agent of the coronavirus disease 2019 (COVID-19) pandemic, is now a global problem and has infected more than 180 countries, including Iran. The only known receptor for this virus in the human body is angiotensin-converting enzyme 2 (ACE2), the same known receptor for the SARS virus (1). The spike protein of SARS-CoV-2 tends to bind to the ACE2 receptor, expressed on the surface of human cells, facilitating its entry and thus replication (2). Since the ACE2 gene is most abundantly expressed in the heart, lung, and kidney tissues (3), many of the symptoms and problems of patients with COVID-19 are related to the interaction of the virus with the ACE2 protein in the same sites, including cardiovascular and pulmonary complications (4). There have been numerous suggestions for therapeutic options regarding the new coronavirus, among which a significant number are based on the interaction between ACE2 and the viral spike protein. For instance, hydroxychloroquine, one of the first approved medications for COVID-19, alters a portion of the ACE2 molecule and prevents the binding of the spike protein of the virus (5). Blocking the ACE2 receptor and using soluble ACE2 (sACE2) to bind to and neutralize the viral spike protein (4) are further examples of how the mentioned interaction could lead to therapeutical strategies. Additionally, alterations in ACE2 expression brought on by heart disease (6), kidney disease (7), diabetes (8), and an aging population (9) may also contribute to the greater susceptibility of these patient populations to SARS-CoV-2.

Despite the absence of the anchored portion of the ACE2 molecule, soluble ACE2 contains the same functional domain as ACE2 receptors that bind to the spike protein of SARS-CoV-2 (10, 11). This enables the soluble ACE2 molecules to circulate in the bloodstream, while simultaneously neutralizing the virus particles, inhibiting them from entering host cells. Also, with the soluble ACE2 being derived from ACE2 receptors in the body (10), measuring the serum soluble ACE2 would give us a clue about the quantity of ACE2 receptors expressed in different tissues.

With this background in mind, we aimed to monitor the level of soluble ACE2 in recently diagnosed COVID-19 patients.

Measurement of serum soluble ACE2 in this study is performed using the sACE2 ELISA kit. By comparing serum ACE2 levels in newly admitted patients with sACE2 levels within 48 hours of the same patients, the role of sACE2 levels in determining the prognosis of COVID-19 patients could be valued. Therefore, this study aimed to use serum ACE2 levels to determine the prognosis of patients with COVID-19.

Methods

In this single-center cross-sectional study, 38 hospitalized adult (≥18 years) patients with laboratory-confirmed COVID-19 were identified in the infectious disease ward in Imam Khomeini hospital. Sampling was performed from June to December 2020. Eligible patients were included in the study and observed until discharge or death. No costs were imposed on patients, and all laboratory costs were paid by the researchers. Inclusion criteria were as follows: patients aged 18 to 75 years old without organ failure and nonintubation. Exclusion criteria were as follows: critically ill patients, end-stage cancer patients, and patients treated in other clinical trial studies. This study has also been approved by the ethics committee of Tehran University of Medical Sciences (ethic code: 99/11/101/16529). When the patient was admitted to the hospital, one blood sample was obtained, and a second one was taken 48 hours later. Blood samples are frozen at -80 °C. After complete collection of samples, the ACE2 level of the samples was measured using the serum sACE2 detection ELISA kit (Human Angiotensin Converting Enzyme 2 [ACE2] ELISA Kit-Bioassay Technology Laboratory-SHANGHI KORAIN BIOTECH CO, LTD.)

All serum sACE2 and other lab tests were collected 24, 72 hours, and 7 days after patients' hospitalization.

Data Analysis

The data were analyzed using SPSS 26 (IBM Corp). Descriptive statistical analysis was used to describe items included in the survey. Data were expressed as medians (interquartile ranges [IQRs]) for continuous variables. For bivariate analysis, the Mann–Whitney U test or the t test was used for continuous variables, and the 2 or the Fisher exact test for categorical variables. A P value of 0.05 was considered statistically significant when a 2-tailed test was performed. The Kolmogorov-Smirnov test was used to evaluate the normal distribution of the data. An analysis of covariance (ANCOVA) was performed to examine the mean differences in day 7 serum ACE2 concentration among the 2 groups after adjusting for the baseline serum ACE2 concentration. The 1-way multivariate analysis of variance (1-way MANOVA) was used to determine whether there were any differences between independent groups (mechanical ventilation yes/no) on serum ACE2 levels at 3 different times.

Results

The mean age of the patients was 64.13 ± 16.49 years, 21 patients (55.3%) were men, 16 patients (42%) were polymerase chain reaction test positive, and 15 patients (39.5%) died. The most common presenting symptoms were dyspnea (66.7%), fever (33.3%), cough (26.7%), myalgias (20%), and nausea/vomiting (16.7%). During hospitalization, 13.2% of patients (3 women and 2 men) needed mechanical ventilation. In addition, lung involvement was visible in 35 patients' chest computed tomography scans (92.1%). Underlying diseases and organ failure are listed in Tables 1 and 2.

A comparison between the 2 groups of patients who died and were discharged showed that serum ACE2 at first

Table 1. Comorbidities and organ failures

Comorbidity	N (%)
Diabetes mellitus	11 (28.9)
Ischemic heart disease	8 (21.1)
Hypertension	10 (26.3)
Hypothyroidism	3 (7.9)
End-stage renal disease/ chronic kidney disease	3 (7.9)
Heart Failure	3 (7.9)
Cerebrovascular accident	3 (7.9)
Asthma	2 (5.3)
Thalassemia	2 (5.3)
HIV/AIDS	1 (2.6)
HCV	1 (2.6)
Cirrhosis	1 (2.6)
Organ failures	
Acute Kidney Injury	3 (7.9)
Acute respiratory distress syndrome	12 (31.6)
Liver failure	4 (10.5)

(p=0.033) and third measurement (7th day) was statistically different (p=0.026). Compared to patients who died, survivors exhibited higher mean serum ACE2 levels (Table 3).

An ANCOVA was performed to examine the mean differences in day 7 serum ACE2 concentration among the 2 groups after adjusting for the baseline serum ACE2 concentration. The results indicated that the day 7 serum ACE2 concentration did significantly differ among the 2 groups after controlling for the baseline serum ACE2 concentration (p=0.023). The model explained about 73.61% of the variance in the day 7 serum ACE2 concentration. Specifically, after adjusting for the baseline concentration, survived patients had the lowest level of serum ACE2 concentration (1 ± 0.65) on the 7th day compared with the deceased patient group (2.83 ± 1.12) (Figs. 1 & 2).

According to a patient's demand for mechanical ventilation, the results of the MANOVA test (Table 4) revealed a statistically significant difference in soluble serum ACE2 (p=0.029; Wilk's Λ =0.49; partial η^2 =0.50). After adjusting for baseline concentration, patients who required mechanical breathing had greater serum ACE2 levels on the second and third measurement days.

Table 2. Categorical variables according to patients' outcome

Variable		Outcome			
		Death		Survived	
		N	%	N	%
Sex	Male	9	42.9	12	57.1
	Female	6	35.3	11	64.7
PCR	Negative	10	45.5	12	54.5
	Positive	5	31.3	11	68.8
Chest CT scan involvement	No	0	0.0	3	100.0
	Yes	15	42.9	20	57.1
Diabetes	No	11	40.7	16	59.3
	Yes	4	36.4	7	63.6
Ischemic heart disease	No	12	40.0	18	60.0
	Yes	3	37.5	5	62.5
Hypertension	No	11	39.3	17	60.7
	Yes	4	40.0	6	60.0
Hypothyroidism	No	13	37.1	22	62.9
	Yes	2	66.7	1	33.3
ESRD_CKD	No	13	37.1	22	62.9
	Yes	2	66.7	1	33.3
Heart failure	No	13	37.1	22	62.9
	Yes	2	66.7	1	33.3
Asthma	No	15	41.7	21	58.3
	Yes	0	0.0	2	100.0
CVA	No	13	37.1	22	62.9
	Yes	2	66.7	1	33.3
Thalassemia	No	15	41.7	21	58.3
	Yes	0	0.0	2	100.0
HIV_AIDS	No	15	40.5	22	59.5
	Yes	0	0.0	1	100.0
HCV	No	14	38.9	22	61.1
	Yes	1	50.0	1	50.0
Cirrhosis	No	15	40.5	22	59.5
	Yes	0	0.0	1	100.0
Fever	No	10	50.0	10	50.0
	Yes	2	20.0	8	80.0
Cough	No	11	50.0	11	50.0
	Yes	1	12.5	7	87.5
Myalgia	No	12	50.0	12	50.0
	Yes	0	0.0	6	100.0
Dyspnea	No	3	30.0	7	70.0
	Yes	9	45.0	11	55.0
Headache	No	12	40.0	18	60.0
	Yes	0	0.0	0	0.0
Nausea and vomiting	No	11	44.0	14	56.0
	Yes	1	20.0	4	80.0

Table 3. Laboratory findings of 2 groups of patients

Variable	Outcome	N	Mean	SD	*P* Value
Age	Death	14	71.93	15.608	0.005
	Survived	22	56.82	14.748	
BMI	Death	14	26.2483	3.59292	0.860
	Survived	22	27.1421	4.84955	
1st ACE2	Death	11	2.6727	2.64517	0.033
	Survived	17	1.9524	2.30787	
2nd ACE2	Death	13	2.2946	1.07574	0.115
	Survived	18	2.6539	3.53895	
3rd ACE2	Death	7	2.6457	1.14401	0.026
	Survived	11	1.8764	2.99450	
Mean ACE	Death	14	2.34452	1.334231	0.790
	Survived	22	2.45083	3.014812	
T-1	Death	12	36.992	0.6815	0.843
	Survived	17	37.029	0.5850	
T-2	Death	12	37.058	0.4295	0.080
	Survived	17	36.824	0.2705	
T-3	Death	6	36.717	0.1941	0.562
	Survived	12	36.783	0.2406	
PR-1	Death	12	95.00	18.640	0.520
	Survived	18	91.17	14.284	
PR-2	Death	12	92.17	15.573	0.088
	Survived	18	83.78	10.525	
PR-3	Death	6	92.00	6.693	0.097
	Survived	13	83.85	10.319	
RR-1	Death	12	26.50	6.762	0.145
	Survived	18	23.17	5.393	
RR-2	Death	12	24.08	6.855	0.057
	Survived	18	20.44	3.091	
RR-3	Death	6	23.50	2.429	0.003
	Survived	13	17.77	3.320	
SBP-1	Death	12	112.08	24.119	0.491
	Survived	18	117.61	19.458	
DBP-1	Death	12	72.83	11.456	0.760
	Survived	18	74.00	9.677	
SBP-2	Death	12	117.50	18.471	0.390
	Survived	18	123.44	18.131	
DBP-2	Death	12	73.83	13.496	0.550
	Survived	18	76.33	9.530	
SBP-3	Death	5	111.00	21.331	0.372
	Survived	13	119.00	14.543	
DBP-3	Death	5	67.40	15.534	0.356
	Survived	13	75.00	7.360	
WBC-1	Death	12	12.033	7.2895	0.981
	Survived	17	11.971	6.7492	
WBC-2	Death	11	10.464	3.3013	0.895
	Survived	16	10.162	6.7647	
WBC-3	Death	8	13.775	4.6154	0.033
	Survived	10	9.860	2.9527	
Hgb-1	Death	12	11.425	2.6527	0.291
	Survived	18	12.511	2.8044	
Hgb-2	Death	11	11.036	3.5345	0.402
	Survived	17	11.941	2.1909	
Hgb-3	Death	8	11.463	3.2191	0.960
	Survived	11	11.409	1.6121	
Plt-1	Death	12	194.00	99.671	0.082
	Survived	18	266.83	113.639	
Plt-2	Death	11	173.91	92.658	0.052
	Survived	17	285.06	163.767	
Plt-3	Death	8	177.75	127.175	0.421
	Survived	11	244.73	113.465	
Urea-1	Death	12	124.25	96.481	0.018
	Survived	18	54.17	28.981	
Urea-2	Death	11	104.64	55.552	0.029
	Survived	17	62.65	29.927	
Urea-3	Death	8	83.00	48.238	0.048
	Survived	11	53.00	19.235	

The results of cross-tabulation (Tables 5 & 6) indicated that diabetic patients are more likely to develop AKI. A history of hypothyroidism significantly increases the odds of liver failure, need for mechanical ventilation, and ARDS. History of kidney diseases (ESRD/CKD) also significantly increased the odds of developing AKI and the

Table 3. Continued

Variable	Outcome	N	Mean	SD	*P* Value
Cr-1	Death	12	2.775	2.6718	0. 104
	Survived	18	1.611	1.0380	
Cr-2	Death	11	2.782	1.8362	0.005
	Survived	17	1.229	0.7042	
Cr-3	Death	8	2.275	1.7153	0.030
	Survived	12	1.008	0.2275	
Na-1	Death	12	141.00	6.325	0.241
	Survived	18	138.78	4.052	
Na-2	Death	11	142.18	8.424	0.765
	Survived	17	141.47	3.659	
Na-3	Death	8	137.50	4.660	0.130
	Survived	13	140.31	3.637	
K-1	Death	12	4.592	1.0783	0.580
	Survived	18	4.411	0.7267	
K-2	Death	11	4.291	0.7204	0.413
	Survived	17	4.112	0.4241	
K-3	Death	8	4.613	1.0643	0.061
	Survived	13	3.962	0.4214	
Mg-1	Death	11	2.355	0.4803	0.431
	Survived	15	2.233	0.3039	
Mg-2	Death	8	2.088	0.2588	0.220
	Survived	10	2.250	0.2759	
Mg-3	Death	3	2.067	0.4509	0.851
	Survived	4	2.125	0.3304	
Ca-1	Death	12	7.933	0.7278	0.014
	Survived	15	8.587	0.6289	
Ca-2	Death	7	7.571	0.6873	0.174
	Survived	11	8.136	0.9047	
Ca-3	Death	3	7.700	0.4000	0.144
	Survived	4	8.225	0.3862	
P-1	Death	12	4.458	1.1642	0.570
	Survived	15	4.127	1.7339	
P-2	Death	7	4.414	1.8942	0.270
	Survived	10	3.510	1.3747	
P-3	Death	3	4.067	1.6258	0.258
	Survived	3	2.667	0.7767	
CRP-1	Death	12	38.58	26.301	0.033
	Survived	18	79.11	58.415	
CRP-2	Death	3	103.67	50.817	0.260
	Survived	14	71.00	42.863	
CRP-3	Death	4	54.75	18.626	0.850
	Survived	7	61.00	61.172	
ESR-1	Death	11	62.27	33.782	0.860
	Survived	17	64.71	38.459	
ESR-2	Death	3	84.67	49.319	0.640
	Survived	11	75.45	23.624	
ESR-3	Death	1	94.00	0	0.350
	Survived	4	77.25	13.598	
BS-1	Death	11	117.91	83.185	0.005
	Survived	18	173.50	79.394	
BS-2	Death	9	132.56	53.843	0.068
	Survived	12	201.67	95.989	
BS-3	Death	6	136.17	61.727	0.521
	Survived	5	112.40	56.880	
ALT-1	Death	12	54.83	55.604	0.543
	Survived	15	89.07	183.861	
ALT-2	Death	5	48.40	20.804	0.880
	Survived	7	53.14	56.858	
ALT-3	Death	2	151.50	181.726	N/A
	Survived	0[a]	0	0	
AST-1	Death	12	66.67	79.529	0.431
	Survived	15	49.40	23.018	
AST-2	Death	5	58.80	34.845	0.880
	Survived	8	54.88	54.465	
AST-3	Death	2	86.00	90.510	N/A
	Survived	0[a]	0	0	

need for mechanical ventilation.

Among the 3 detected organ failures, liver failure and ARDS significantly increased in-hospital mortality. Also, all 3 organ failures significantly increased the risk of the need for mechanical ventilation.

Analysis of the receiver operating characteristic curve

Table 3. Continued

Variable	Outcome	N	Mean	SD	*P* Value
Alp-1	Death	11	401.364	517.8807	0.351
	Survived	16	269.513	185.9658	
Alp-2	Death	5	437.80	585.130	0.340
	Survived	7	215.57	105.978	
Alp-3	Death	2	220.00	127.279	N/A
	Survived	0[a]	0	0	
Bili-T-1	Death	11	1.618	3.1530	0.481
	Survived	15	1.027	0.7353	
Bili-T-2	Death	5	2.540	3.8850	0.505
	Survived	6	1.400	1.0714	
Bili-T-3	Death	2	1.550	0.9192	N/A
	Survived	0[a]	0	0	
Bili-D-1	Death	12	0.883	1.8369	0.405
	Survived	15	0.480	0.2336	
Bili-D-2	Death	5	1.560	2.3755	0.291
	Survived	6	0.483	0.2563	
Bili-D-3	Death	2	0.650	0.0707	N/A
	Survived	0[a]	0	0	
GCS-1	Death	9	14.56	0.882	0.060
	Survived	15	15.00	0.000	
GCS-2	Death	7	14.71	0.756	0.167
	Survived	14	15.00	0.000	
GCS-3	Death	3	15.00	0.000	0.990
	Survived	8	15.00	0.000	
O2SAT-1	Death	11	83.82	10.989	0.561
	Survived	16	85.81	6.863	
O2SAT-2	Death	11	86.00	7.239	0.080
	Survived	15	90.20	4.459	
O2SAT-3	Death	6	86.00	6.663	0.027
	Survived	11	92.45	6.170	
pH-1	Death	11	7.4382	0.1008	0.205
	Survived	7	7.3729	0.1038	
pH-2	Death	8	7.4263	0.0542	0.810
	Survived	7	7.4343	0.0761	
pH-3	Death	5	7.4320	0.0759	0.906
	Survived	4	7.4250	0.0943	
PCO2-1	Death	11	38.655	16.3697	0.650
	Survived	7	41.686	6.5481	
PCO2-2	Death	8	35.888	10.9662	0.612
	Survived	7	38.657	9.9341	
PCO2-3	Death	5	35.160	10.0868	0.350
	Survived	4	41.700	9.5251	
HCO3-1	Death	11	25.282	7.7796	0.655
	Survived	7	26.857	5.7956	
HCO3-2	Death	8	24.088	8.0717	0.697
	Survived	7	25.857	5.0921	
HCO3-3	Death	5	25.140	8.4860	0.490
	Survived	4	28.925	6.6775	
PT-1	Death	12	16.733	4.0005	0.890
	Survived	17	17.065	8.0590	
PT-2	Death	11	17.045	3.0995	0.840
	Survived	9	16.667	5.4818	
PT-3	Death	7	18.086	6.7627	0.441
	Survived	5	15.640	1.3390	
PTT-1	Death	12	34.58	5.915	0.950
	Survived	17	34.41	8.155	
PTT-2	Death	10	43.90	27.990	0.389
	Survived	9	35.33	6.964	
PTT-3	Death	7	46.43	14.211	0.170
	Survived	5	36.40	5.814	

(ROC) (Fig. 3) to predict mortality based on patients' serum ACE2 levels showed that serum ACE2 measured in the first and third rounds are most accurate in predicting patient mortality (Table 7).

The cut-off for the serum ACE2 level was found for the first time at 1.405, the second time at 1.375, the third time at 1.755, and the mean of the serum ACE2 level was 2.773.

Discussion

There are numerous facts linking ACE2 to SARS-CoV-2, with the first and most important of them being that ACE2 is the receptor for the spike protein of SARS-CoV-2, which facilitates the virus entry into the host cell. This very fact, which has been revealed in the early months of the COVID-19 pandemic (12), has made ACE2 a perfectly suitable therapeutical target for developing an effective

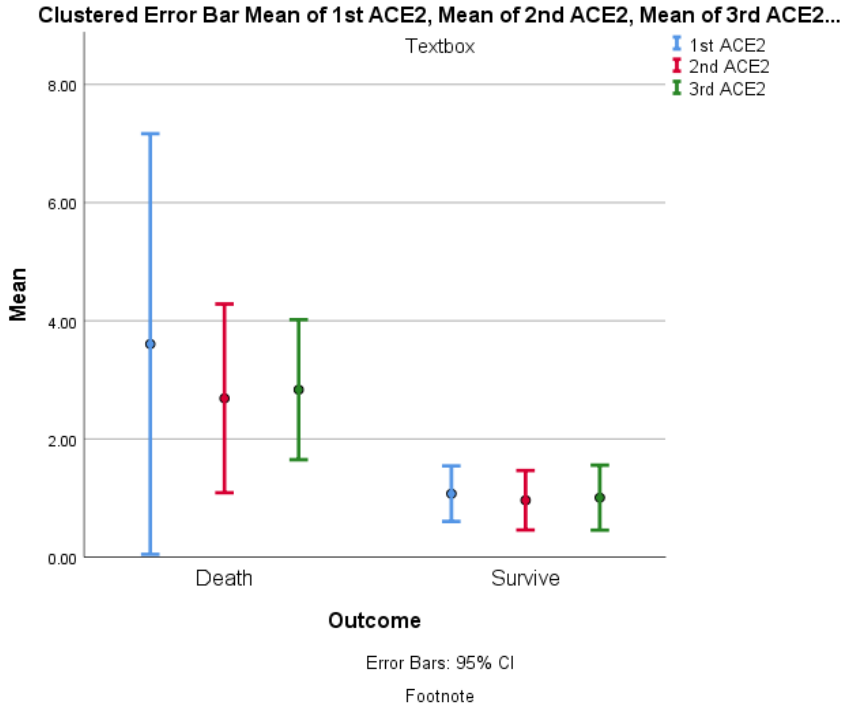

Fig. 1. Comparison of the serum ACE2 level between deceased and discharged patients

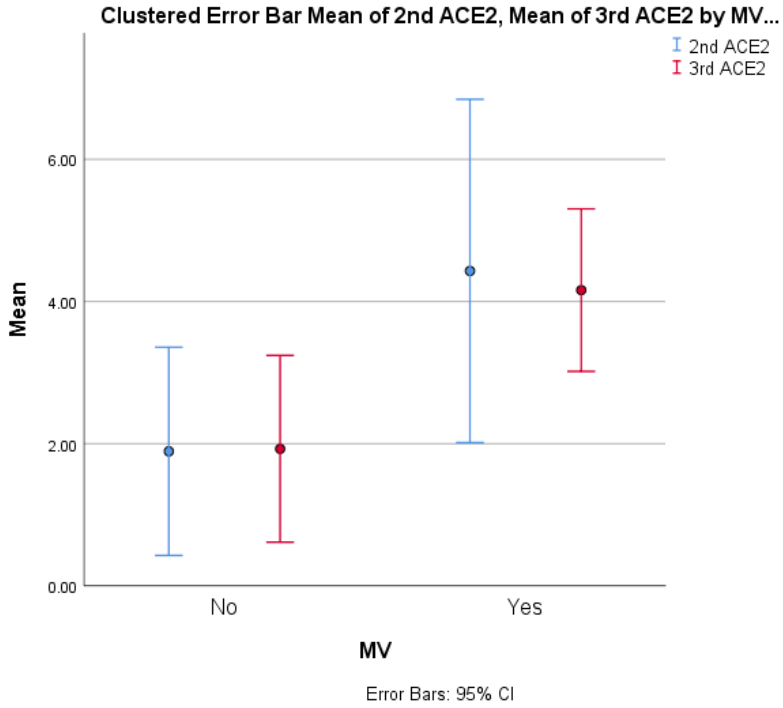

Fig. 2. Comparison of the serum ACE2 level between patients with/without mechanical ventilation

treatment for COVID-19 (13). Researchers have been able to examine a variety of aspects of this global health problem, including the susceptibility to COVID-19 based on ACE2 receptor polymorphism (14) and the pattern of COVID-19 complications using the distribution of ACE2 among tissues (15). They have also been able to demonstrate an association between ACE2 expression and COVID-19 mortality (16). Soon enough, the physiologically relevant soluble ACE2 gathered attention. Soluble ACE2 has been suggested to be of great importance in the fields of therapy and prognosis of COVID-19 (17). It is simpler and more practical to test the soluble form of the ACE2 receptor since it may be broken down into soluble ACE2 and circulate in the bloodstream (10). The balance

Table 4. Result of MANOVA

Variable	MV	
	Yes	No
Second ACE2	4.43±0.26	1.24±0.78
Third ACE2	4.16±0.12	1.39±0.83

Table 5. Cross tabulation of comorbidities and clinical complications

Risk factors	Mortality (Death)	Mechanical Ventilation	AKI	ARDS	Liver Failure
Diabetes mellitus	OR = 1.2, p=0.805	OR=1.7, p=0.601	OR=1.37, p=0.020	OR=2.3, p=0.201	OR=0.8, p=0.307
Ischemic heart disease	OR=1.1, p=0.948	OR=3, p=0.210	OR=2, p=0.545	OR=1.4, p=0.685	OR=1.2, p=0.966
Hypertension	OR=0.97, p=0.935	OR=2., p=0.546	OR=1.4, p=0.955	OR=3, p=0.275	OR=0.92, p=0.945
Hypothyroidism	OR=1.8, p=0.508	OR=21.3, p=0.041	OR=0.91, p=0.900	OR=n/a, p=0.021	OR=33, p=0.020
End-stage renal disease/ chronic kidney disease	OR=1.8, p=0.501	OR=21.3, p=0.041	OR=68, p=0.013	OR=5, p=0.201	OR=0.8, p=0.900

Table 6. Cross of organ failures with mortality and mechanical ventilation

	Death (n=15)	*P* value	Mechanical ventilation (n=5)	*P* value
AKI (n=3)	OR=n/a	0.054	OR=21.3	0.04
Liver failure (n=4)	OR=n/a	0.018	OR=48	0.005
ARDS (n=12)	OR=3.07	0.004	OR=1.74	0.002

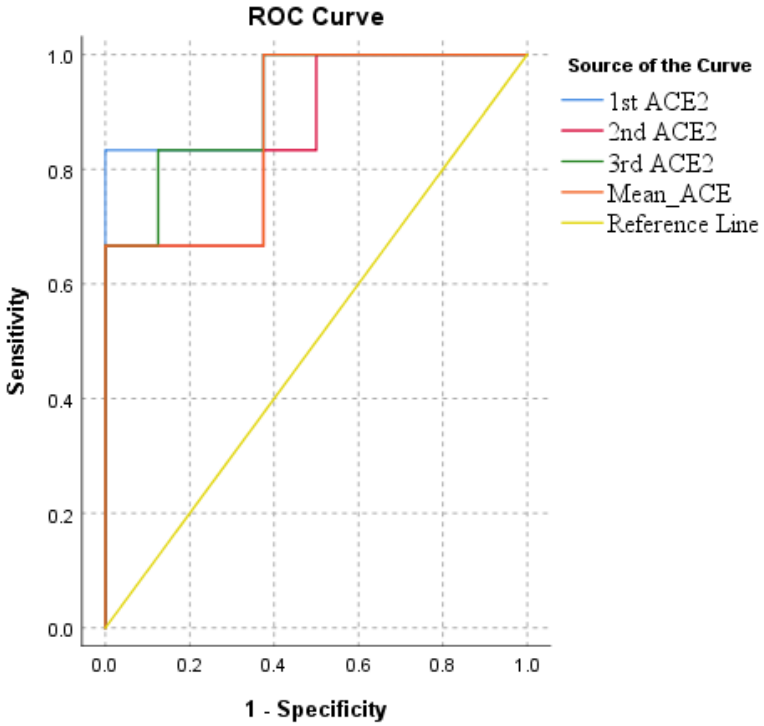

Fig. 3. Analysis of the receiver operating characteristic curve for predicting death based on the level of serum ACE2

Table 7. Area Under the receiver operating characteristic Curve

Variable	Area	Cut-off	*P* Value	95% CI	
First ACE2	0.938	1.405	<0.001	0.802	1.073
Second ACE2	0.854	1.375	0.001	0.644	1.064
Third ACE2	0.917	1.755	<0.001	0.767	1.066
Mean ACE2	0.875	2.773	<0.001	0.683	1.067

and equation between the ACE2 receptor and its soluble form, which possibly plays an important role in the pathogenesis of COVID-19 is yet to be fully understood. However, the measuring is not the only advantage of soluble ACE2 over the ACE2 receptor; many suggested therapies for COVID-19 are based on the fact that SARS-CoV-2 tends to attach to soluble ACE2 the same way it attaches to ACE2 receptors. Additionally, despite the ACE2 receptors, attachment of the spike protein of SARS-CoV-2 to soluble ACE2 would not result in cell entry. Thus, it could drain the viruses of the bloodstream, helping to prevent the infection or expedite the healing process of COVID-19 patients. The approach used in this paper also applies to binding recombinant soluble ACE2 to the spike protein of

SARS-CoV-2 to neutralize it (18).

We conducted this study to evaluate the possible correlation between serum soluble ACE2 levels of COVID-19 patients and their prognosis. Some studies hypothesized that a significant increase in serum ACE2 activity may act as an endogenous nonspecific protective mechanism against SARS-CoV-2 infection that preceded the recovery of patients (19, 20). A study by Emilsson et al (21) suggested that the upregulation of ACE2 expression may reflect the severity of the outcome in COVID-19. Besides, it was reported that serum ACE2 activity on admission does not reflect disease severity (22). Clarifying the physiological balance between ACE2 receptors and soluble ACE2 would simplify the interpretation of these findings. Another aspect that is yet to be discussed is the reason causing the serum soluble ACE2 to rise. The most probable sources would either be the upregulated expression of ACE2 receptors (20) in different tissues or extra cleavage of ACE2. In each case, a bold connection is to be noticed between the rise of soluble ACE2 in serum and the susceptibility to COVID-19 or immunity against it.

As the most important result of this study, the soluble ACE2 samples of the third day (taken 7 days after admission) showed a significant difference between discharged and expired groups, despite the first- and second-day samples (taken on the day of admission and 3 days later). This makes a relatively reliable background, upon which the predictive value of soluble ACE2 in COVID-19 would be assessed. The measured soluble ACE2 in the serum of patients in the deceased and discharged group had a mean of 2.6457 and 1.8764 pg/mL, respectively. It is obvious that the higher levels of soluble ACE2 found in the serum of patients who later died do not rule out or disprove the potential neutralizing or protective effects of therapeutic and synthetic soluble ACE2. Nevertheless, it is helpful to have a clearer understanding of how soluble ACE2 levels might be used as a tool in the management of COVID-19 patients.

The fact that soluble ACE2 level changes in patient's bloodstream in a specific way that leads to this phenomenon, raises the question of how the virus affects the ACE2 receptors so that a significant change appears after this interval; one would look at the pathophysiological changes during the course of COVID-19 to answer this question.

Align with this change, other measured biochemical and clinical indicators measured in our study have also responded to the course of the disease. Patients' calcium levels showed a significant difference between the 2 groups on day 1, but not on days 2 or 3. Similar to this, the blood sugar of patients was only significantly different between the deceased and discharged group on day 1. Moreover, the creatin level was more significantly higher in the deceased group on day 3 compared with day 2. Among the clinical items, the respiratory rate of patients followed the same pattern as described for creatin levels; the respiratory rate of expired patients has been higher than the discharged group each day, but the difference gradually increased from day 1 to day 3; interestingly, both discharged and deceased groups had lower respiratory rates in each day compared with the last measuring. These statistically different values could be useful in the management of critically ill COVID-19 patients. Since many of the markers measured in this study are covered in routine laboratory data of hospitalized patients, it would be more practical to screen the patient's soluble ACE2 level while considering how it changes compared with other biochemical and clinical markers.

Given that the soluble ACE2 was much greater in those who were intubated, it is fair to prepare the patient for intubation in addition to closer monitoring for patients who have a higher risk of developing critical conditions based on their soluble ACE2 levels. What seems to be the most practical is having a cut-off level for soluble ACE2. Monitoring both the soluble ACE2 level in the serum of COVID-19 patients and its changes after a certain interval simultaneously could provide a new clinical approach, through which those patients at higher risk would be treated accordingly. However, to come across a reliable cut-off for this purpose, greater sample size is required, which we recommend be taken into account for future studies.

Conclusion

Our data showed that soluble ACE2 in the serum of COVID-19 patients who died, later on, were significantly higher than the discharged patients when the samples were taken 7 days after admission. We suggest that the serum soluble ACE2 level be used as a prognostic factor for COVID-19 patients' outcomes and also their need for mechanical ventilation. To overcome the limitations we encountered in this study, additional investigations are required to determine the appropriate cut-off and time interval for soluble ACE2 monitoring in COVID-19 patients.

Acknowledgment

The authors would like to thank all medical staff for their effort in COVID-19 patient care.

Conflict of Interests

The authors declare that they have no competing interests.

References

1. Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Nat Microbiol. 2020;5(4):562–9.
2. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020;181(2):271–80.
3. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci. 2020;12(1):1–5.
4. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. Nat Rev Cardiol. 2020;17(5):259–60.
5. Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J. 2005;2(1):1–10.
6. Burrell LM, Risvanis J, Kubota E, Dean RG, MacDonald PS, Lu S, et al. Myocardial infarction increases ACE2 expression in rat and humans. Eur Heart J. 2005;26(4):369–75.
7. Lely AT, Hamming I, van Goor H, Navis GJ. Renal ACE2 expression in human kidney disease. J Pathol A J Pathol Soc Gt Britain Irel.

2004;204(5):587–93.
8. Mizuiri S, Hemmi H, Arita M, Ohashi Y, Tanaka Y, Miyagi M, et al. Expression of ACE and ACE2 in individuals with diabetic kidney disease and healthy controls. Am J Kidney Dis. 2008;51(4):613–23.
9. Schulman IH, Zhou MS, Treuer A V, Chadipiralla K, Hare JM, Raij L. Altered renal expression of angiotensin II receptors, renin receptor, and ACE-2 precede the development of renal fibrosis in aging rats. Am J Nephrol. 2010;32(3):249–61.
10. Batlle D, Wysocki J, Satchell K. Soluble angiotensin-converting enzyme 2: a potential approach for coronavirus infection therapy? Clin Sci. 2020;134(5):543–5.
11. Wysocki J, Ye M, Rodriguez E, González-Pacheco FR, Barrios C, Evora K, et al. Targeting the degradation of angiotensin II with recombinant angiotensin-converting enzyme 2: prevention of angiotensin II–dependent hypertension. Hypertension. 2010;55(1):90–8.
12. Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature. 2020;581(7807):215–20.
13. Jia H, Neptune E, Cui H. Targeting ACE2 for COVID-19 Therapy: Opportunities and Challenges. Am J Respir Cell Mol Biol. 2021;64(4):416–25.
14. Devaux CA, Rolain J-M, Raoult D. ACE2 receptor polymorphism: Susceptibility to SARS-CoV-2, hypertension, multi-organ failure, and COVID-19 disease outcome. J Microbiol Immunol Infect. 2020;53(3):425–35.
15. Li G, He X, Zhang L, Ran Q, Wang J, Xiong A, et al. Assessing ACE2 expression patterns in lung tissues in the pathogenesis of COVID-19. J Autoimmun. 2020;112:102463.
16. Al-Benna S. Association of high level gene expression of ACE2 in adipose tissue with mortality of COVID-19 infection in obese patients. Obes Med. 2020;19:100283.
17. Rahman MM, Hasan M, Ahmed A. Potential detrimental role of soluble ACE2 in severe COVID□19 comorbid patients. Rev Med Virol. 2021.
18. Lei C, Qian K, Li T, Zhang S, Fu W, Ding M, et al. Neutralization of SARS-CoV-2 spike pseudotyped virus by recombinant ACE2-Ig. Nat Commun. 2020;11(1):2070.
19. Rieder M, Wirth L, Pollmeier L, Jeserich M, Goller I, Baldus N, et al. Serum ACE2, Angiotensin II, and Aldosterone Levels Are Unchanged in Patients With COVID-19. Am J Hypertens. 2021 Mar 1;34(3):278–81.
20. Nagy Jr B, Fejes Z, Szentkereszty Z, Sütő R, Várkonyi I, Ajzner É, et al. A dramatic rise in serum ACE2 activity in a critically ill COVID-19 patient. Int J Infect Dis. 2021;103:412–4.
21. Emilsson V, Gudmundsson EF, Aspelund T, Jonsson BG, Gudjonsson A, Launer LJ, et al. ACE2 levels are altered in comorbidities linked to severe outcome in COVID-19. MedRxiv. 2020;
22. Avanoglu Guler A, Tombul N, Aysert Yıldız P, Özger HS, Hızel K, Gulbahar O, et al. The assessment of serum ACE activity in COVID-19 and its association with clinical features and severity of the disease. Scand J Clin Lab Invest. 2021;81(2):160–5.