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Clinical Research Article

A Randomized Trial of Sitagliptin and Spironolactone With Combination Therapy in Hospitalized Adults With COVID-19

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Abstract

Context: COVID-19 may cause respiratory distress syndrome and death. Treatment of COVID-19 to prevent complications remains a priority.

Objective: Our investigation sought to determine whether combination of spironolactone and sitagliptin could reduce mortality for inpatients with SARS-CoV-2 infection.

Methods: This single-blind, 4-arm, prospective randomized clinical trial was conducted at Shiraz and Bushehr University of Medical Sciences hospitals between December 2020 and April 2021. We randomized hospitalized adult patients with COVID-19 pneumonia into 4 groups: control, combination therapy, sitagliptin add-on, or spironolactone add-on. The primary outcome was the clinical improvement of the patients in the hospital as measured on an 8-point numerical scale. The secondary outcomes included intubation, ICU admission, end organ damages, CT findings, and paraclinical information.

Results: A total of 263 admitted patients were randomly assigned to control group (87 patients), combination group (60 patients), sitagliptin group (66 patients), and spironolactone group (50 patients). There were no significant differences in baseline characteristics, except for higher age in control group. The intervention groups, especially combination therapy, had better clinical outcomes (clinical score on fifth day of admission: 3.11 ± 2.45 for controls, 1.33 ± 0.50 for combination, 1.68 ± 1.02 for sitagliptin, and 1.64 ± 0.81 for spironolactone; $P = 0.004$). However, the mortality rate was lower in patients who received spironolactone (21.84% control, 13.33% combination, 13.64% sitagliptin, 10.00% spironolactone; $P = 0.275$). Our intervention reduced lung infiltration but not the area of involvement in lungs.

Conclusion: Sitagliptin and spironolactone can potentially improve clinical outcomes of hospitalized COVID-19 patients.

Key Words: COVID-19, ACE2, spironolactone, DPP4 inhibitor

Abbreviations: ACE2, angiotensin-converting enzyme 2; ADAM17, disintegrin and metalloproteinase domain-containing protein 17; BMI, body mass index; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; CT, computed tomography; DPP4, dipeptidyl peptidase-4; ESR, erythrocyte sedimentation rate; ICU, intensive care unit; IL-6, interleukin-6; INR, international normalized ratio; LDH, lactate dehydrogenase; PCR, polymerase chain reaction; PT, prothrombin time; PTT, partial thromboplastin time; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane protease serine 2, WHO, World Health Organization.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of coronavirus disease 2019 (COVID-19), has generated huge workloads for healthcare facilities since late 2019. It also has produced devastating global economic impacts. Despite the remarkable progress that has been made in treating COVID-19 patients, we are still facing significant mortality and hospitalization. The convalescent plasma transfusion therapy seemed to have promising results [1, 2], but its benefits on outcomes are still controversial [3-5].

The SARS-CoV-2 can bind to human cells primarily through the transmembrane protein angiotensin-converting enzyme 2 (ACE2) [6] and, presumably, dipeptidyl peptidase 4 (DPP4) [7, 8]. The main barrier of virus replication is cell entry. The affinity of the virus to cell receptors is an important

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factor that determines infectivity, viral replication, and severity of the disease in humans [9]. In addition, reducing the coronavirus viral load in organs such as lungs or other tissues can potentially reduce the disease severity and mortality, either by applying antiviral therapy, namely remdesivir [10], or blocking viral entrance through ACE2 and DPP4.

ACE2 is the main receptor of SARS-CoV-2, and it has 2 forms—soluble and membrane bound. The host proteases, including transmembrane serine protease 2 (TMPRSS2), play a crucial role in priming through proteolytic activation of the viral spike protein, which is an important step of virus entry after binding to ACE2 receptor [11, 12]. Furthermore, the binding of SARS-CoV-2 to soluble ACE2 changes the biophysics of the viral particles by increasing their weight and radius, leading to a higher chance of virus entry into the cells [13]. Generally, virus entry into the host cells and further replication are major determinants of SARS-CoV-2 infectivity and clinical deterioration. Therefore, targeting soluble ACE2 and entry cofactors can potentially mitigate the risk of virus entry into the cells and improve clinical outcomes. In terms of membrane-bound ACE2 function, it is important to mention that the AT1 inhibitor losartan could reduce inflammation due to spike protein [14]. The entrance of SARS-CoV-2 into the cells is associated with reduced ACE2 expression and increased inflammatory responses [15, 16]. ACE2 on cell membrane reduces the amount of angiotensin-II (AT1R stimulator) and increases Ang [1-7], which has similar effects as losartan. As a result, ACE2 on cell membranes seems to have a protective role against SARS-CoV-2-mediated lung injury by reducing inflammation.

Spironolactone is a mineralocorticoid receptor blocker that reduces ACE2 plasma level [17] but upregulates ACE2 expression on cell membranes [18]. It also has an anti-androgenic action that may affect the expression of TMPRSS2 entry cofactors [19, 20]. Thus, spironolactone can potentially reduce viral entry by reducing soluble ACE2 and antagonizing TMPRSS2, in addition to protecting the cell membrane from further damage by increasing ACE2 expression.

Moreover, DPP4 inhibitors are oral medications for diabetes that have immunomodulatory roles [21]. The interaction between spike glycoprotein of SARS-CoV-2 and DPP4 (also known as CD26) may signify the role of DPP4 inhibitors (eg, sitagliptin) in preventing this interaction and improving clinical outcomes of COVID-19 [7, 22]. Although the affinity of SARS-CoV-2 for DPP4 is not as high as its affinity to ACE2 [23], during the acute infection viral replication may overwhelm the ACE2 receptors and attach to DPP4, resulting in further replication and tissue injury. It has been reported that DPP4 inhibitors could reduce mortality and intubation risk in COVID-19 patients with diabetes [24, 25].

In this study, given the roles of ACE2 and DPP4 in coronavirus cell entry, we hypothesized that the mineralocorticoid receptor blocker spironolactone in combination with sitagliptin could potentially impede the entrance of the coronavirus into the cells without serious complications, and thus reduce mortality and complications of COVID-19.

Methods

Study Design and Population

Our study was a single-blind, 4-arm, prospective randomized clinical trial (IRCT registration number: IRCT20201003048904N2), conducted at Shiraz University of Medical Sciences (SUMS) Hospital (Faghihi Hospital) and Bushehr University of Medical Sciences (BUMS) Hospital (Shohadaye Khalije Fars Hospital) between December 2020 and April 2021. These hospitals are institutionalized, and care was provided by attending physicians, residents, and medical students. The residents and medical students closely observed the patients during their hospital stay. The attending physicians visited the patients daily and supervised the staff, including residents and medical students. We enrolled adult patients, at least 20 years of age, who were admitted to the hospital for COVID-19 treatment. The eligible patients had laboratory-confirmed SARS-CoV-2 infection (nasal/throat swabs positive for SARS-CoV-2 by reverse transcriptase polymerase chain reaction [PCR]) or positive history of exposure to COVID-19 patients besides typical pattern of viral pneumonia on high-resolution computed tomography (CT) and characteristic clinical manifestations. We excluded patients who needed intubation or intensive care unit (ICU) admission at the presentation and those who had active malignancy or severe immune deficiency. In addition, we excluded patients who were taking spironolactone (or other mineralocorticoid blockers) and/or DPP4 inhibitors before hospital admission. We did not exclude patients with organ failure, such as cirrhosis, end-stage renal disease, etc. Upon the decision of the research physicians (Yasaman Mansoori and Mehdi Hajiani at the Shiraz University of Medical Sciences and Farzan Azodi, Shayesteh Davoudi and Farzana Rezaei at the Bushehr University of Medical Sciences), the eligible patients were identified through screening and randomization was made with an online software (https://www.random.org/). Eligible patients were randomized into 4 intervention groups (A, B, C, D). All groups received the standard treatment for COVID-19 (dexamethasone, methylprednisolone, remdesivir, colchicine, antiplatelet and/or anticoagulants) according to the protocol designed by their institutions. Group A received standard treatment, Group B received standard treatment plus spironolactone 100 mg daily and sitagliptin 100 mg daily, Group C received standard treatment plus sitagliptin 100 mg daily, and Group D received standard treatment and spironolactone 100 mg daily. We compared the clinical outcomes, including mortality, intubation, ICU admission, end organ damages, and duration of hospitalization, between the groups. The attending physicians (Mohammad Ali Davarpanah, Mohsen Moghadami and Farhad Abbasi) supervised the enrollment process and eligibility. We aimed to treat the patients for at least 2 days and assess the clinical conditions on the fourth to fifth day of admission to correlate the relationship between intervention and outcome. Therefore, patients were disqualified and removed from the study when they stopped medications in less than 2 days or had already left the hospital without improvement and against medical advice in less than 4 days (Fig. 1). The patients were generally treated until recovered and discharged from the hospital.

Clinical Data

The research physicians were trained before the study procedures. The research physician collected and recorded baseline characteristics, medical history, physical exams, medications, comorbidities, clinical conditions, hospital courses, complications, and deaths using Microsoft Excel. We extracted data through medical records, or by history provided by the patients,

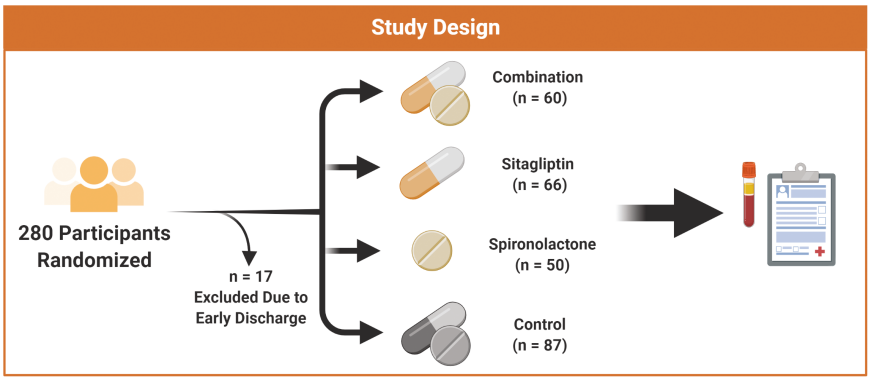

Figure 1. Flow chart of study. A total of 280 (204 from Shiraz and 76 from Bushehr) participants were enrolled. We excluded 17 patients because of early discharge. Combination group had 60 (51 from Shiraz and 9 from Bushehr), sitagliptin group had 66 (38 from Shiraz and 28 from Bushehr), spironolactone group had 50 (39 from Shiraz and 11 from Bushehr) and control group had 87 (59 from Shiraz and 28 from Bushehr) participants. Figure was created by BioRender.com.

or through direct observation of research/attending physicians. The comorbidities included obesity (body mass index [BMI] > 30 kg/m^2), dyslipidemia (on medication, low-density lipoprotein > 100 mg/dL or triglyceride > 200 mg/dL), diabetes (on medication or hemoglobin A1C > 6.5%), hypertension (on medication or blood pressure > 140/90 mmHg), renal disease, liver disease, lung disease, heart disease, nervous system disease, immune deficiency, malignancy, thyroid disease (hyperthyroidism or hypothyroidism), polycystic ovary syndrome, hypogonadism, sleep apnea, or other medical problems. The research/attending physicians evaluated the admitted patients during the study on clinical endpoints. We used a modified World Health Organization (WHO) clinical progression scale [26] to determine the severity of clinical illness from uninfected to death on the first and fifth day of admission. The research physicians scored the patients ranged from 0 (uninfected) to 8 (death) (Table 1).

Paraclinical Data

The routine laboratory measurements, including complete blood count (none missing on the first day and 1 missing on the fifth day), white blood cell differential (1 missing on the first day and 1 missing on the fifth day), complete metabolic panel (1 missing on the first day and 2 missing on the fifth day), lactate dehydrogenase (LDH) (2 missing on the first day), creatine kinase (creatinine phosphokinase; CPK) (3 missing on the first day), erythrocyte sedimentation rate (ESR) (27 missing on the first day), C-reactive protein (CRP) (1 missing on the first day and 53 missing on the fifth day), prothrombin time (PT) (2 missing on the first day), partial thromboplastin time (PTT) (3 missing on the first day), international normalized ratio (INR) (2 missing on the first day), oxygen saturation (none missing), PaO_2 (2 missing on the first day), $PaCO_2$ (2 missing on the first day), D-dimer (9 missing on the first day and 55 missing on the fifth day) were done at the hospital laboratories. The research physicians monitored data collection and data validation. We measured D-dimer using an IMMULITE 2000 Systems Analyzers, solid-phase, two-site, chemiluminescent enzyme immunometric assay (Siemens, Catalog # L2KDD2, RRID: AB_2904264, https://scicrunch.org/resources/Any/search?q=AB_2904264&l=AB_2904264). The reportable range was 100 to 15 000 ng fibrinogen equivalent units (FEU)/mL. We measured cytokine interleukin 6 (IL-6) (3 missing on the first day and 67 missing on the fifth day) in the serum of COVID-19 patients using an IMMULITE 2000 Systems Analyzers, solid-phase, enzyme-labeled, chemiluminescent sequential immunometric assay (Siemens, Catalog # L2K6P2, RRID: AB_2904178, https://scicrunch.org/resources/Any/search?q=AB_2904178&l=AB_2904178). The calibration range was up to 1000 pg/mL and analytical sensitivity was 2 pg/mL. All data were measured on the day of admission and 5 days after receiving any of the treatment regimen, except for LDH, CPK, ESR, PT, PTT, INR, and $PaCO_2$, which were only measured on the day of admission (Table 2).

CT Findings

The high-resolution CT was performed at the radiology department of the hospital at the time of admission (27 missing) and 4 to 6 days after treatment (43 missing). A total of 199 patients had both CT scans (123 patients in Shiraz and 76 patients in Bushehr) (Table 2). The radiology attending (Sepideh Sefidbakht) at the Shiraz University of Medical Sciences (Faghihi Hospital) reported the percentage of involved area, including ground-glass opacifications (hazy areas of increased attenuation), crazy-paving pattern, consolidations (homogeneous opacification of the parenchyma), and linear opacities (coarse linear, curvilinear opacities, subpleural reticulation, interlobular septal thickening, parenchymal reticulation, fibrosis, and bronchial wall thickening), before and after treatment. Among 187 enrolled patients, 126 individuals (67.4%) had both CT scans (38 control, 37 combination, 27 sitagliptin, and 24 spironolactone). The infectious diseases attending (Farhad Abbasi) at the Bushehr University of Medical Sciences (Shohadaye Khalije Fars Hospital) reported the percentage of involved area with opacifications, either ground-glass opacities, crazy-paving pattern, or consolidation, before and after treatment. All 76 patients had both CT scans (28 control, 9 combination, 28 sitagliptin, and 11 spironolactone). Both CT readers estimated the visualized area of involvement and reported the CT results blindly without knowing which group the patients were in.

The ethics committees of Shiraz University of Medical Sciences (IR.SUMS.MED.REC.1399.550) and Bushehr University of Medical Sciences (IR.BPUMS.REC.1399.140) approved the study. We followed the declaration of Helsinki and Iranian national guidelines for ethics in research to design the study. The research physicians de-identified the patients'

Table 1. Clinical progression scale

Patient condition	Description	Score
Uninfected	No clinical or virological evidence of infection	0
Ambulatory with PCR + or exposure besides CT finding consistent with COVID-19	No limitation of activities	1
	Limitation of activities	2
Hospitalized mild disease	No oxygen therapy	3
	Oxygen by mask or nasal prongs	4
Hospitalized severe disease	Noninvasive ventilation or high flow oxygen	5
	Intubation and mechanical ventilation	6
	ECMO	7
	Death	8

Abbreviations: CT, computed tomography; ECMO, extracorporeal membrane oxygenation; PCR, polymerase chain reaction.

Table 2. Missing data (number of cases without the test on the first or fifth day of admission)

Variable	First day, missing cases	Fifth day, missing cases
Chest CT	27	64
Complete blood count	0	1
White blood cell differential	1	1
Complete metabolic panel	1	2
LDH	2	Not measured
CPK	3	Not measured
ESR	27	Not measured
CRP	1	53
D-dimer	9	55
IL-6	3	67
PT	2	Not measured
PTT	3	Not measured
INR	2	Not measured
Oxygen saturation	0	0
PaO_2	2	0
$PaCO_2$	2	Not measured

Abbreviations: CPK, creatine kinase (creatinine phosphokinase); CRP, C-reactive protein; CT, computed tomography; ECMO, extracorporeal membrane oxygenation; ESR, erythrocyte sedimentation rate; IL-6, interleukin-6; INR, international normalized ratio; LDH, lactate dehydrogenase; PCR, polymerase chain reaction; PT, prothrombin time; PTT, partial thromboplastin time.

information after collecting the data. The enrollees had a code to re-identify. The University of Kentucky received the de-identified information for statistical analysis and writing the manuscript.

The research physicians had routinely collected a written formal informed consent at the time of admission. They had explained the purpose of study, benefits and risks of medications and any kind of interventions. We excluded patients who had not signed the formal informed consent, refused taking medications, and declined blood sampling, imaging, or any kind of participation, or who expressed opposition to data collection.

The Faghihi Hospital at the Shiraz University of Medical Sciences, Shohadaye Khalije Fars Hospital at Bushehr University of Medical Sciences sponsored the study.

Statistical Analysis

We compared baseline characteristics of the admitted patients using chi-square tests, Fisher's exact tests, or ANOVA tests, as appropriate, to determine whether the randomization into the treatment groups were unbalanced at baseline. These characteristics were considered for use in covariate-adjusted modeling techniques to determine which variables explained the survival or hospital discharge after being admitted into the hospital. The main outcome for this analysis is survival, which is characterized by hospital discharge. A patient may get well and be discharged, or a patients' condition may worsen and require admission into the ICU, potentially leading to intubation, death, or recovery. The analysis of clinical outcomes 5 days after admission into the hospital were presented. Kaplan–Meier survival curves for time to hospital discharge were generated comparing the treatment groups with the standard therapy (control intervention).

We implemented a logistic regression model for hospital discharge accounting for covariates that may influence survival of patients receiving any of the intervention treatments (standard therapy, combination, sitagliptin, and spironolactone). We presented the odds ratio and its 95% CI with its associated *P* value for each of the covariates in the model. To account for the number of days the admitted patients were hospitalized and the

Table 3. Baseline characteristics of the study population

Characteristics	Control group, standard therapy (87): Mean ± SE or n (%)	Combination group, spironolactone + sitagliptin + standard therapy (60): Mean ± SE or n (%)	Sitagliptin group, sitagliptin + standard therapy (66): Mean ± SE or n (%)	Spironolactone group, spironolactone + standard therapy (50): Mean ± SE or n (%)	*P* value
Age (years)	**60.91 ± 15.98**	53.73 ± 15.98	58.68 ± 17.10	53.14 ± 17.35	**0.018**
≥ 70 years of age, n (%)	24 (27.59)	10 (16.67)	16 (24.24)	10 (20.00)	0.438
Male sex, n (%)	44 (50.57)	29 (48.33)	30 (45.45)	30 (60.00)	0.459
Clinical Score (0-8)[a]	4.23 ± 0.64	4.28 ± 0.52	4.18 ± 0.63	4.18 ± 0.48	0.743
Time from symptom onset, days	8.90 ± 4.70	9.22 ± 6.76	8.25 ± 3.91	8.22 ± 4.41	0.627
BMI (kg/m^2)	26.22 ± 5.04	28.21 ± 6.56	26.46 ± 4.36	26.66 ± 5.59	0.144
Smoking, n (%)	4 (4.60)	6 (10.00)	11 (16.67)	10 (20.00)	0.026
Alcohol consumption, n (%)[b]	2 (2.30)	0	1 (2.00)	1 (2.00)	0.836
Coexisting conditions, n (%)[c]					
BMI > 30	13 (14.94)	16 (26.67)	15 (27.73)	10 (20.00)	0.354
Diabetes	24 (27.59)	13 (21.67)	17 (25.76)	14 (20.59)	0.848
Hypertension	29 (33.33)	23 (38.33)	16 (24.24)	14 (26.00)	0.343
Cardiovascular disease	22 (25.29)	7 (11.65)	14 (21.21)	7 (14.00)	0.148
Chronic kidney disease	7 (8.05)	1 (1.65)	3 (4.56)	3 (6.00)	0.415
Chronic pulmonary disease	6 (6.90)	1 (1.67)	5 (7.58)	6 (12.00)	0.179
Cancer	7 (8.05)	2 (3.33)	3 (4.55)	2 (4.00)	0.659
Immune deficiency (transplant etc.)	4 (4.60)	1 (1.67)	2 (3.03)	2 (4.00)	0.830
Neurologic disorders	7 (8.05)	4 (6.67)	3 (4.55)	1 (2.00)	0.534
Fever (temperature °C), n (%)	36.89 ± 0.80	36.30 ± 3.90	37.07 ± 0.80	37.00 ± 0.92	0.1423
Respiratory rate (breaths/min) on admission	20.48 ± 3.46	20.58 ± 2.99	20.62 ± 3.31	20.24 ± 2.19	0.440
Heart rate (beat/min)	91.48 ± 14.80	93.13 ± 14.43	91.35 ± 15.69	89.50 ± 14.61	0.655
Hypotension (systolic blood pressure ≤ 90 mmHg), n (%)	5 (5.75)	3 (5.00)	3 (4.55)	1 (2.00)	0.826
Mean O_2 saturation on admission	85.37 ± 8.20	84.55 ± 7.70	85.68 ± 7.77	85.30 ± 6.58	0.867
Percentage of chest CT involvement on admission[d] (Shiraz)	38.11 ± 23.82	45.00 ± 26.98	45.56 ± 18.47	41.74 ± 20.87	0.528
Percentage of chest CT involvement on admission[d] (Bushehr)	39.29 ± 22.60	**75.00 ± 11.99**	55.00 ± 21.82	44.55 ± 19.55	**< 0.001**
Medications					
Glucose-lowering medications, n (%)					
Metformin	12 (13.79)	5 (8.33)	9 (13.64)	6 (12.00)	0.759
Insulin	5 (5.75)	2 (3.33)	6 (9.09)	3 (6.00)	0.653
Other oral antidiabetic agents (DPP4 inhibitors excluded)		1 patient	2 (3.03)	2 patients	0.676
Antihypertensive drugs, n (%)					
ACE inhibitors	6 (6.90)	4 (6.67)	8 (12.12)	4 (8.00)	0.893
ARB	13 (14.94)	10 (16.67)	4 (6.06)	8 (16.00)	0.502
Beta-blockers	11 (12.64)	4 (6.67)	1 (1.52)	5 (10.00)	0.550

Table 3. Continued

Characteristics	Control group, standard therapy (87): Mean ± SE or n (%)	Combination group, spironolactone + sitagliptin + standard therapy (60): Mean ± SE or n (%)	Sitagliptin group, sitagliptin + standard therapy (66): Mean ± SE or n (%)	Spironolactone group, spironolactone + standard therapy (50): Mean ± SE or n (%)	*P* value
Diuretics (spironolactone excluded)	3 (3.45)	1 (1.67)	13 (19.70)	3 (6.00)	0.497
Antiplatelet drugs, n (%)	15 (17.24)	6 (10.00)	1	8 (16.00)	-
Anticoagulant drugs, n (%)	0	0	8 (12.12)	0	0.744
Statin drugs, n (%)	16 (18.39)	8 (13.33)		7 (14.00)	
Laboratory findings on 5th day of admission					
Glycemia (mg/dL)[e]	145.05 ± 85.80	145.65 ± 96.71	150.47 ± 87.15	146.12 ± 79.84	0.983
Serum creatinine (mg/dL)	1.33 ±1.28	1.04 ± 0.34	1.12 ± 0.58	1.30 ± 1.73	0.357
Hemoglobin g/dL	12.61 ± 1.90	12.63 ± 2.07	12.44 ± 2.03	12.35 ± 2.18	0.896
White blood cell count, (× 10^{-9}/L)	9.32 ± 10.31	7.46 ± 4.08	8.68 ± 4.42	8.18 ± 4.24	0.432
Neutrophil percentage	75.84 ± 18.18	74.58 ± 16.86	74.68 ± 17.21	78.70 ± 9.45	0.535
Lymphocyte percentage	16.25 ± 18.04	18.12 ± 13.93	16.28 ± 9.36	14.64 ± 7.83	0.443
Platelet count (× 10^{-9}/L)	215.57 ± 81.73	214.27 ± 90.45	239.39 ± 106.48	223.08 ± 80.19	0.346
INR	1.23 ± 0.24	1.23 ± 0.20	1.17 ± 0.20	1.30 ± 0.34	0.065
AST (units/L)	52.15 ± 25.80	53.83 ± 27.39	52.25 ± 28.07	54.02 ± 28.25	0.968
ALT (units/L)	51.24 ± 37.32	55.12 ± 66.33	45.92 ± 28.30	56.28 ± 40.99	0.578
CPK (units/L)	183.26 ± 220.05	233.43 ± 233.61	182.03 ± 192.06	157.20 ± 207.18	0.294
LDH (units/L)	668.10 ± 300.24	709.42 ± 241.62	632.76 ± 298.71	628.67 ± 273.63	0.378
ESR (mm/h)	49.62 ± 28.10	52.91 ± 30.99	52.32 ± 30.03	46.42 ± 25.85	0.679
CRP (mg/L)	62.55 ± 28.62	65.96 ± 25.39	66.09 ± 38.79	59.49 ± 26.45	0.618
D-dimer (mg/mL)	818.04 ± 1405.91	1048.50 ± 1710.93	535.83 ± 631.28	**1257.70 ± 1840.31**	**0.049**
Interleukin-6 (ng/L)	48.80 ± 151.44	27.93 ± 56.46	28.12 ± 46.07	28.01 ± 43.22	0.441

Data are mean ± SEM unless otherwise indicated.
Abbreviations: ACE, angiotensin-converting enzyme; ALT, alanine aminotransferase; ARB, angiotensin receptor blocker; AST, aspartate aminotransferase; BMI, body mass index; CPK, creatine kinase (creatinine phosphokinase); CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; IU, international units; n, number.
[a]Modified WHO clinical scores (Table 1).
[b]Alcohol drinking is defined as consuming ≥ 5 drinks per week for men and ≥ 4 drinks per week for women.
[c]The coexisting disorders include obesity (BMI > 30), diabetes (on medication or hemoglobin A1C > 6.5), hypertension (on medication or blood pressure > 140/90), renal disease, liver disease, lung disease, heart disease, nervous system disease, immune deficiency, malignancy, or other diseases (hypothyroidism, hyperthyroidism, polycystic ovary syndrome, hypogonadism, sleep apnea, etc.).
[d]Percentage of chest CT involvement: Shiraz area of involvement with and without opacification, Bushehr area of involvement with opacification.
[e]Glycemia (mg/dL) is mean blood glucose level that was calculated based on the first day measurements of random blood glucose.

effect of other covariates, we fitted Cox proportional hazards models to estimate the risk of hospital death from SARS-CoV-2 among the treatment groups through hazard ratios (HR). We implemented a Cox proportional hazard survival model for the time-to-event variable: hospital death after SARS-CoV-2 hospitalization that is right-censored by hospital discharge. That is, the time it takes the hospitalized Covid patients (who were randomized into 4 treatment regimens) to be discharged from the hospital as survivors. The general form of the Cox proportional hazard model at time t is: $\lambda_i(t) = \lambda_0(t) \cdot e^{-X_i'\beta}$ where $\lambda_0(t)$ is the baseline hazard at time t, X is the set of covariates, β is the set of parameters corresponding to each covariate X and $\lambda_i(t)$ is the hazard for an individual i at time t. The Cox model examines the effects of the covariates on hospital survival after admission. Positive coefficients indicate lower mortality risk (higher survival) and negative coefficients indicate higher mortality risk (lower survival).

The models were fitted using SAS logistics and PHreg procedures. All analyses were performed using SAS Version 9.4 (TS1M1 SAS Institute Inc., Cary, NC) and R statistical software. We used standard 5% significance level for testing our entire hypothesis. This means that we reject the null hypothesis for small values. The study was approved by the Institutional Review Boards of the University of Kentucky, the Bushehr University Medical Sciences, and Shiraz University of Medical Sciences.

Results

Patient Characteristics

A total of 263 patients were admitted with COVID-19 infection (187 Shiraz and 76 Bushehr). The majority of them had positive PCR test results for COVID-19, except for 15 (5.7%) patients who had history of exposure to COVID-19 with typical

clinical and radiologic findings consistent with COVID-19 infection. Of the total patients, 176 were treated with sitagliptin, spironolactone, or a combination of both, as add-on therapy to standard therapy, while 87 patients received just standard therapy (Fig. 1). The baseline characteristics of the 4 groups are shown in Table 3. The groups did not have major differences in terms of demographic characteristics, except for mean age, which was higher in the control group, percentage of chest CT involvement with opacification, which was higher in the combination group in Bushehr, and D-dimer, which was higher in the spironolactone group. All patients had respiratory symptoms and were enrolled into the study if they were eligible based on the decision made by the attending physicians. However, we ended up excluding 17 patients as they stopped medications or left the hospital against medical advice in fewer than 4 days, which means the noncompliance rate was 6%. (Fig. 1). No differences were observed with regard to comorbidities, clinical findings, inflammatory markers, and medication history, which can affect the outcomes of diseases (Table 3). Assessment of clinical score by research physicians and time of onset of symptoms at the time of admission did not show significant differences among the groups. There were no statistically significant differences in parameters such as fever, respiratory rates, heart rate, and oxygen saturation on the first day.

Clinical Outcomes

Patient treated with spironolactone, sitagliptin, or combination (intervention groups) added on to standard therapy (control group) had better clinical outcomes than with standard therapy alone: the intervention group had significantly better clinical scores after 5 days ($P = 0.004$). In addition, mortality ($P = 0.275$), ICU admission rate ($P = 0.469$), intubation rate ($P = 0.405$), and the incidence of end organ damage (acute respiratory failure, acute kidney injury, and elevated liver enzymes), were lower in the intervention group, but without significant P values. The intervention group also had higher oxygen saturation on the fifth day of admission ($P = 0.174$) (Table 4). Mortality was lower in patients who received spironolactone compared to sitagliptin and combination. However, other clinical parameters, such as clinical score, ICU admission, and intubation rate were more improved in the combination therapy group than in the other intervention groups. Duration of hospitalization was not significantly different between the groups (Table 4).

Laboratory Analysis and CT Findings

The sitagliptin recipients had lower levels of D-dimer ($P = 0.005$), as well as CRP ($P = 0.09$) and IL-6 ($P = 0.185$), but no significant differences in the complete blood count were seen among the groups. With regard to CT findings, Shiraz investigators reported the percentage of involvement with or without opacification and there was no significant improvement in the intervention group after 4 to 6 days of intervention ($P = 0.735$). However, Bushehr investigators estimated the area of involvement with opacification, and they saw significant improvement in all intervention groups, especially the combination group ($P < 0.001$).

Subgroup Analysis and Group Comparison

A subgroup analysis showed that patients who received spironolactone ($P = 0.028$), sitagliptin ($P = 0.157$) and combination therapy ($P = 0.220$) had better clinical outcomes, respectively, than the control group (Table 5 and Fig. 2). Compared to the standard therapy, those on combination, sitagliptin, and spironolactone treatments had better survival within the first 10 days of hospitalization period (Fig. 2). The probability of death was lower in the intervention groups during the hospital course and spironolactone appeared to be better at reducing death (Tables 4 and 5), especially within the first 10 days of hospitalization (Fig. 2). As the number of hospitalization days increases, survival decreases. By day 30, the probability of survival is close to zero (Fig. 2). In addition, we found in multivariate logistic regression model that older age (odds ratio [OR] 0.960 [95% CI 0.933, 0.987]; $P = 0.004$), male sex (OR 0.592 [95% CI 0.267, 1.314]; $P = 0.085$), higher BMI (OR 0.822 [95% CI 0.273, 2.478]; $P = 0.864$), cardiovascular disease (OR 0.428 [95% CI 0.157, 1.168]; $P = 0.159$), or cancer (OR 0.683 [95% CI 0.104, 4.470]; $P = 0.798$) were associated with lower survival. We present the estimates for the hazard ratio (Table 5) and OR when comparing the intervention groups while adjusting for risk factors (smoking, BMI > 30, diabetes mellitus, hypertension, cardiovascular disease, chronic obstructive pulmonary disease, cancer, immune deficiency, and neurologic disorders).

Discussion

The pandemic of COVID-19 has severely affected many countries, including Iran. Vaccination could effectively reduce COVID-19 cases, hospitalization, and deaths [27]. However, there is a concern regarding the availability of vaccines in developing countries and their effectiveness against certain variants of SARS-CoV-2 strains [28]. Furthermore, a large proportion of the population has not yet been exposed to the virus, which highlights the importance of an efficient therapeutic approach to reduce mortality and complication of COVID-19 [29]. Transfusion of convalescent plasma could reduce mortality through antiviral and immunomodulatory effects. Technically, the convalescent plasma carries neutralizing antibodies that have antiviral effects and can block virus entry into the cells. The immunomodulatory effects of convalescent plasma through cytokines and complement result in inhibition of immune system overactivity, cytokine storm, and hypercoagulability [30]. However, convalescent plasma therapy is recommended for severely ill patients and its therapeutic or prophylactic roles need further investigation. Moreover, it is associated with limitations, such as accessibility, adverse side effects, and the necessity of a coordinated approach involving clinical teams, blood banks, and sophisticated laboratories [31, 32]. As a result, effective and safe medications are necessary to reduce mortality and hospitalization of COVID-19.

In our study, spironolactone and combination spironolactone/sitagliptin therapy reduced mortality, ICU admission, intubation rate, and end organ damage but without statistical significance. However, they improved WHO score significantly. Soluble ACE2 seems to have detrimental role on infectivity and progression of COVID-19. It is known that increase in weight and radius of viral particle potentiates virus entry into the cells [13]. Infusion of the human recombinant soluble ACE2, which was shorter than native soluble ACE2, could effectively reduce the severity of disease [33, 34]. This indicates that shorter bioengineered soluble ACE2 competes with native soluble ACE2 in attaching to the virus, which leads to lighter and smaller viral

Table 4. Clinical outcomes in patients evaluated on fifth day of admission

Characteristics	Control group standard therapy (87): Mean ± SE or n (%)	Combination group spironolactone + sitagliptin + standard therapy (60): Mean ± SE or n (%)	Sitagliptin group sitagliptin + standard therapy (66): Mean ± SE or n (%)	Spironolactone group spironolactone + standard therapy (51): Mean ± SE or n (%)	*P* value
Mortality, n (%)	19 (21.84)	8 (13.33)	9 (13.64)	5 (10.00)	0.275
Clinical Score on 5th day of admission (0-8)[a]	**3.11 ± 2.45**	**1.33 ± 0.50**	**1.68 ± 1.02**	**1.64 ± 0.81**	**0.004**
Duration of hospitalization, days	9.44 ± 4.73	8.65 ± 5.03	8.77 ± 4.62	8.54 ± 5.70	0.690
ICU admission, n (%)	21 (24.14)	9 (15.00)	10 (15.38)	10 (20.00)	0.469
ICU average duration per person (days/person)	8.33 ± 6.48	7.44 ± 4.42	9.60 ± 6.11	10.00 ± 9.40	0.824
Intubation required, n (%)	18 (20.69)	7 (11.67)	8 (12.12)	7 (7 14.00)	0.405
Respiratory rate (breaths/min) on 5th day of admission	19.21 ± 2.23	19.56 ± 2.49	18.63 ± 3.11	19.12 ± 2.36	0.253
Hypotension (SBP ≤ 90) on 5th day of admission, n (%)	3 (3.45)	1 (1.67)	4 (6.06)	1 (2.00)	0.593
Mean O_2 saturation on 5th day of admission	85.69 ± 11.28	88.43 ± 10.09	88.32 ± 11.63	89.60 ± 9.86	0.174
Shock state[b]	3 (3.45)	2 (3.33)	-	-	0.277
Acute respiratory failure[c]	18 (20.69)	7 (11.67)	9 (13.64)	3 (6.00)	0.110
Acute kidney injury[d]	6 (6.90)	1 (1.67)	3 (4.55)	2 (4.00)	0.555
Elevated liver enzymes[e]	38 (43.68)	20 (33.33)	20 (30.30)	21 (42.00)	0.296
Percentage of chest CT involvement on 5th day of admission[f] Shiraz	40.12 ± 26.18	40.32 ± 26.71	48.45 ± 24.24	42.33 ± 24.56	0.529
Percentage of chest CT involvement on 5th day of admission[f] Bushehr	**35.89 ± 25.31**	**25.56 ± 11.30**	**20.71 ± 11.03**	**19.55 ± 4.72**	**0.007**
Percentage of chest CT changes between 1st and 5th day of admission[f] Shiraz	1.49 ± 16.41	-1.38 ± 14.38	-0.56 ± 14.63	2.39 ± 12.24	0.735
Percentage of chest CT changes between 1st and 5th day of admission[f] Bushehr	**-3.39 ± 21.56**	**-49.44 ± 12.86**	**-34.29 ± 19.04**	**-25.00 ± 18.71**	**<0.001**
Laboratory findings on 5th day of admission					
Serum creatinine (mg/dL)	1.36 ± 1.65	0.93 ± 0.26	1.08 ± 0.72	1.10 ± 1.37	0.172
Hemoglobin g/dl	13.42 ± 13.95	12.66 ± 3.02	12.16 ± 1.82	13.04 ± 2.07	0.818
White blood cell count, (× 10^{-9}/L)	11.07 ± 8.97	10.49 ± 4.07	11.62 ± 11.68	9.64 ± 3.37	0.613
Neutrophil percent	86.52 ± 89.99	77.01 ± 17.01	73.88 ± 19.01	76.87 ± 10.07	0.483
Lymphocyte percent	15.18 ± 12.40	14.52 ± 14.31	16.45 ± 9.45	15.28 ± 8.82	0.822
Platelet count (× 10^{-9}/L)	274.33 ± 119.47	299.13 ± 135.72	266.15 ± 114.56	305.90 ± 103.20	0.202
CRP (mg/L)	35.12 ± 38.18	35.80 ± 53.88	19.54 ± 24.06	34.06 ± 32.52	0.090
D-dimer (mg/mL)	**1099.41 ± 1820.44**	**1685.80 ± 2528.23**	**481.78 ± 810.27**	**673.49 ± 1138.43**	**0.005**
Interleukin-6 (ng/L)	21.03 ± 42.37	42.83 v 111.40	14.86 ± 34.55	22.90 ± 51.39	0.185

Data are mean ± SEM unless otherwise indicated.
Abbreviations: CRP, C-reactive protein; CT, computed tomography; LDH, lactate dehydrogenase; IU, international units; n, number; SBP, systolic blood pressure.
[a]Modified WHO clinical scores (Table 1).
[b]Shock state means low systolic blood pressure that required IV hydration, pack cell infusion, or vasopressors
[c]Acute respiratory failure means low oxygen saturation that required noninvasive ventilation (eg, nasal mask, face mask, or nasal plugs) or an invasive intervention (endotracheal tube, tracheostomy).
[d]Acute kidney injury means elevated creatinine ≥1.5 times the baseline value during the hospital course
[e]Elevated liver enzymes means elevated AST/ALT ≥3 times above normal value during the hospital course
[f]Percentage of chest CT involvement: Shiraz area of involvement with and without infiltration, Bushehr area of involvement with infiltration.

particles with less potency for cell entry. ACE2 shedding occurs mainly through activity of ADAM17 (disintegrin and metalloproteinase domain-containing protein 17; also known as TNF-alpha converting enzyme [TACE]) and blocking this enzyme can reduce ACE2 activity in plasma [35] and other body secretion. Mineralocorticoid receptor

Table 5. Cox proportional hazard model for COVID-19 patients admitted to hospital for 4 treatment groups

Variable	Estimate (SE)	*P* value	Hazard ratio
Combination vs Control	0.260(0.213)	0.220	1.297
Sitagliptin vs Control	0.266 (0.188)	0.157	1.305
Spironolactone vs Control	**0.442(0.201)**	**0.028**	**1.556**
Age	**-0.025(0.005)**	**<0.001**	**0.976**
Smoking	0.183(0.220)	0.405	1.201
BMI > 30	-0.223(0.177)	0.207	0.800
DM	0.238(0.174)	0.170	1.269
COPD	0.554(0.294)	0.059	1.741
Neurologic disorders	0.222(0.305)	0.466	1.249
Colchicine receiver	-0.242(0.257)	0.347	0.785
Heparin receiver	-0.170(0.212)	0.422	0.844
Antiviral receiver	-0.118(0.150)	0.431	0.889
Interferon receiver	-0.468(0.249)	0.060	0.626
Intubation	**-1.130(0.484)**	**0.020**	**0.323**
ICU duration	**-0.160(0.034)**	**<0.001**	**0.852**

A hazard ratio higher (lower) than 1 indicates higher (lower) survival.
Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; ICU, intensive care unit.

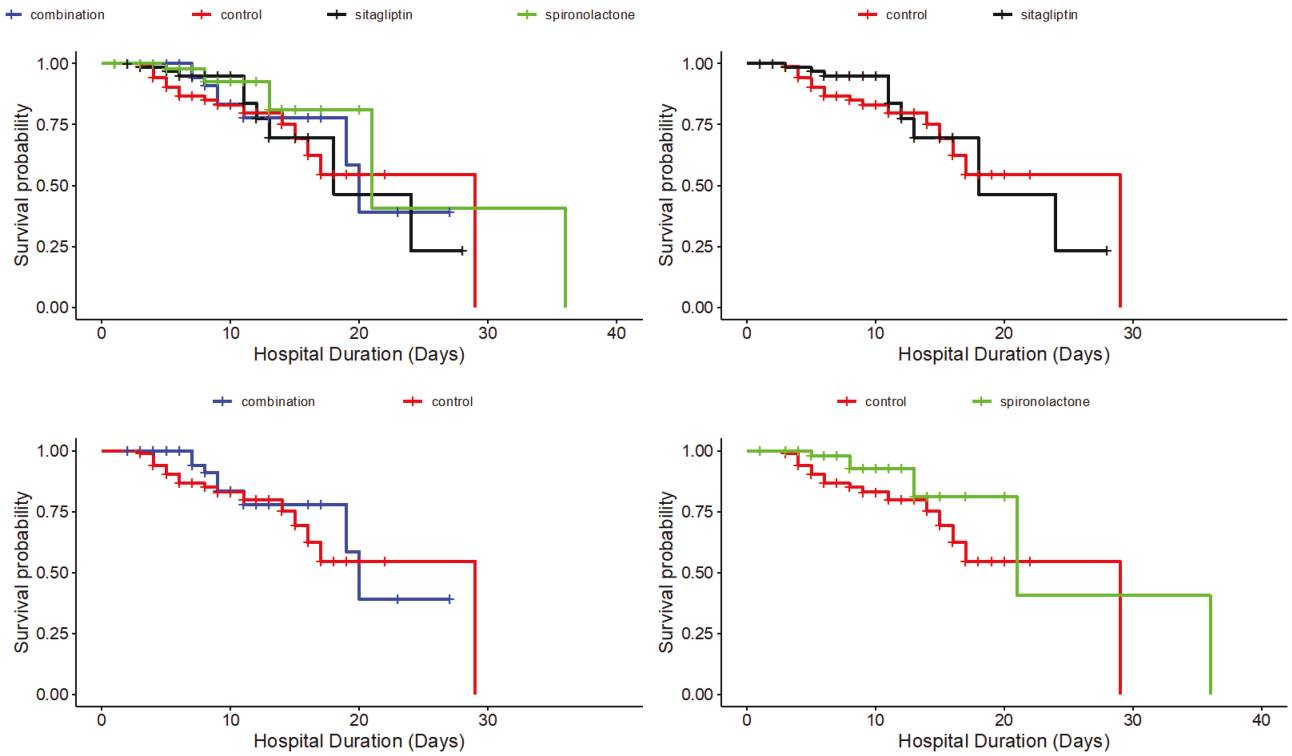

Figure 2. The Kaplan–Meier survival curves intersect, indicating that the difference between the survivals across the 4 therapies did not remain constant over the duration of patient hospitalization. It could also be inferred that the survival curves for patients on control treatment were mostly lower than the survival curves for patients on other therapies. Additionally, patients on sitagliptin, spironolactone, and combination therapies had a better survival within the first 10 days of hospitalization. Beyond 10 days of hospitalization, there were no significant differences in survival.

blockers can inhibit the ACE2 shedding by ADAM17 [36], reducing ACE2 plasma level [17], but increasing ACE2 expression on cell membrane by blocking aldosterone [37]. Thus, spironolactone can reduce virus entry by reducing soluble ACE2 and affecting the expression of TMPRSS2, an entry cofactor. In addition, it has a protective role against the SARS-CoV-2-mediated cell membrane injury by increasing ACE2 expression on membrane [14-16]. Accordingly, it has been reported that patients with liver cirrhosis who developed COVID-19 have had less exposure to spironolactone. It seems that spironolactone reduces susceptibility to COVID-19 in cirrhotic patients [38]. In addition, spironolactone and bromhexine combination shortened clinical recovery endpoints, such as temperature normalization,

hospitalization, and viral elimination times, better than the control group. However, there were no significant changes in CRP, lung damage on CT, and D-dimer values [39].

The use of sitagliptin, a DPP4 inhibitor, showed statistically nonsignificant improvements in clinical end points, such as mortality, WHO score, ICU admission, intubation rate, and end organ damage, without being superior to spironolactone and combination therapy. However, those who received sitagliptin therapy had reduced inflammatory markers. First, sitagliptin may improve the clinical outcomes by preventing the interaction between SARS-CoV-2 S-glycoprotein and DPP4 receptors. The structure analysis of binding site for SARS-CoV-2 spike protein predicted the possible interaction of many residues of COVID-19 spike glycoprotein with DPP4 (CD26) sequences [7, 8]. Second, DPP4 inhibitors have immunomodulatory roles. They have regulatory effects on immune functions, anti-inflammatory properties, and controversial effects on autoimmune and inflammatory diseases [21]. Thus, DPP4 inhibitors may antagonize the SARS-CoV-2 associated inflammation, as suggested by reduction of CRP and IL-6. In addition, the protective immune response to SARS-CoV-2, besides blocking viral entry into the cells, could potentially prevent the development of cytokine storm and massive destruction of tissues [24, 40].

The CT involvement in treated patients (in the combination group more than the others) improved in Bushehr. They have reported the involvement based on opacities, including ground-glass opacities, crazy-paving pattern or consolidation, which indicates that combination therapy could reduce the alveolar exudative lesions faster. However, the changes in CT findings in Shiraz patients were not remarkable. They have considered any kind of involvement, including any consolidation and linear opacities, which means the recovery process for interstitial edema and post-inflammatory fibrosis is much slower [41] and our intervention did not change the extent of involvement significantly. Chest CT findings are generally helpful for diagnosing and severity assessment in COVID-19 patients [42]. However, the current literature has limitations in terms of diagnosis and prognosis determination for pneumonia due to COVID-19 by applying chest radiographs and CT scans [43].

We must mention that our study has several limitations, including not having a placebo or double-blind design, the significant difference in age among the groups, higher D-dimer in spironolactone group, and higher percentage of involvement with opacification in chest CT in combination group on the first day of admission. Additionally, 15 (5.7%) patients had history of exposure to COVID-19 with typical manifestations but did not actually have positive test results for COVID-19. Moreover, the inequality of the number of participants among the groups, not having enough participants to achieve significant *P* values for secondary outcomes, including mortality, ICU admission, intubation rate, and end organ damage are further limitations. Likewise, the lack of some paraclinical data that were missing for some patients creates shortcomings for analysis. In particular, data for inflammatory markers and CT scans were important but could not be completely collected.

In conclusion, sitagliptin and spironolactone can possibly reduce mortality of admitted COVID-19 patients and improve the clinical outcomes.

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Author Contributions

Kamyar Asadipooya proposed the idea, designed the study, and wrote the manuscript. Farhad Abbasi and Mohammad Ali Davarpanah helped designi the study. Reuben Adatorwovor provided statistical analysis, wrote the statistical portions, and revised the manuscript. Sepideh Sefidbakht and Farhad abbasi read the CT scan results. Yasaman Mansoori, Mehdi Hajiani, Farzan Azodi, Shayesteh Davoudi, and Farzana Rezaei collected the data. Shayan Mohammadmoradi helped with designing the study and editing the manuscript. All authors approved the final version of the manuscript.

Disclosures

The authors have declared that no conflict of interest exists.

Iranian Registry of Clinical Trial Information

Iranian Registry of Clinical Trial registration number: IRCT20201003048904N2; registration date: December 10, 2020.

Data Availability

Shiraz University (M.A.D., Y.M., and M.H.) and Bushehr University (F.A., F.A., S.D., and F.R.) generated the data. University of Kentucky (S.M. and K.A.) had de-identified data and analyzed (R.A.) the data. The de-identified data are available for further investigations.

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