A Randomized Trial of Combination Therapy, Sitagliptin and Spironolactone, in Hospitalized Adult Patients with COVID-19

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Conflicts of Interest: The authors have declared that no conflict of interest exists.

Abstract Manuscript total Iranian Registry of Clinical Trial: IRCT registration number: IRCT20201003048904N2, Registration date: December 10, 2020.

Abbreviations

ACE2, angiotensin-converting enzyme 2; ADAM17, Disintegrin and metalloproteinase domain-containing protein 17; ARB, angiotensin receptor blocker; ARDS, acute respiratory distress syndrome; AT1R, Angiotensin II receptor type 1; BMI, body mass index; BUMS, Bushehr University of Medical Sciences, COVID-19, coronavirus disease 2019; CRP, C-reactive protein; DPP4, dipeptidyl peptidase-4; DIC, disseminated intravascular coagulation; LDH, lactate dehydrogenase; MR, mineralocorticoid receptor; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SUMS, Shiraz University of Medical Sciences; TMPRSS2, transmembrane protease serine 2, TNF, tumor necrosis factor.
Abstract

**Background:** COVID-19 may cause respiratory distress syndrome and death. Treatment of COVID-19 to prevent complications remains a priority. Our investigation sought to determine whether combination of spironolactone and sitagliptin could reduce mortality for inpatient with SARS-CoV-2 infection.

**Methods:** This single blind, 4-arms, 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 four 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 eight-point numerical scale. The secondary outcomes included intubation, ICU admission, end organ damages, CT findings and paraclinical information.

**Results:** 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 5th day of admission was 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; with p-value = 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-value = 0.275). Our intervention reduced lung infiltration but not the area of involvement in lung.

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

**Keywords:** COVID-19, ACE2, Spironolactone, DPP4 inhibitor
Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of coronavirus disease 2019 (COVID-19), has produced huge workloads for healthcare facilities since late 2019. It carries devastating global economic impacts too. Despite the remarkable progress that were 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 cell 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 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, which has two forms of soluble and membrane bound. The host proteases, including transmembrane serine protease 2 or 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 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 membrane seems to have 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 membrane (18). It also has 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 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 (CD26) may signify the role of DPP4 inhibitors (sitagliptin) in preventing this interaction and improving clinical outcomes of COVID-19 (7,22). Although the affinity of SARS-CoV-2 to DPP4 is not as high as its affinity to ACE2 (23), during the acute infection, viral replication may overwhelm the ACE2 receptors and endure through attaching to DPP4, which results 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 spironolactone (mineralocorticoid receptor blockers) and sitagliptin could potentially impede the entrance of the coronavirus into the cells without serious complications, reducing mortality and complications of COVID-19.

**Materials and Methods**

**Study design and population.** A single blind, 4-arms, 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, 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 RT-PCR) or positive history of exposure to COVID-19 patients besides typical pattern of viral pneumonia on high-resolution CT and characteristic clinical manifestations. We excluded patients who needed intubation or ICU admission at the
presentation, 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 four 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 4-5th 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 (Figure 1). The patient 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 mortalities using Microsoft excel. We extracted data through medical records, or by history provided by the patients or through direct observation of research/attending physicians. The comorbidities include obesity (BMI > 30), dyslipidemia (on medication, LDL > 100 or triglyceride > 200), 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, 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 modified 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 (no missing on first day and one missing on fifth day), white blood cell differential (one missing on first day and one missing on fifth day), complete metabolic panel (one missing on first day and two missing on fifth day), LDH (two missing on first day), CPK (three missing on first day), ESR (twenty seven missing on first day), CRP (one missing on first day and fifty three missing on fifth day), PT (two missing on first day), PTT (three missing on first day), INR (two missing on first day), oxygen saturation (no missing), PaO$_2$ (two missing on first day), PaCO$_2$ (two missing on first day), D-dimer (nine missing on first day and fifty five missing on fifth day) are done at the hospital laboratories. The research physicians monitored data collection and data validation. We measured D-Dimer using IMMULITE 2000 Systems Analyzers, solid-phase, two-site, chemiluminescent enzyme immunometric assay (Siemens, Catalog # L2KDD2, RRID: AB_2904264). The reportable range was 100 – 15,000 ng FEU/mL. We measured cytokine IL-6 (three missing on first day and sixty-seven missing on fifth day) in serum of COVID-19 patients using IMMULITE 2000 Systems Analyzers, solid-phase, enzyme-labeled, chemiluminescent sequential immunometric assay (Siemens, Catalog # L2K6P2, RRID: AB_2904178). The calibration range up to 1000 pg/ml and analytical sensitivity 2 pg/ml. All data were measured on the day of admission and 5 days after receiving any of the treatment regimen, except for complete metabolic panel, 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 (HRCT) was done at the radiology department of the hospital at the time of admission (27 missing) and 4-6 days after treatment (43 missing). On hundred and ninety-nine patients had both CT scans (123 patient 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 the patients’ group.

The ethics committee 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’ 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, 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 be further admitted 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% confidence interval 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 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 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 hospitalized covid patients (who were randomized into four treatment regimen) to survive and be discharged from the hospital. 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, \( \beta \) 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 examine the effects of the covariates on hospital survival after admission. Positive coefficients indicates 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 admitted with COVID-19 infection (187 Shiraz and 76 Bushehr). 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. The patients (176 persons) were treated with sitagliptin, spironolactone or combination of both as add-on therapy to standard therapy, while 87 patients received just standard therapy (Figure 1). The baseline characteristics of the four 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 advices in less than 4 days, which means the noncompliance rate of 6%. (Figure 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) add-on standard therapy (control group) had better clinical outcomes. The intervention group had better clinical scores after 5 days (P-value 0.004). They had lower mortality (P-value 0.275), ICU admission rate (P-value 0.469), intubation rate (P-value 0.405) and incidence of end organ damage (acute respiratory failure, acute kidney injury and elevated liver enzymes), but without significant P-values. They also had higher oxygen saturation on the 5th day of admission (P-value 0.174). 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 improved in combination therapy group better than other intervention groups. Duration of hospitalization was not significantly different between the groups (Table 4).

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

**Subgroup Analysis and Group Comparison.** A subgroup analysis showed that patients who received spironolactone (P-value 0.028), sitagliptin (P-value 0.157) and combination therapy (P-value 0.220) had better clinical outcomes respectively than control group (**Table 5 and Figure 2**). Compared to the standard therapy, those on combination, sitagliptin, and spironolactone treatments had better survival within the first 10 days of hospitalization period (**Figure 2**). The probability of death was lower in intervention groups during the hospital course and spironolactone seems to be better (**Table 4 and 5**), especially within the first 10 days of hospitalization (**Figure 2**). As the number of hospitalization dates increases, the instantaneous survival decreases. By day 30, the probability of survival is close to zero (**Figure 2**). In addition, we found in multivariate logistic regression model that older age (odds ratio 0.960 with 95% CI 0.933, 0.987; P-Value 0.004), male sex (odds ratio 0.592 with 95% CI 0.267, 1.314; P-Value 0.085), higher BMI (odds ratio 0.822 with 95% CI 0.273, 2.478; P-Value 0.864), cardiovascular disease (odds ratio 0.428 with 95% CI 0.157, 1.168; P-Value 0.159) or cancer (odds ratio 0.683 with 95% CI 0.104, 4.470; P-Value 0.798) were accompanied with lower survival. We presented the estimates for the hazard ratio (**Table 5**) and odds ratio when comparing the intervention groups while adjusting for the risk factors (smoking, BMI>30, DM, HTN, CVD, COPD, 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 bank and sophisticated labs (31,32). As a result, it is necessary to introduce an effective and safe medication to reduce mortality and hospitalization of COVID-19.

In our study, spironolactone and combination therapy reduced mortality, ICU admission, intubation rate and end organ damage but without significant P-values. 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 particles with less potency for cell entry. ACE2 shedding occurs mainly through activity of ADAM17 (TNF-alpha converting enzyme) and blocking this enzyme can reduce ACE2 activity in plasma (35) and other body secretion. Mineralocorticoid receptor 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, could improve the clinical end points, such as mortality, WHO score, ICU admission, intubation rate and end organ damage, but without significant P-value and not 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-CoV2 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). Then, DPP4 inhibitors can 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 (combination group more than 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 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, fifteen (5.7%) patients had history of exposure to COVID-19 with typical manifestations but did not actually have COVID-19 positive test results. 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 more limitations. Likewise, lack of some paraclinical data that were missed for some patients are further shortcomings for analysis. In particular, data for inflammatory markers and CT scans were important but could not be collected completely.
In conclusion, sitagliptin and spironolactone can possibly reduce mortality of admitted COVID-19 patients and improve the clinical outcomes.
Acknowledgments
The authors express their gratitude to Drs. Mohammadreza Kalantarhormozi, Katayoun Vahdat, Mehdi Mahmudpour at the Bushehr University for helping with data collection. They also acknowledge Drs. Mohsen Moghadami, Zahra Nasiri, Halimeh Arjomand, Razieh Rahmati, Somayeh Rezaee, Omid Roosta, Amir Javidnia, Saeed Limoee, Samira Bazrafshan, Mohammad Rasool Rajabi) at Shiraz University for their efforts in data gathering. They appreciate the efforts of Professor Philip A. Kern (University of Kentucky) for his editorial contributions and Artin Asadipooya for writing edition.

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Source of funding: This project is supported by Vice-Chancellor for Research at the Shiraz University of Medical Sciences, Bushehr University Medical Sciences, Faghihi Hospital and Shohadaye_Khalije_Fars Hospital.
Authors' contributions: Kamyar Asadipooya propose the idea, designed the study, and wrote the manuscript. Farhad Abbasi and Mohammad Ali Davarpanah helped designing study. Reuben Adatorwovor provided statistical analysis, wrote statistical part and revised the manuscript. Sepideh Sefidbakht and Farhad Abbasi read the CT scan results. Yasaman Mansoori, Mehdi Hajiani, Farzán Azodi, Shayesteh Davoudi, Farzana Rezaei collected the data. Shayan Mohammadmoradi helped designing the study and editing the manuscript. All authors approved the final version of the manuscript.
Data Availability: Shiraz University (MAD, YM, and MH) and Bushehr University (FA, FA, SD, and FR) generated the data. University of Kentucky (SM and KA) has de-identified data and analyzed (RA) the data. The de-identified data are available for further investigations.
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**Figure Legends:**

**Figure 1.** Flow chart of study.

**Figure 2.** The Kaplan-Meier survival curves intersect indicates that the difference between the survivals across the four therapies did not remain constant over the duration of patient hospitalization. It could also be inferred that the survival curve 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 between survivals.
| 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     |
Table 2. Missing data indicates the number of cases that did not have the investigation 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                        |
| PaO₂                           | 2                        | 0                        |
| PaCO₂                          | 2                        | Not measured             |
| Characteristics                                      | Control Group | Combinatio Group | Sitaglipti Group | Spironolacton Group | P-value |
|------------------------------------------------------|---------------|------------------|------------------|---------------------|---------|
|                                                      | Standard therapy (87) Mean ± SE or n (%) | Spironolactone + Sitagliptin + Standard therapy (60) Mean ± SE or n (%) | Sitagliptin + Standard therapy (66) Mean ± SE or n (%) | Spironolactone + Standard therapy (50), Mean ± SE or n (%) |         |
|                                                      | 60.91 ± 15.98 | 53.73 ± 15.98    | 58.68 ± 17.10    | 53.14 ± 17.35       | 0.018   |
| Age (years)                                          | 60.91 ± 15.98 | 53.73 ± 15.98    | 58.68 ± 17.10    | 53.14 ± 17.35       | 0.018   |
| Elderly patient ≥ 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)                                 | 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/m²)                                          | 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 (%)                           | 2 (2.30)      | 0                | 1 (2.00)         | 1 (2.00)            | 0.836   |
| Coexisting conditions, n (%)                         | 13 (14.94)    | 16 (26.67)       | 15 (27.73)       | 10 (20.00)          | 0.354   |
| BMI > 30                                             | 24 (27.59)    | 13 (21.67)       | 17 (25.76)       | 14 (26.00)          | 0.848   |
| Diabetes                                             | 29 (33.33)    | 23 (38.33)       | 16 (24.24)       | 14 (26.00)          | 0.343   |
| Hypertension                                         | 22 (25.29)    | 7 (11.65)        | 14 (21.21)       | 7 (14.00)           | 0.148   |
| Cardiovascular disease                                | 7 (8.05)      | 1 (1.65)         | 3 (4.56)         | 3 (6.00)            | 0.415   |
| Chronic kidney disease                               | 6 (6.90)      | 1 (1.67)         | 5 (7.58)         | 6 (12.00)           | 0.179   |
| Chronic Pulmonary Disease                            | 7 (8.05)      | 2 (3.33)         | 3 (4.55)         | 2 (4.00)            | 0.659   |
| Cancer                                               | 4 (4.60)      | 1 (1.67)         | 2 (3.03)         | 2 (4.00)            | 0.830   |
| Immune deficiency (transplant etc.)                  | 7 (8.05)      | 4 (6.67)         | 3 (4.55)         | 1 (2.00)            | 0.534   |
| Neurologic disorders                                 | 38.11 ± 23.82 | 45.00 ± 26.98    | 45.56 ± 18.47    | 41.74 ± 20.87       | <0.001  |
| Fever (temperature °C), n (%)                        | 36.89 ± 0.80  | 36.30 ± 3.90     | 37.07 ± 0.80     | 37.00 ± 0.92        | 0.142   |
| 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), n (%)    | 5 (5.75)      | 3 (5.00)         | 3 (4.55)         | 1 (2.00)            | 0.826   |
| Mean O2 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 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               | 39.29 ± 22.60 | 75.00 ± 11.99    | 55.00 ± 21.82    | 44.55 ± 19.55       | <0.001  |
| Medications                        | 4 admission, n (%) | 8 admission, n (%) | 12 admission, n (%) | 16 admission, n (%) | 18 admission, n (%) |
|-----------------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| 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 excluded) | 1 patient          |                    |                    |                    |                    |
| Antihypertensive drugs, n (%)     |                    |                    |                    |                    |                    |
| ACE inhibitors                    | 15 (17.24)         | 6 (10.00)          | 13 (19.70)         | 8 (16.00)          | 0.497              |
| ARB                               | 0                  | 0                  | 1                  | 0                  | -                  |
| Beta-Blockers                     |                    |                    |                    |                    |                    |
| Diuretics (Spironolactone excluded) |                    |                    |                    |                    |                    |
| Antiplatelet drugs, n (%)         |                    |                    |                    |                    |                    |
| Anticoagulant drugs, n (%)        |                    |                    |                    |                    |                    |
| Statin drugs, n (%)               |                    |                    |                    |                    |                    |
| Laboratory findings on 5th day of admission |                    |                    |                    |                    |                    |
| Glycemia (mg/dL)                  | 145.05 ± 145.65 ± 96.71 | 150.47 ± 87.15 | 146.12 ± 79.84 | 0.983              |
| Serum creatinine (mg/dL)          | 1.33 ± 1.28        | 12.63 ± 2.07       | 12.44 ± 2.03       | 1.30 ± 1.73        | 0.357              |
| Hemoglobin g/dl                   | 12.61 ± 12.46 ± 4.08 | 7.48 ± 4.08        | 8.68 ± 4.42        | 12.35 ± 2.18       | 0.896              |
| White blood cell count, (× 10⁹/L) | 1.90 ± 74.58 ± 16.86 | 74.68 ± 17.21      | 78.70 ± 9.45       | 8.18 ± 4.24        | 0.432              |
| Neutrophil percentage             | 9.32 ± 18.12 ± 13.93 | 16.28 ± 9.36       | 14.64 ± 7.83       | 5.04 ± 2.85        | 0.443              |
| Lymphocyte percentage             | 10.31 ± 21.47 ± 90.45 | 239.39 ± 239.39    | 223.08 ± 80.19     | 5.62 ± 28.09       | 0.346              |
| Platelet count (× 10⁹/L)          | 18.18 ± 53.83 ± 27.39 | 1.17 ± 0.20       | 1.30 ± 0.34        | 5.02 ± 28.25       | 0.065              |
| INR                               | 16.25 ± 16.5 ± 66.33 | 52.25 ± 28.07      | 56.28 ± 40.99      | 5.78 ± 24.58       | 0.578              |
| AST (units/L)                     | 81.73 ± 233.43 ± 233.61 | 45.92 ± 28.30     | 157.20 ± 207.18    | 5.94 ± 28.52       | 0.294              |
| ALT (units/L)                     | 215.57 ± 709.42 ± 241.62 | 182.03 ± 182.03   | 628.67 ± 273.63    | 5.78 ± 24.58       | 0.378              |
| CPK (units/L)                     | 81.73 ± 233.43 ± 233.61 | 45.92 ± 28.30     | 157.20 ± 207.18    | 5.94 ± 28.52       | 0.294              |
| LDH (units/L)                     | 52.15 ± 1048.50 ± 170.93 | 298.71 ± 298.71   | 1257.70 ± 1840.31  | 0.049              |
| ESR (mm/hr)                       | 25.80 ± 27.93 ± 56.46 | 52.32 ± 52.32 ± 30.03 | 28.01 ± 43.22     | 5.35 ± 30.03 ± 18.03 | 0.441              |
| CRP (mg/L)                        | 51.24 ± 51.24 ± 51.24 | 66.09 ± 66.09 ± 38.79 | 535.83 ± 535.83     | 531.28 ± 531.28   | 0.441              |
| D-dimer (mg/mL)                   | 37.32              | 28.12 ± 28.12      | 28.12 ± 28.12      | 28.12 ± 28.12      | 0.441              |
| Interleukin-6 (ng/L)              | 183.26 ± 183.26 ± 183.26 | 631.28 ± 631.28   | 6.12 ± 6.12        | 6.12 ± 6.12        | 0.441              |

Data are mean ± SEM unless otherwise indicated. LDH, lactate dehydrogenase; IU, international units; n, number.
1 Modified WHO clinical scores (table 1).

2 Alcohol drinking is defined as consuming ≥ 5 drinks per week for men and ≥ 4 drinks per week for women.

3 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.).

4 Percentage of chest CT involvement: Shiraz area of involvement with and without opacification, Bushehr area of involvement with opacification.

5 Glycemia (mg/dL) is mean blood glucose level that was calculated based on the first day measurements of random blood glucose.
Table 4. The clinical outcomes in patients evaluated at 5\textsuperscript{th} day of admission

| Characteristics                                                                 | Control Group                                                                 | Combination group                                                                 | Sitagliptin group                                                                 | Spironolactone group                                                                 | P-Value |
|---------------------------------------------------------------------------------|-------------------------------------------------------------------------------|----------------------------------------------------------------------------------|---------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|---------|
|                                                                                 | Standard therapy (87) Mean ± SE or n (%)                                      | Spironolactone + Sitagliptin + Standard therapy (60) Mean ± SE or n (%)          | Sitagliptin + Standard therapy (66) Mean ± SE or n (%)                           | Spironolactone + Standard therapy (51), Mean ± SE or n (%)                         |         |
| Mortality, n (%)                                                                | 19 (21.84)                                                                    | 8 (13.33)                                                                        | 9 (13.64)                                                                        | 5 (10.00)                                                                           | 0.275   |
| Clinical Score on 5\textsuperscript{th} day of admission (0-8)\textsuperscript{1} | 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 (14.00)                                                                           | 0.405   |
| Respiratory rate (breaths/min) on 5\textsuperscript{th} day of admission       | 19.21 ± 2.23                                                                  | 19.56 ± 2.49                                                                     | 18.63 ± 3.11                                                                     | 19.12 ± 2.36                                                                        | 0.253   |
| Hypotension (SBP \(\leq 90\)) on 5\textsuperscript{th} day of admission      | 3 (3.45)                                                                      | 1 (1.67)                                                                         | 4 (6.06)                                                                         | 1 (2.00)                                                                            | 0.593   |
| Mean O2 saturation on 5\textsuperscript{th} day of admission                   | 85.69 ± 11.28                                                                 | 88.43 ± 10.09                                                                    | 88.32 ± 11.63                                                                    | 89.60 ± 9.86                                                                        | 0.174   |
| Shock state\textsuperscript{2}                                                  | 3 (3.45)                                                                      | 2 (3.33)                                                                         | -                                                                                | -                                                                                  | 0.277   |
| Acute respiratory failure\textsuperscript{3}                                   | 18 (20.69)                                                                    | 7 (11.67)                                                                        | 9 (13.64)                                                                        | 3 (6.00)                                                                            | 0.110   |
| Acute kidney injury\textsuperscript{4}                                          | 6 (6.90)                                                                      | 1 (1.67)                                                                         | 3 (4.55)                                                                         | 2 (4.00)                                                                            | 0.555   |
| Elevated liver enzymes | 38 (43.68) | 20 (33.33) | 20 (30.30) | 21 (42.00) | 0.296 |
|------------------------|------------|------------|------------|------------|-------|
| Percentage of chest CT involvement on 5th day of admission 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 Shiraz | 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 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 Shiraz | -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 | 12.66 ± 3.02 | 12.16 ± 1.82 | 1.08 ± 0.72 |
| Hemoglobin (g/dL) | 13.42 ± 13.95 | 11.07 ± 8.97 | 77.01 ± 17.01 | 11.62 ± 11.68 | 1.10 ± 1.37 |
| White blood cell count, (× 10^9/L) | 86.52 ± 89.99 | 15.18 ± 12.40 | 14.52 ± 14.31 | 73.88 ± 19.01 | 9.64 ± 3.37 |
| Neutrophil percent | 274.33 ± 119.47 | 35.12 ± 38.18 | 299.13 ± 135.72 | 16.45 ± 9.45 | 76.87 ± 10.07 |
| Lymphocyte percent | 1099.41 ± 1820.44 | 42.83 ± 111.40 | 1685.80 ± 2528.23 | 266.15 ± 114.56 | 15.28 ± 8.82 |
| Platelet count (× 10^9/L) | | | | | 305.90 ± 103.20 |
| CRP (mg/L) | 21.03 ± 42.37 | | | | 34.06 ± 32.52 |
| D-dimer | | | | | 673.49 ± 1138.43 |
| | | | | | 22.90 ± 51.39 |
| | | | | | 0.005 |
| | | | | | 0.185 |
(mg/mL)  
Interleukin -6 (ng/L)

|       |       |       |       |
|-------|-------|-------|-------|

Data are mean ± SEM unless otherwise indicated. LDH, lactate dehydrogenase; IU, international units; n, number; SBP, systolic blood pressure.

1 Modified WHO clinical scores (table 1).

2 Shock state means low systolic blood pressure that required IV hydration, pack cell infusion or vasopressors.

3 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).

4 Acute kidney injury means elevated creatinine more than ≥1.5 times the baseline value during the hospital course.

5 Elevated liver enzymes means elevated AST/ALT more than ≥3 times above normal value during the hospital course.

6 Percentage of chest CT involvement: Shiraz area of involvement with and without infiltration, Bushehr area of involvement with infiltration.
Table 5. Cox Proportional Hazard Model for COVID-19 Patients Admitted to Hospital for Four Treatment Groups. A hazard ratio higher (lower) than 1 means higher (lower) survival.

| 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        |
Figure 1

204 Participants from Shiraz
76 Participants from Bushehr

17 excluded with early discharge

263 Participants Randomized

Control Group
- 87 Participants
  - 59 Shiraz
  - 28 Bushehr

Combination Group
- 60 Participants
  - 51 Shiraz
  - 9 Bushehr

Sitagliptin Group
- 66 Participants
  - 38 Shiraz
  - 28 Bushehr

Spironolactone Group
- 50 Participants
  - 39 Shiraz
  - 11 Bushehr
