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*Original Article*

# Evaluation of Possible Association between Serum Levels of Aldosterone and Cortisol with Clinical Symptoms Progression in COVID-19 Suspicious Outpatients Tested for SARS-CoV2 RT-PCR: An Analytical Cross-Sectional Study

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**Abstract**

Aldosterone is a key component of Renin-Angiotensin-Aldosterone System (RAAS). The RAAS could play a substantial role in the pathophysiology of coronavirus disease 2019 (COVID-19). Moreover, the dynamics of the Hypothalamic-Pituitary-Adrenal (HPA) axis may have changed in COVID-19. Cortisol, as an important factor in assessing immune system activity, is an important part of this axis. The present study compared the serum levels of aldosterone and cortisol in COVID-19 outpatients with those of potentially non-infected participants. It was also aimed to assess the possible association between serum levels of aldosterone and cortisol with clinical symptoms progression in COVID-19 outpatients. Demographic characteristics (i.e., gender and age) and clinical data (i.e., oxygen saturation [$SPO_2$], respiratory rate [RR], and heart rate) were collected. Serum cortisol and aldosterone measurements were conducted using the ELISA technique. Clinical symptoms of the positive polymerase chain reaction (PCR) group were followed up on for 28 days in weekly intervals. $SPO_2$ was significantly lower in the positive PCR group; however, the RR was significantly higher ($P$=0.03 and $P$=0.001, respectively). Significantly higher levels of aldosterone were found in males of the negative PCR group, compared to females ($P$=0.05). Cortisol (OR=0.937, $P$=0.033) and aldosterone (OR=1.005, $P$=0.020) levels had a decreasing and increasing effect on the chances of respiratory symptoms occurring over time, respectively. Furthermore, over time, women were twice as likely as men to develop neurologic symptoms (OR=0.530, $P$=0.015). According to the findings of this study, cortisol and aldosterone are associated with the chance of respiratory symptoms occurring over time. However, the levels of these two markers do not seem to be related to the progression of clinical symptoms of lower grades of COVID-19.

**Keywords:** Aldosterone, Cortisol, Clinical symptoms progression, COVID-19

## 1. Introduction

For the third time in two decades, an outbreak has been linked to the family of coronaviruses, causing a global pandemic leaving many countries in a state of despair (1). Acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for coronavirus disease

2019 (COVID-19), which became a pandemic in 2020 (2). Coronaviruses are ribonucleic acid (RNA)-coated viruses of the Coronavirinae subfamily of the Coronaviridae family of the Nidovirales category. Birds are infected with gamma and delta coronaviruses (3). Infectious Bronchitis Virus is one of the Coronaviruses infecting birds which causes infectious bronchitis, a ubiquitous and highly contagious disease of chickens (4). Mammals are infected with beta and alpha coronaviruses, which entail six different types of coronaviruses and have been reported to infect humans (3). On January 7, 2020, a new coronavirus was extracted from pneumonia patients infected with the virus. In February 2020, the World Health Organization identified the virus as the cause of COVID-19 (5). SARS-CoV-2 diagnosis is based on clinical features, imaging studies (peripheral ground-glass opacities in chest radiograph), and reverse transcription-polymerase chain reaction (RT-PCR) (6). Currently, RT-PCR testing for viral nucleic acid is the most common diagnostic method for COVID-19. Acute respiratory syndrome coronavirus 1 (SARS-CoV-1) and SARS-CoV-2, which were responsible for the SARS epidemic from 2002 to 2004, as well as the newer 2019 coronavirus (COVID-19), all bind to the Renin-Angiotensin-Aldosterone system (RAAS) via Angiotensin-Converting Enzyme 2 (ACE2) (7). ACE2 is the host receptor for COVID-19 (8). Patients on ACE inhibitors or angiotensin receptor blockers could be at greater risk due to the mechanism by which SARS-CoV-2 enters the cells (9).

The role of the RAAS in COVID-19 infection has become a controversial topic of discussion (8). COVID-19 has stimulated the study of the RAAS, and how it can be manipulated to treat COVID-19 (10). Clinical trials are underway to evaluate the safety and efficacy of RAAS modulators in the treatment of COVID-19 (7). The results of studies conducted so far on the role of this system in the pathophysiology of COVID-19 are diverse and contradictory, and like many other factors involved in the pathophysiology of this emerging disease, need more detailed study (11).

Often overlooked in the consideration of the limited RAAS, is aldosterone, which is a component of the wider RAAS. The formation of angiotensin II out of angiotensin I is mediated by ACE. Angiotensin II promotes the secretion of aldosterone from the adrenal gland (12). Antagonists of aldosterone have also been found to increase ACE2 levels in human macrophages. Aldosterone could be detrimental in COVID-19 infection by renal tubular actions to produce sodium retention, as well as by tissue actions, including endothelial alterations and immune system activation, resulting in pro-inflammatory actions (10).

In animal models, the use of spironolactone was an important drug in the prevention of pulmonary fibrosis. Through its dual action as a mineralocorticoid receptor (MR) antagonist and an androgenic inhibitor, spironolactone can provide significant benefits when used to treat COVID-19 infections. The primary effect of spironolactone in reducing pulmonary edema may also be beneficial in COVID-19 acute respiratory distress syndrome (ARDS).

It has been shown that activation of the MR in immune cells promotes the hyper-inflammatory response. In macrophages, MR activation causes polarization towards the M1 pro-inflammatory phenotype. In CD4+lymphocytes, the activation of the MR facilitates differentiation towards pro-inflammatory Th17 cells while enhancing Th17-mediated immunity influences dendritic cells' functioning, which is crucial for immunological tolerance and homeostasis. It also induces cytotoxic IFNγ+-CD8+T lymphocytes. This is particularly important since COVID-19 infections are characterized by a cytokine storm and hyper-inflammatory state, with Th17 T cells increased and increased CD8+cells cytotoxicity.

It has been shown that *in vitro* effects of glucocorticoids are immunosuppressive; however, it is becoming increasingly evident that the *in vivo* effects of glucocorticoids are frequently different from *in vitro* treatment or treatment with synthetic glucocorticoids, such as dexamethasone (13). Large-scale

epidemiological reports on COVID-19 have underlined that apart from age and co-morbidities, additional risk factors include obesity, hypertension, and male gender, all of which have been associated with mineralocorticoid action (14). Glucocorticoids are widely used to treat various inflammatory lung diseases but are often associated with significant side effects. Published guidelines suggest that the systemic administration of low-dose and short-term glucocorticoids may be beneficial for COVID-19 patients for whom the disease progresses rapidly. However, the evidence is still limited.

COVID-19 may also affect the Hypothalamic-Pituitary-Adrenal (HPA) axis. It has been shown that hypothalamic and pituitary tissues also express the ACE2 enzyme, and therefore, can be the target tissue of the virus. The measurement of the serum cortisol is one of the methods used to assess the status of the HPA activity axis and immune system activity during COVID-19 (15).

A systematic review investigated the efficacy and safety of glucocorticoids for the treatment of patients with COVID-19. A total of 23 studies were reviewed, one of which was a randomized controlled trial, and the other 22 of which were cohort studies. The total number of patients studied in these studies was 13,815. Studies have shown that in adults with COVID-19, the systemic use of glucocorticoids does not decrease mortality (RR=2.00, 95% CI: 0.69-5.75, IU=90.9%) or the course of lung inflammation (WMD=-1 days, 95% CI: -2.91 to 0.91). Meanwhile, a significant decrease was observed in the duration of fevers. It was also found that systemic use of therapeutic glucocorticoids prolongs the length of hospital stay. The researchers concluded that glucocorticoid therapy reduced the duration of fever but did not reduce mortality, length of hospital stay, or absorption of lung inflammation.

The long-term use of high-dose glucocorticoids increases the risk of side effects, such as co-infections. Therefore, the routine use of systemic glucocorticoids is not recommended for patients with COVID-19. There are generally conflicting opinions about using glucocorticoids in the treatment of COVID-19 patients (16). ACE2 has also been shown to be expressed on adrenal gland endothelial cells. The dynamics of cortisol may have changed in patients with COVID-19. However, there are very few studies in this area.

Considering the importance of the HPA axis and RAAS in the pathophysiology of COVID-19, it is vital to evaluate and understand the mechanisms and systems involved in the pathophysiology of COVID-19 to better understand this disease and provide appropriate preventive and therapeutic solutions. However, diverse and sometimes contradictory results have been obtained from research on each of the mentioned factors in various previous studies. Therefore, this study aimed to screen the possible association between serum levels of aldosterone and cortisol with clinical symptoms progression in COVID-19 suspicious outpatients tested for SARS-CoV-2 RT-PCR referred to 16-hour comprehensive health centers in Abadan, Iran.

## 2. Materials and Methods

### 2.1. Participants and Study Protocol

To examine the potential association between on-admission serum levels of aldosterone/cortisol and the progression of the clinical symptoms of COVID-19 among the clients of a health care setting, this epidemiological health service center-based cross-sectional-analytical study was designed and conducted at Abadan University of Medical Sciences, Abadan, Khuzestan Province (southwestern Iran) between the beginning of April and the end of July 2020. With the coordination between the vice-chancellor for education and the vice-chancellor for the health of Abadan University of Medical Sciences, a list of outpatients was obtained from the deputy minister of health. Patients on the list had been referred to 16-hour comprehensive health centers in Abadan for COVID-19 RT-PCR testing (COVITECH, Tehran, Iran TR-

PCR kits) and had clear (negative/positive) PCR results.

By telephone contact with these outpatients, those who met the criteria for inclusion (male and female participants ≥11 years of age, a clear [positive, negative] PCR result, willingness to participate in the study, the ability to understand the relevant information, and completion of the informed consent form) in the study and provided informed consent to participate were selected and assigned into two groups of negative and positive PCR (n=52 in each group) (17).

On the other hand, outpatients with known hypothalamic, pituitary, adrenal, or severe hepatic diseases, as well as those on corticosteroid treatment or other medications affecting adrenal function in the preceding three months, were excluded from the study. Furthermore, those who had known RAAS disorders or were on any medications which interfered with the activity of this system during the three last months were excluded. In addition, pregnancy and lactation in women, unclear PCR test results, PCR testing for the second time, smoking during the test period, high-risk jobs, including healthcare staff and public transport drivers (for the control group) were grounds for exclusion, as were any other circumstances that the researcher did not consider to justify the participation of individuals.

The study protocol was approved by the Ethics Committee of Abadan University of Medical Sciences, Abadan, Iran (Ethics Code: IR.ABADANUMS.REC. 1399.079) following the Declaration of Helsinki for medical research involving human subjects.

With prior coordination, the participants were referred to Imam Khomeini Health Center. All participants were asked to go to the Imam Khomeini Center in a fasting state between 7:00 a.m. and 9:00 a.m. Patients' medical histories were retrospectively reviewed, and their demographic characteristics (age and gender) and clinical data (respiratory rate [RR], pulse rate, oxygen saturation [$SPO_2$]) were collected. Additionally, the participant's clinical symptoms were recorded in four groups of symptoms, including general (fever, fatigue, night sweats, shivering, and asthenia), respiratory (cough and dyspnea), gastrointestinal (nausea, constipation, and diarrhea), and neurologic (muscle/joint pain, loss of taste/smell, headache) (18) via self-reports. The data of clinical symptoms were collected from both asymptomatic and symptomatic COVID-19-infected patients.

The participants were then referred to the Abadan Private Health Laboratory, where a blood sample was collected from each person and stored for biochemical tests. Around 5 mL of blood was collected following 8 h of fasting. Routine laboratory investigations including serum levels of aldosterone and cortisol were performed within the first 24 h after laboratory admission. These included the assessment of serum levels of cortisol (Cortisol ELISA Kit; Monobind Inc.) and serum levels of aldosterone (Aldosterone ELISA Kit; Monobind Inc.). All experiments were performed in a clinical laboratory (Abadan Salamat Laboratory) having a quality control certificate from Iran Health Reference Laboratory. The day of referral to Imam Khomeini Health Center was considered the first day of the study for each person. The clinical symptoms of positive-PCR outpatients were followed up with and recorded over 28 days (in one-week intervals) by telephone.

**2.2. Data analysis and Report**

The normality of the data was assessed using the Kolmogorov-Smirnov test, and all data were shown to be normal. The results were displayed as mean±standard deviation (SD) for quantitative variables and number (percent) for qualitative variables. Data were compared between the study groups using an independent sample t-test. The correlation of baseline serum biomarkers with demographic and clinical parameters was measured by bivariate analysis to obtain the Pearson correlation coefficient (r) for quantitative variables, and the Chi-square test was employed to analyze categorical variables. According to the longitudinal data, there are repeated outcomes within one individual; therefore, the

generalized estimating equations technique (GEE) model was used with unstructured correlation to analyze a longitudinal dataset with four measurements (gender, age, serum levels of cortisol, and serum levels of aldosterone) on a positive PCR group (n=52) for each of the four dichotomous outcome variables (pulmonary, general, gastrointestinal, and neurologic symptoms), separately. The odds ratio (OR) and confidence interval values (95% CI) for OR were reported for each model. All statistical analyses were performed using IBM SPSS software (version 26.0), and a *P*-value less than 0.05 was considered statistically significant.

## 3. Results

### 3.1. General Characteristics and Clinical Presentations

From April 2020 to July 2020, a total of 104 participants were enrolled including 36 (67.9%) males and 17 (32.1%) females in the positive PCR group, and 38 (71.7%) males and 15 (28.3%) females in the negative PCR group. The mean ages of the outpatients were 41.22±1.80 and 39.89±1.85 years for positive and negative PCR groups, respectively.

A significant difference was found in the mean RR and $SPO_2$ between negative and positive PCR groups. $SPO_2$ was significantly lower in the positive PCR (96.88±0.18) group than the negative (97.43±0.16) group (*P*=0.03). However, the mean RR was significantly higher in the positive (14.10±0.22) PCR group, compared to the negative (12.96±0.26) group (*P*=0.001).

General characteristics and clinical presentations are provided in table 1.

### 3.2. Biochemical and Laboratory Evaluations

The results of the between-group analysis of serum levels of cortisol are shown in table 1. Serum levels of cortisol did not show any significant differences between the two groups (14.40±13.56 for negative and 15.98±5.37 for positive PCR groups, respectively, *P*=0.4). Serum levels of aldosterone also did not show any significant differences between the two groups (148.34±95.5 for negative and 172.86±100.9 for positive PCR groups, *P*=0.2). Table 1 also tabulates the results of a comparison between the mean serum aldosterone levels of negative and positive PCR groups.

### 3.3. Correlation among on-Admission Serum Levels of Cortisol and Aldosterone with Demographic and Clinical Variables Measured on the Day of Admission

The relationship of serum levels of cortisol and aldosterone with various demographic and clinical variables in negative and positive PCR groups was assessed (Tables 2 and 3, respectively). An evaluation of the relationship of age, clinical variables of $SPO_2$, mean respiratory, and pulse rates with baseline levels of cortisol and aldosterone showed no significant association in either the positive or negative PCR group. The investigation of the relationship between gender and basal serum levels of cortisol and aldosterone in the positive PCR group did not show any significant correlation.

Our results also showed a significantly higher level of aldosterone in males (132.2±82.7) of the negative PCR group, compared to females (189.1±115.3) (*P*=0.05).

### 3.4. Association of Estimated Parameters (age, gender, serum levels of aldosterone and cortisol) with the Chances of Different Groups of Clinical Symptoms Occurring during the Follow-Up Period

The association of gender, age, baseline cortisol level, and baseline aldosterone level with the chances of clinical symptoms occurring over time is presented in table 4. In terms of the gastrointestinal and general categories of clinical symptoms, our analysis indicated no association among any of the examined parameters and the chances of symptoms occurring.

Meanwhile, a significant relationship of the baseline serum cortisol and aldosterone levels with the chances of respiratory symptoms occurring over time was observed in this study. Serum cortisol levels (OR=0.937, *P*=0.033) had a decreasing effect on the outcome measure (chance of respiratory symptoms

occurring over time). However, serum aldosterone levels (OR=1.005, *P*=0.020) had an increasing effect on the chance of respiratory symptoms occurring over time. In the neurologic category of symptoms, a significant relationship was found between gender (OR=0.530, *P*=0.015) and the outcome variable. Over time, women are twice as likely as men to develop neurologic symptoms.

**Table 1.** Demographic characteristics, clinical presentations, as well as serum levels of cortisol and aldosterone in COVID-19 suspicious outpatients, tested for SARS-CoV-2 by RT-PCR*

| Variables | | Negative PCR group (n=52) | Positive PCR group (n=52) | *P*-value |
|---|---|---|---|---|
| Age (mean) | | 39.89±1.85 | 41.22±1.80 | 0.6 |
| **Gender** | Male (%) | 71.7% | 67.9% | 0.4 |
| | Female (%) | 28.3% | 32.1% | |
| Mean respiratory rate (number/min) | | 12.96±0.26 | 14.10±0.22 | 0.001 |
| Mean pulse rate (number/min) | | 87.23±1.84 | 90.71±2.60 | 0.3 |
| Cortisol (µg/dL) | | 14.40±13.56 | 15.98±5.37 | 0.4 |
| Aldosterone (pg/ml) | | 148.34±95.5 | 172.86±100.9 | 0.2 |
| $SPO_2$ (%) | | 97.43±0.16 | 96.88±0.18 | 0.03 |

*The results are shown as mean±SD for quantitative and number (percent) for qualitative data. Independent sample t-test and chi-square test were applied to compare the study groups. $SPO_2$: oxygen saturation levels

**Table 2.** Correlation of baseline serum biomarkers with demographic characteristics and clinical variables measured on the day of admission in the negative PCR group*

| Variables | | Cortisol | | Aldosterone | |
|---|---|---|---|---|---|
| | | r/mean± SD | *P*-Value | r/mean± SD | *P*- Value |
| Age | | 0.2 | 0.1 | -0.2 | 0.2 |
| $SPO_2$ | | -0.2 | 0.1 | 0.05 | 0.7 |
| Mean respiratory Rate | | -0.05 | 0.7 | -0.1 | 0.5 |
| Mean pulse Rate | | 0.08 | 0.6 | 0.2 | 0.2 |
| Gender | Male | 12.5±4.9 | 0.1 | 132.2±82.7 | 0.05 |
| | Female | 19.3±24.2 | | 189.1±115.3 | |

*Correlations among quantitative variables have been presented by Pearson correlation coefficient (r) measured by bivariate analysis. The comparison between categorical variables in terms of cortisol and aldosterone serum levels has been analyzed by independent sample t-test and ANOVA

**Table 3**. Correlation of baseline serum biomarkers with demographic characteristics and clinical variables measured on the day of admission in the positive PCR group*

| Variables | | Cortisol | | Aldosterone | |
|---|---|---|---|---|---|
| | | r/mean±SD | *P*-Value | r/mean±SD | *P*-Value |
| Age | | 0.2 | 0.2 | -0.2 | 0.1 |
| $SPO_2$ | | 0.02 | 0.9 | 0.03 | 0.8 |
| Mean respiratory Rate | | -0.002 | 1 | 0.1 | 0.4 |
| Mean pulse Rate | | 0.1 | 0.3 | -0.07 | 0.6 |
| Gender | Male | 15.9±5 | 0.9 | 155.8±77.3 | 0.07 |
| | Female | 16.1±6.3 | | 209±134.2 | |

*Correlations among quantitative variables have been presented by Pearson correlation coefficient (r) measured by bivariate analysis. The comparison between categorical variables in terms of cortisol and aldosterone serum levels has been analyzed by independent sample t-test and ANOVA

**Table 4.** Odds ratio and 95% confidence interval estimated by GEE analysis with an unstructured model to determine the observed symptoms progression of COVID-19 and the associations of demographic characteristics with laboratory parameters among infected patients

| Parameters | Clinical Symptoms Category* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | General | | Pulmonary | | Gastrointestinal | | Neurologic | |
| | OR (95% CI) | *P*-value | OR (95% CI) | *P*-value | OR (95% CI) | *P*-value | OR (95% CI) | *P*-value |
| Male | 1.013 (0.509-2.018) | 0.970 | 0.625 (0.306-1.277) | 0.197 | 1.230 (0.424-3.568) | 0.703 | 0.530 (0.317-0.886) | 0.015 |
| Female | 1 (Ref) | | 1 (Ref) | | 1 (Ref) | | 1 (Ref) | |
| Age | 1.011 (0.992-1.031) | 0.255 | 1.009 (0.976-1.042) | 0.601 | 0.966 (0.933-1.001) | 0.056 | 0.990 (0.967-1.013) | 0.381 |
| Cortisol | 0.959 (0.881-1.043) | 0.327 | 0.937 (0.883-0.995) | 0.033 | 1.018 (0.926-1.118) | 0.717 | 0.985 (0.929-1.045) | 0.622 |
| Aldosterone | 1.002 (1.000-1.004) | 0.116 | 1.005 (1.001-1.008) | 0.020 | 1.003 (0.997-1.008) | 0.316 | 1.000 (0.997-1.003) | 0.971 |

*Dependent Variables
OR: Odds ratio
95% CI: 95% Confidence Interval

## 4. Discussion

This is likely the first study that compared the serum levels of aldosterone and cortisol in COVID-19 outpatients with those of potentially non-infected participants in Abadan County and characterized the association of on-admission serum levels of aldosterone and cortisol with the progression of clinical symptoms of patients with low grades of COVID-19. In this study, 52 outpatients with RT-PCR confirmed COVID-19 and 52 potentially non-infected participants for whom the COVID-19 was excluded by RT-PCR were included to compare the demographic characteristics, clinical variables, serum levels of aldosterone, and serum levels of cortisol. In addition, the possible associations between the serum concentrations of aldosterone and cortisol and the progression of clinical symptoms among infected patients were evaluated by the GEE model.

Our findings showed that $SPO_2$ was significantly lower in the positive PCR group; however, the RR was significantly higher. Moreover, significantly higher levels of aldosterone were found in males of the negative PCR group, compared to females. Although the results of the between-group analysis of serum levels of cortisol and aldosterone did not show any significant differences between the negative and positive-PCR groups, the GEE analysis revealed that cortisol and aldosterone levels had a decreasing and increasing effect on the chances of respiratory symptoms occurring over time, respectively. Furthermore, women were twice as likely as men to develop neurologic symptoms during the follow-up period.

The COVID-19 pandemic has presented multiple challenges regarding clinical management. Accurate clinical monitoring is fundamental to inform both patient safety and management decisions. Of particular importance is the monitoring of blood oxygen saturation due to the direct impact of the disease on the respiratory system and complications, such as thromboembolic disease. Oximetry is an indirect way of measuring the oxygen concentration in the blood (i.e., what percentage of the blood is carrying oxygen). Oxygen levels can be easily and quickly measured using a pulse oximeter, and it can also determine whether an individual needs to seek medical help, which is the case when their $SPO_2$ is lower than 92%. Oximetry is a quick and non-invasive method of estimating oxygenation and has other benefits, such as being continuous, meaning it can highlight sudden changes in a patient's clinical status (19).

During the ongoing COVID-19 pandemic, reports in social media and the lay press indicate that a subset of patients is presenting severe hypoxemia in the absence of dyspnea, a problem unofficially referred to as "silent hypoxemia" (20). Oxygen is an essential aspect of treatment for patients with COVID-19 pneumonia.

Indeed, the major mechanism for injury and death in COVID-19 is related to hypoxia (21).

Our results, in line with recent findings, certify the importance of $SPO_2$ levels in the diagnosis of patient safety and management decisions. COVID-19 predominantly affects the respiratory system. It shows a wide range of clinical presentations ranging from asymptomatic/mild symptoms (fever, cough, dyspnea, myalgia, fatigue, anosmia, dysgeusia, and diarrhea) to severe illnesses, such as ARDS, arterial and venous thrombosis, myocarditis, and varieties of neurological manifestations (22). COVID-19 can cause shortness of breath, lung damage, and impaired respiratory function.

RR is a vital sign that is related to, and therefore regulated by, multiple physiological and neural factors. Moreover, it has an important role in the detection of various cardiovascular and respiratory diseases, as well as relevant clinical events. The variation of RR reflects the deterioration of respiratory diseases and other clinical conditions, including fever, infection, and drug overdose (23). It is a common screening tool used to identify lower respiratory tract infections in clinical settings. Given that COVID-19 impairs and damages the respiratory system, it is reasonable to suggest that changes in respiratory efficiency, and therefore, resting respiratory rate, might occur in the early stages of infection (24), which is in line with our results.

The reliable monitoring of RR is also very important for the treatment and management of other respiratory issues, such as chronic obstructive pulmonary disease (23). There was no significant difference in terms of age, gender, and mean pulse rate between the two evaluated groups ($P>0.05$). In line with our results, a case-control study comparing the COVID-19 infected patients and healthy matched controls reported that vital parameters, including heart rate, as well as systolic and diastolic blood pressure, showed no difference between the study groups (22).

Immune system response plays a crucial role in controlling and resolving viral infection. Therefore, cortisol is linked to the immune system and viral infection as part of the neuroendocrine stress axis. Exogenous or endogenous glucocorticoid excess is characterized by increased susceptibility to infections due to impairments of the innate and adaptive immune systems. Therefore, patients with chronic glucocorticoid excess may be at high risk of developing COVID-19 with a severe clinical course (25). Investigations have shown that the effects of different drugs, opioids, and pathogens on the immune system could be induced through three mechanisms, including the direct effect of these substances on immune cells in peripheral blood, effect on the HPA axis which indirectly induces cortisol increase and activates sympathetic nervous system which causes circulating levels of epinephrine from the adrenal medulla, as well as norepinephrine from sympathetic nerve terminal leading to increase in the catecholamines (13).

In line with our results, a descriptive and analytical cross-sectional study was conducted on a population of patients infected with COVID-19 in Cameroon. The researchers found no statistically significant association between serum cortisol and disease severity. Similarly, they concluded that the absence of a marked rise of cortisol during COVID-19 suggests the possible involvement of the HPA axis in this infection. However, their study did not have a non-COVID control group (26).

Another study compared baseline cortisol concentrations between COVID-19 patients and controls. Contrary to our results, they found that patients with COVID-19 presented a marked and appropriate acute cortisol stress response; moreover, this response was significantly higher in this patient cohort than in individuals without COVID-19 (27).

The observed differences, as well as individual differences in stress responses, might have arisen because COVID patients were not the same in the two studies. Specifically, they were at different stages of the disease. It should be mentioned that our study was conducted on asymptomatic/mild and moderate outpatients of COVID-19.

COVID-19 and the RAAS are closely linked both in infection and in possible post-infection inflammatory cascades (10). Not only is ACE2 the target of

COVID-19 for penetration of cells, but it is also inactivated by COVID-19. The binding of COVID-19 to ACE2 reduces the availability of ACE2 to result in diminished production of anti-inflammatory products of ACE2, especially Ang (1-7), which results in a dominance of the pro-inflammatory products (10). Pasquale Campana (28) reported that increased levels of aldosterone might be associated with severe forms of COVID-19. This report can confirm the results obtained in our study because lower grades of the disease (mild and moderate) were evaluated in our study.

The results of a study by Henry, Benoit (29), which included 30 COVID-19 patients and controls, are in line with our findings. They compared plasma concentrations of aldosterone between patients and controls using the Mann-Whitney U test and reported that aldosterone concentrations were comparable between patients with and without COVID-19 (8.9 [IQR:5.8-16.2] vs. 9.0 (IQR:7.4-12.2) ng/dL, *P*=0.865) (29).

Consistent with our results, in a prospective single-center study from Germany, Marina Rieder (12) analyzed serum samples from 24 SARS-CoV-2 positive and 61 SARS-CoV-2 negative patients. All-comers admitted to the department of emergency medicine of the University Medical Center, University of Freiburg, Freiburg, Germany, undergoing a PCR testing for SARS-CoV-2 from throat swab were included in their study. They discovered no significant difference between the SARS-CoV-2 positive and the control group regarding the mean serum concentration of aldosterone (151 pg/ml vs. 185 pg/ml) (12).

Based on the findings, it can be concluded that the absence of a marked rise in the serum levels of cortisol and aldosterone in low grades of COVID-19 outpatients would suggest the possible low involvement of the HPA axis and RAAS in the pathogenesis of the early stages of this infection.

## Authors' Contribution

S. G. contributed to conceptualization, project administration, methodology, and writing the original draft. M. N. contributed to the methodology and writing the original draft. M. A., S. M., and M. P. contributed to the methodology, data collection, and investigation. M. ST. and F. M. contributed to data analysis. S. G. contributed to funding acquisition, formal analysis, supervision, writing, review, and editing.

## Ethics

The Ethics Committee of the Abadan University of Medical Sciences, Abadan, Iran (Ethics code: IR.ABADANUMS.REC. 1399.079) approved this study.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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## Data Availability

Due to the confidential nature of the data (patient information) used in this study, raw data is confidential and will not be shared.

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