Evaluation of the renin-angiotensin-aldosterone system and neuroendocrine stress axis in COVID-19 suspicious outpatients tested for SARS-CoV2 RT-PCR referred to 16-hour comprehensive health centers in Abadan County: A cross-sectional case-control study

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Abstract

RAAS could play a substantial role in the pathophysiology of COVID-19. Also, the dynamics of the HPA axis may have changed in COVID-19. So, we aimed to assess RAAS and the HPA axis in COVID-19 suspicious outpatients referred to 16-hour comprehensive health centers in Abadan. Demographic and clinical data were collected. Serum cortisol and aldosterone measurements and blood grouping were done. Clinical symptoms of the positive PCR group were followed up on for four weeks. SPO2 was significantly lower in the positive PCR group, but the respiratory rate was significantly higher (\(P=0.03\) and \(P=0.001\), respectively). Outpatients with the O blood group showed higher levels of cortisol in comparison to those with A and AB blood groups (\(P=0.003\) and \(P=0.03\) respectively) in the positive PCR group. Negative PCR individuals with the AB blood type had significantly higher levels of cortisol compared with those who had A (\(P=0.02\)) and O (\(P=0.03\)) blood types. We saw significantly higher levels of aldosterone in males of the negative PCR group in comparison with 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. Also, over time, women were twice as likely as men to develop neurologic symptoms (OR= 0.530, \(P=0.015\)). 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 lower grades of COVID-19.

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**Keywords:** SARS-CoV2, RT-PCR, COVID-19, Aldosterone, Cortisol, ABO system of blood groups

What is already known about this topic?

RAAS could play a substantial role in the pathophysiology of COVID-19

The dynamics of the HPA axis may have changed in COVID-19

What does this article add?

Cortisol and aldosterone are associated with the chance of respiratory symptoms occurring overtime in the early stages of COVID-19

The levels of Cortisol and aldosterone do not seem to be related to the lower grades of COVID-19

**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-CoV2) is responsible for coronavirus 2019 (COVID-19), which became a pandemic in 2020(2). On January 7, 2020, a new coronavirus was extracted from pneumonia patients infected with the virus. In February 2020, the WHO identified the virus as the cause of COVID-19(3). Currently, RT-PCR testing for viral nucleic acid is the most common diagnostic method for COVID-19. Acute respiratory syndrome coronavirus 1 (SARS-CoV1) and SARS-CoV2—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)(4). Patients on ACE inhibitors or angiotensin receptor blockers (ARB) could be at greater risk due to the mechanism by which SARS-CoV-2 enters the cell(5).

Clinical trials are underway to evaluate the safety and efficacy of RAAS modulators in treating COVID-19(4). 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(6).

Often overlooked in consideration of the limited RAS, is aldosterone, which is a component of the wider RAAS. 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(7).

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 ARDS.

It has been shown that activation of MR in immune cells promotes the hyperinflammatory 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 IFN$\gamma$+CD8+ T lymphocytes. This is particularly important since COVID-19 infections are characterized by a cytokine storm and hyperinflammatory state, with Th17 T cells increased and increased CD8+ cells cytotoxicity.

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(8). 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, short-term glucocorticoids may be beneficial for COVID-19 patients for whom the disease progresses rapidly. However, the evidence is still limited.

In a systematic review, the efficacy and safety of glucocorticoids for the treatment of patients with COVID-19 were investigated. Twenty-three studies were reviewed, one of which was randomized controlled trial (RCT) 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 to 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, 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(9). 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.

COVID-19 may also affect the 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. Measuring serum cortisol is one of the methods used to assess the status of the HPA activity axis and immune system activity during COVID-19(10).

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, our aim was to screen RAAS and the HPA (neuroendocrine stress) axis in COVID-19 suspicious outpatients tested for SARS-Co2 RT-PCR referred to 16-hour comprehensive health centers in Abadan.

MATERIALS AND METHODS
Participants and study protocol
This epidemiological cross-sectional-analytical study was conducted at Abadan University of Medical Sciences, Abadan, Khuzestan Province, Iran. With the coordination between the vice-chancellor for education and the vice-chancellor for 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 PCR testing between the beginning of April and the end of July 2020 and had definitive (negative/positive) PCR results.

By telephone contact with these outpatients, those who met the criteria for inclusion (all ages, male and female gender) in the study and provided informed consent to participate were selected and assigned to two groups: negative and positive PCR (N = 52 in each group).
The study protocol was approved by the Ethics Committee of Abadan University of Medical Sciences (Ethics Code: IR.ABADANUMS.REC. 1399.079) following the Declaration of Helsinki for medical research involving human subjects. 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. Also, those who had known RAAS disorders or were on any medications which interfere with the activity of this system during the three last months were excluded. Also, 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.

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 information (age and sex) and clinical data (respiratory rate, pulse rate, SPO2) were collected. Also, the participant’s clinical symptoms were recorded in four groups of symptoms: 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)(11) via self-reports.

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. Routine laboratory investigations were performed within the first 24 hours after laboratory admission. These included blood groupings using commercially prepared monoclonal anti-A, anti-B, and anti-D antisera (Agappe Diagnostics Ltd., India), assessment of serum levels of cortisol (Cortisol ELIZA Kit; Monobind Inc.), and serum levels of aldosterone (Aldosterone ELIZA Kit; Monobind Inc.). The day of referral to Imam Khomeini Health Center was considered the first day of the study for each person. The clinical symptoms of PCR-positive outpatients were followed up with and recorded over 28 days (in one-week intervals) by telephone.

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 among 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 five measurements (sex, blood group type, age, serum levels of cortisol, and serum levels of aldosterone) on a positive PCR group (52 subjects) 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 Statistics version 26.0 and the significance level was considered as 0.05.

RESULTS AND DISCUSSION

General characteristics and clinical presentations

A total of 104 outpatients were tested for SARS-CoV2 RT-PCR, 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 age of the outpatients was 41.22±1.80 and 39.89±1.85 years for positive and negative PCR groups, respectively. General characteristics and clinical presentations are provided in Table 1.

We found a significant difference in the mean respiratory rate and SPO2 between negative and positive PCR groups. SPO2 was significantly lower in the positive PCR group than in the negative group (P = 0.03).
However, the mean respiratory rate was significantly higher in the positive PCR group ($P = 0.001$).

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). Using a pulse oximeter, we can quickly and easily measure oxygen levels and determine whether an individual needs to seek medical help, which is the case when their SPO2 is lower than 92%. Oximetry is a quick, 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(12).

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”(13). 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(14).

Our results, in line with recent findings, certify the importance of SPO2 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 like acute respiratory distress syndrome (ARDS), arterial and venous thrombosis, myocarditis, and varieties of neurological manifestations(15). COVID-19 can cause shortness of breath, lung damage, and impaired respiratory function.

Respiratory rate 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(16), which is in line with our results.

The reliable monitoring of respiratory rate is also very important for the treatment and management of other respiratory issues like chronic obstructive pulmonary disease (COPD)(17). There was no significant difference in terms of age, sex, 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 like heart rate, systolic, and diastolic blood pressure showed no difference between the study groups(15).

Biochemical and laboratory evaluations

We assessed blood group types of participants. The distribution of the ABO blood groups system in COVID-19 suspicious outpatients tested for SARS-CoV2 RT-PCR is presented in Table 1. We did not find any significant difference in terms of blood group type between negative and positive PCR groups ($P = 0.4$).

In contrast with our results, some recent studies identified associations between ABO blood groups and COVID-19. In one previous study, 397 patients with confirmed diagnoses of COVID-19 were admitted to Imam Khomeini Hospital Complex, Tehran, Iran. Also, 500 individuals were selected to form the control group, all of whom had been disclosed to the same medical center in June 2019, before the onset of the outbreak. The results demonstrated ABO histo-blood phenotypes are correlated with patients’ susceptibility to the infection. Specifically, a higher rate of infection was observed among patients with the AB histo-blood group, while patients with the O histo-blood group had shown a lower rate of infection(18).

Also, a study on COVID-19 patients in Wuhan and Shenzhen, China, discovered associations between ABO blood types and infection. They found that the odds of having COVID-19 were higher among the A blood group and lower among the O blood group relative to the general populations of Wuhan and Shenzhen(19).

Previous work has identified similar associations between ABO blood groups and different infections or disease severity following infections, including SARS-CoV1(20), P. falciparum(21), H. pylori(22), Norwalk
virus(23), hepatitis B virus(24), and N. gonorrhoeae(25). The difference between our observations and the results obtained in the mentioned studies may be due to differences in the population (especially in terms of race and ethnicity) or in the severity and stage of the disease.

On the other hand, there are studies whose results are in line with the results of our study. For instance, one study found insufficient evidence to conclude that the blood group distribution among all individuals tested for SARS-CoV2 is different from the general population at NYP/CUIMC (ABO: $P =0.64$, Rh: $P =0.36$)(26). Therefore, the results obtained regarding the possible relationship between blood group type and the incidence and severity of COVID-19, as well as the type of possible relationship, are still contradictory.

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 ($P =0.4$). We also categorized serum levels of cortisol as hypocortisolism ($<5 \mu g/dL$), norm cortisol ($5-25 \mu g/dL$), and hypercortisolism ($>25 \mu g/dL$).

Cortisol serum level categories are also shown in Table 1. Our results revealed that 3.8%, 92.5%, and 3.8% of the negative PCR group presented hypo, norm, and hypercortisolism, respectively. In the positive PCR group, 7.5%, 88.7%, and 3.8% were hypo, norm, and hypercortisolism, respectively.

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. Thus, patients with chronic glucocorticoid excess may be at high risk of developing COVID-19 with a severe clinical course(27).

In line with our results, a descriptive and analytical cross-sectional study was conducted in a population of patients infected with 2019-nCoV in Cameroon. The researchers found no statistically significant association between serum cortisol and disease severity. Like us, they concluded that the absence of a marked rise of cortisol during COVID-19 suggests the possible involvement of the hypothalamic-pituitary-adrenal axis in this infection. However, their study did not have a non-COVID control group(28).

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 and that this response is significantly higher in this patient cohort than in individuals without COVID-19(29).

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—our study was conducted on asymptomatic/mild and moderate outpatients of COVID-19. Serum levels of aldosterone also did not show any significant differences between the two groups ($P =0.2$).

Table 1 shows the results of a comparison between the mean serum aldosterone levels of negative and positive PCR groups. COVID-19 and the RAAS are closely linked both in infection and in possible post-infection inflammatory cascades(7). Campana et al. reported that increased levels of aldosterone might be associated with severe forms of COVID-19(30). This report can confirm the result 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 et al., which included 30 COVID-19 patients and controls, align 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, $P =0.865$)(31).

Correlation among baseline levels of tested biomarkers and demographic, clinical, and laboratory variables measured on the day of admission

The relationship of serum levels of cortisol and aldosterone with various demographic, clinical, and laboratory variables in negative and positive PCR groups was assessed (Tables 2 and 3). An evaluation of the relationship
of age and clinical variables of SPO2 and mean respiratory and pulse rates with baseline levels of cortisol and aldosterone showed no significant relationship in either the positive or negative PCR group. The investigation of the relationship between sex and basal serum levels of cortisol and aldosterone in the positive PCR group did not show any significant relationship.

However, ANOVA and post-hoc LSD analysis showed a significant difference between outpatients with A and O blood compared with those with AB and O blood in terms of cortisol serum levels. Specifically, outpatients with O blood had higher levels of serum cortisol in comparison to those with A and AB blood ($P = 0.003$ and $P = 0.03$, respectively).

There was no significant relationship between blood group type and aldosterone serum levels in the positive PCR group. In the negative PCR group, ANOVA analysis did not show a significant difference between individuals with different types of blood in terms of serum levels of aldosterone or cortisol. However, LSD analysis showed that negative PCR individuals with blood type AB had significantly higher levels of serum cortisol compared with those with blood type A ($P = 0.02$) and O ($P = 0.03$). Our results also showed a significantly higher level of aldosterone in males of the negative PCR group in comparison with females ($P = 0.05$).

Association of estimated parameters with the chances of different groups of clinical symptoms occurring over time

Association of sex, age, blood group type, baseline cortisol level, and baseline aldosterone level with the chances of clinical symptoms occurring over time are presented in Table 4. In terms of the gastrointestinal and general categories of clinical symptoms, our analysis indicated no association between any of the examined parameters and the chances of symptoms occurring.

Meanwhile, we observed a significant relationship between baseline serum cortisol and aldosterone levels with the chances of respiratory symptoms occurring over time. 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 level (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, we saw a significant relationship between sex (OR = 0.530, $P = 0.015$) and the outcome variable. Over time, women are twice as likely as men to develop neurologic symptoms.

Conclusion

The absence of a marked rise in serum levels of cortisol and aldosterone during low grades of COVID-19 would suggest the possible involvement of the HPA axis and RAAS during the early stages of this infection.

References


Table 1. General characteristics, clinical presentations, serum levels of cortisol and aldosterone, and ABO blood group system distribution in COVID-19 suspicious outpatients tested for SARS-CoV2 RT-PCR*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Variables</th>
<th>Variables</th>
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</thead>
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<tr>
<td>Age (mean)</td>
<td>Age (mean)</td>
<td>Age (mean)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male (%)</td>
<td>Female (%)</td>
</tr>
<tr>
<td>Mean Respiratory rate (number/min)</td>
<td>Mean Respiratory rate (number/min)</td>
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<tr>
<td>Mean Pulse rate (number/min)</td>
<td>Mean Pulse rate (number/min)</td>
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<tr>
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<td>δρτισολ (μγ/δΑ)</td>
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<tr>
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<td>SPO2 (%)</td>
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</tr>
<tr>
<td>Blood Groups (%)</td>
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*The results were shown as mean± standard deviation (SD) for quantitative and number (percent) for qualitative data. Independent sample T and chi square tests were applied to compare study groups. SPO2, oxygen saturation levels.

Table 2. Correlation among baseline serum biomarkers and demographic, clinical, and laboratory variables measured on the day of admission in negative PCR Group*
Correlations between 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 tests.

Table 3. Correlation among baseline serum biomarkers and demographic, clinical, and laboratory variables measured on the day of admission in positive PCR Group*

<table>
<thead>
<tr>
<th>Demographic and Laboratory Variables</th>
<th>Cortisol</th>
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<tr>
<td></td>
<td>r /mean± SD</td>
<td>P- Value</td>
<td>r /mean± SD</td>
<td>P- Value</td>
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<td>14.2±5.3</td>
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<td>0.3</td>
<td>142.4</td>
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<tr>
<td>O</td>
<td>13.3±4</td>
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*Correlations between 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 tests.

Table 4. Odds ratio and 95% confidence interval (95% CI) estimated by GEE analysis with unstructured model to determine the observed symptoms progression of COVID-19 and the associations with demographic, blood groups, and laboratory parameters among infected patients
<table>
<thead>
<tr>
<th></th>
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<th>p-value</th>
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<td>Group O</td>
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<td>(0.143-2.806)</td>
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<td>(0.933-1.001)</td>
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*Dependent Variables*

Hosted file