

Original Article

Molecular and Physiological Study in Patients with Coronavirus in Thi-Qar Province Iraq

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Abstract

The current study aimed to study the effect of Covid-19 disease on some physiological parameters for assessing the physiological effect of Covid-19. The current study included 100 patients, 50 males, and 50 females, whose ages ranged from 10 – 73 years infected with Covid-19 molecularly diagnosed at AL-Imam Hussein Teaching Hospital in Thi-Qar Province from April to August 2021 and 50 people as a control group. The results illustrated a significant decrease in the level of hemoglobin, lymphocyte, and platelet in the patient's group, whereas a significant increase in the count of total white blood cells (WBC) was recorded in patients compared with the control group. The results showed that the hemoglobin level, WBC, and platelet increased significantly in males compared to females, while the lymphocytes increased significantly in female patients. The results of age groups showed non-significant differences in the hematological parameters. The current results illustrated a significant increase in the level of blood urea, serum creatinine, and random blood sugar in the patient's group. According to the gender of patients, the blood urea increased significantly in the female group, while the blood sugar increased significantly in the male group. On the other hand, the serum creatinine had no significant difference. According to age groups, a significant increase in blood urea was recorded in patients over 70 years, but no differences in both creatinine and random blood sugar level were noted. The current study recorded that the concentration of CRP, ferritin, and D. Dimer was significantly higher in patients; according to gender, the level of CRP and ferritin increased non-significantly compared with a male group of patients, while D. Dimer increased significantly in the female group. According to age group, only CRP increased significantly in the first age group compared with other age groups. The sequencing analysis was performed for 10 isolated Covid-19, and the result indicates that only one isolated sample has a sequencing identity of 99,5% with Covid-19 in Iran.

Keywords: Covid-19, Hematological, spike protein, Angiotensin 2, ACE2

1. Introduction

Coronavirus is a significant infection that primarily affects the respiratory system of humans. Previous coronavirus (CoV) outbreaks include the severe acute respiratory syndrome (SARS)-CoV and the Middle East respiratory syndrome (MERS)-CoV, both of which have been labeled as major public health threats. A group of patients was admitted to hospitals

in late December 2019 with an initial diagnosis of pneumonia of uncertain cause. These cases were related epidemiologically to a wholesale market for seafood and wet animals in Wuhan, Hubei Province, China (1).

SARS-CoV, also known as SARS-CoV-1, is a zoonotic virus (2). Four varieties (OC43, 229E, NL63, and HKU1) cause mild illnesses such as the

common cold and digestive system infections. Due to their zoonotic emergence and breach of species barriers, causing high pathogenic and human mortality levels, and having been highly affected by serious public health fears, the other two are severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East Respiratory Coronavirus (MERS-CoV). SARS- and MERS-CoVs have been passed from bats to palm civets or dromedary camels to humans (3). Both SARS and MERS CoVs are highly pathogenic, generating 8096 and 2519 human cases in 2003–2004 and 2012–one-to-one, respectively, with mortality of 9.6% and 34.3 percent (4).

In December 2019, numerous pneumonia patients with unclear etiology were recorded in Wuhan, China. Epidemiological examinations linked most patients to the Huanan Sea Nutrition Wholesale market. This pneumonia was identified as a causative agent of the severe acute coronavirus-2 disease (SARS-CoV-2), also known as the new 2019 coronavirus (2019-nCoV) and the coronavirus disease-2019 (COVID-19) (5). Under electron microscopy, the crown looks to be a coronavirus. They are encased in a single strand of the RNA genome, an RNA virus's lead genome (6). ORF5 follows the 16 non-structural proteins encoded by ORF1a/b for four key structural proteins: spikes, envelopes, membranes, and nucleocapsids (6). In hospitalized individuals with coronavirus disease, the bacterial and fungal co-infection caused by the severe acute breathing syndrome coronavirus 2 (SARS-CoV-2) is unknown (COVID-19).

2. Materials and Methods

Nasal swabs were collected from COVID-19 patients only, and blood was drawn for 100 patients and 50 control groups hospitalized in Al-Hussein Teaching Hospital in Thi-Qar, the isolation unit for patients aged between 20 to 70 years, for the period from the beginning of April to the end of August 2021 for patients diagnosed with COVID-19. The blood sample

was centrifuged and used to estimate physiological parameters. A hemoanalyzer (Mindy) estimated the complete blood count, and cobbas estimated renal function tests. The Primer for the S gene (spike) is as follows:

Forward primer: CAAATCGCTCCAGGGCAAAC

Reverse primer: CCCGCCGAGGAGAATTAGTC

3. Results

3.1. Estimate Hematological Parameters in Patients and Control

The recorded data showed that the blood parameters (Hb, WBC, lymphocyte, and platelets) significantly differed between infected participants and healthy individuals (control group), according to table 1.

3.2. Estimate Renal Function in Patients and Control

When comparing the disease states of people with a corona concerning the blood parameters in terms of blood urea and serum creatinine, it was found that there were significant differences between infected individuals and healthy participants (control group); the results are tabulated in table 2.

3.3. Estimate of RBS in Patients and Control

The results of blood sugar analysis showed that in the infected group with coronavirus, the level of blood sugar (212.04 ± 57.11) significantly increased compared with the healthy control group (99.96 ± 12.99) (Table 3).

3.4. Molecular Identification of Covid-19

The S gene of Covid-19 was detected by gel electrophoresis by the S gene ladder with product size Of 826 base pair.

3.4.1. Sequencing Analysis

The sequencing analysis was performed for ten isolated SARS-2 and compared with sequencing in a gene bank (NCBI), as shown in table 4.

Ten isolated Covid-19 were sent to South Korea for sequencing analysis, and the result indicates that only one isolated sample has a sequencing identity of 99,5% with Covid-19 in Iran.

Table 1. Estimate of Hematological Parameters in Patients and Control

Groups Statistics				
Parameters	Groups	No.	Mean & Std.	P-value
Hb	Patients	100	12.04±2.02	<0.001
	Control	100	13.45±1.27	
WBC	Patients	100	13.90±4.41	<0.001
	Control	100	7.11±1.64	
LYM	Patients	100	2.29±0.60	<0.001
	Control	100	2.65±1.00	
PLT	Patients	100	236.80±77.33	<0.001
	Control	100	240.60±54.61	

Table 2. Estimate of Renal Function in Patients and Control

Groups Statistics				
Parameters	Groups	No.	Mean & Std.	P-value
B. Urea	Patients	100	67.83±19.63	<0.001
	Control	100	29.56±6.73	
S. Creatinine	Patients	100	1.73±0.515	<0.001
	Control	100	0.73±0.193	

Table 3. Estimate of RBS in Patients and Control

Groups Statistics				
Parameters	Groups	No.	Mean & Std.	P-value
RBS	Patients	100	212.04±57.11	<0.001
	Control	100	99.96±12.99	

Table 4. Sequencing analysis for isolated Covid-19

Isolate SARS-2 from patients	Isolates Accession Number	NCBI-BLAST Homology Sequence Identity (%)		
		Gene bank Accession Number	Country	Identity (%)
SARS-CoV-2 Isolate No. 1	OL372605.1	OL701608.1	USA	100%
SARS-CoV-2 Isolate No. 2	OL372606.1	MW321435.1	IRAN	99.55%
SARS-CoV-2 Isolate No. 3	OL372607.1	OL701558.1	USA	100%
SARS-2 Isolate No. 4	OL372608.1	OL701515.1	USA	100%
SARS-CoV-2 Isolate No. 5	OL372609.1	OV092970.1	KSA	100%
SARS-CoV-2 Isolate No. 6	OL372610.1	OL701504.1	USA	100%
SARS-CoV-2 Isolate No. 7	OL372611.1	OL701518.1	USA	100%
SARS-CoV-2 Isolate No. 8	OL372612.1	OV088000.1	KSA	100%
SARS-CoV-2 Isolate No. 9	OL372613.1	OV090880.1	KSA	100%
SARS-CoV-2 Isolate No. 10	OL372614.1	OL701608.1	USA	100%

4. Discussion

4.1. Estimation of Hematological Parameters of SARS-2 Patients and Control Groups

The current study recorded a significant decrease in the level of hemoglobin, percent of lymphocytes, and platelet in the patient group, whereas there was a

significant increase in the count of white blood cells in the patient group compared with the control group. According to the gender of patients, the hemoglobin level, count of white blood cells, and platelet increased significantly in the male group, while the percent of lymphocytes increased significantly in the female

group of patients. According to age groups a non-significant differences in the hematological parameters.

The current study agreed with the study of Asghar, Khan (7); their study investigated that the level of hemoglobin, lymphocyte, and platelet was decreased significantly in the patient's group, and their levels have a negative relation to the severity of the disease. Also recorded, the count of white blood cells was higher than the control group. Their study investigated that the patient's ward in the intensive care unit had lower hemoglobin, platelet, and lymphocyte levels while increased white blood cell count.

The study of Usul, Şan (8), found a different result from what our study found; their study recorded the level of hemoglobin, percentage of lymphocytes, and platelet increased significantly in control groups than SARS-2 patients, while their study agreed with the current study with regarding the count of white blood cell where it was increased in SARS-2 patients. Their study also recorded non-significant differences according to gender except for hemoglobin, but this was a typical result in female hemoglobin levels.

In a study conducted by Anurag, Jha (9), the results suggested that the count of lymphocytes had a positive correlation with disease severity and noted the elevated Neutrophil-lymphocyte ratio to be a poor prognostic marker for patients with SARS CoV-2 infection. A similar study by Lagunas-Rangel (10) also suggested that an increased Neutrophil-lymphocyte ratio predicts poor prognosis in COVID-19.

Several studies have highlighted the importance of B and T lymphocytes, given that neutralizing antibodies and T cell responses are required for effective immunity. In addition, other reports have described that myeloid cells such as macrophages and monocytes play a significant role in the immunity against SARS-CoV-2 and deregulated pro-inflammatory signature that characterizes severe COVID-19 (11). A decrease in the number of lymphocytes (lymphocytopenia) was detected in patients in the critical stage resulting in a higher ratio of platelets to lymphocytes among patients undergoing treatment in intensive care units or

deceased compared to patients in the initial stage. of infection (admission) or accommodation in isolation wards, thus determining the ratio of platelets to lymphocytes as an independent mediator that predicts prognosis and mortality in critical cases that correlate with other diseases (13,12). The low hemoglobin levels may be due to the presence of concomitant diseases or anemia and habits such as smoking cigarettes. The patient files used in this study did not include detailed patient history, so their effect on hemoglobin levels was not accounted for. The average hemoglobin level in females is lower than in males.

4.2. Estimation of Renal Function Test and RBS of SARS-2 Patients and Control Groups

The current results illustrated a significant increase in blood urea, serum creatinine, and random blood sugar level in the patient's group compared with the control group. According to the gender of patients, the blood urea increased significantly in the female group, random blood sugar increased significantly in the male group, while serum creatinine had no significant difference. According to age groups, there was a significant increase in urea level in patients over 70 years, but no notable differences in both creatinine and random blood sugar level.

The current study agreed with the results of a study Ok, Erdogan (14); their study involved the values of blood urea nitrogen/creatinine ratio and their relation to disease severity and concluded that the concentration of blood urea and creatinine increases significantly in patients compared with the control group, also noted the highest level of both parameters in patients with critical status than moderate or severe status. The study of Cheng, Luo (15). Their study involved the effect of covid-19 disease on patients with renal problems, and they concluded an association between kidney involvement and poor outcome in patients with COVID-19. We found that patients with elevated baseline serum creatinine were more likely to be admitted to the intensive care unit and to undergo mechanical ventilation, suggesting that kidney disease on admission represented a higher risk of deterioration.

It has been reported that kidney injury was associated with an increased risk of death in patients with influenza A virus subtype H1N1 and SARS (16).

The study Cheng, Luo (15) agreed with a current study by reporting that patients with covid-19 increased dramatically the concentration of urea and creatinine, and this increase was associated with D. Dimer increased, also noted that the concentration of urea and creatinine in dead patients was higher than patients in the intensive care unit and the last more than moderate status. D-dimer levels play a significant role in diagnostic algorithms to exclude venous thromboembolism; elevated plasma D-dimer levels in adult CAP are associated with increased inflammatory reaction and are a prognostic variable (17). Recent studies reported that D-dimer was significantly associated with the severity of Covid-19. D-dimer is a lab marker that is commonly tested in hospitalized patients. However, the diagnostic performance of serum D-dimer levels in Covid-19 patients has not been reported (18). In a study conducted by Qiu et al. (18) concluded that the severity of the kidneys effect could be related to the amount of virus entering the lungs due to the excessive expression of angiotensin-2 receptors and their effect on the kidneys, and there is a combined effect associated with an increase in the gene expression of angiotensin and an increase in the level of D. dimer.

Angiotensin 2 is an enzyme that physiologically resists activation of RAAS but also acts as a receptor for SARS viruses. ACE-2 is a type I membrane protein expressed in the lung, heart, kidney, and intestine but is primarily associated with cardiovascular disease (19). RNA sequencing data from modern human tissues showed that the expression of ACE-2 in the kidney was approximately 100-fold higher than that in the lung; therefore, kidney disease may be caused by the entry of coronavirus into kidney cells through an ACE2-dependent pathway. Moreover, RAAS activity is increased in patients with chronic kidney disease, so there is a systemic increase in ACE2 receptors that can

be translated into infection SARS-CoV-2 cells are more accessible (20).

The current study agreed with the study of Bhandari, Shaktawat (21), which indicated that patients with covid-19 have higher levels of RBS, even in patients who do not have pre-diabetes and their RBS increases as the disease progress. RBS is an important indicator of mortality risk; a recent study reported that ACE2 receptors are expressed in pancreatic islets and that infection with SARS-CoV-1 causes hyperglycemia in people without diabetes. Persistent hyperglycemia was observed 3 years after recovery from SARS, indicating beta-cell damage in the pancreas. Similar effects may be demonstrated by SARS-CoV-2, resulting in increased blood sugar levels (22).

Authors' Contribution

Study concept and design: M. C.

Acquisition of data: M. C.

Analysis and interpretation of data: M. O. K.

Drafting of the manuscript: E. A. K. J.

Critical revision of the manuscript for important intellectual content: M. O. K.

Statistical analysis: E. A. K. J.

Administrative, technical, and material support: M. O. K.

Ethics

The Review Board at the Ahi Evran University, Kırşehir, Turkey approved this study. Written informed consent was obtained from all participants for this work.

Conflict of Interest

The authors declare that they have no conflict of interest.

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