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Research Article

Serum Cytokine Levels As Critical Parameters in Early Diagnosis of Disease Progression in COVID-19: A Pilot Study

Walaa Mohammedsaeed[1], Ziab Zakey Alahmadey[2], and Nikhat Manzoor[3]

[1]Clinical Biochemistry, Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, Taibah University, Madinah, Saudi Arabia
[2]Laboratory Department, Ohud Hospital Madinah, Saudi Arabia
[3]Medical Mycology Lab, Department of Biosciences, Jamia Millia Islamia, New Delhi, India

ORCID:
Walaa Mohammedsaeed: https://orcid.org/0000-0002-6696-5441

Abstract

Background: The severity of Coronavirus disease 2019 (COVID-19) has been proposed to be associated with cytokine dysregulation. A significant number of patients become serious and need intensive care in hospitals.

Methods: The concentrations of cytokines interleukin (IL-6, IL-10) and tumor necrosis factor (TNF) were estimated using enzyme-linked immunosorbent assay (ELISA) in serum samples of 60 adult patients infected with SARS-CoV-2 along with 50 healthy controls of the same age. The mean age of the subjects was 50-52 years and included an equal number of males and females. The patients were further grouped as severe (38 patients) and non-severe cases (22 patients).

Results: The mean serum cytokine levels were significantly higher in the COVID-19 patients than in the healthy controls. IL-6 was excessively elevated in comparison to IL-10 and TNF. Comparative analysis of severe versus non-severe cases revealed only slight alterations in the cytokine levels: IL-6 being the most elevated in severe cases. The concentration of the liver enzyme ALT was higher than AST in both severe and non-severe cases. The mean concentration of serum electrolytes (Na, K, and Ca) did not vary much between the patients and healthy controls.

Conclusion: There was a significant positive correlation between the levels of cytokines serum biomarkers in COVID-19 patients. It may be suggested that early detection of cytokines, especially IL-6 and serum biomarkers can help predict disease prognosis and severity in COVID-19 patients.

Keywords: COVID-19, Cytokines, disease severity, diagnosis, liver function, kidney function

Corresponding Author: Walaa Mohammedsaeed; email: wmohammed-saeed@taibahu.edu.sa

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1. Introduction

The SARS-CoV-2 infection (COVID-19) has been wreaking havoc since its outbreak in December 2019. More than 660 million infections and 6.7 million deaths have been reported to date. The virus has not only spread worldwide but has also mutated several times [1]. The disease has pneumonia-like symptoms affecting the lungs with varying degrees of severity. From the infected lungs, it may spread to other parts of the body, sometimes leading to multiple organ failure [2]. Acute lung injury in COVID-19 patients may be triggered by the release of inflammatory molecules, activated immune cells, overproduction of cytokines, and the presence of adhesins [3]. The damage caused to the lungs and other body organs has been linked to the abnormally strong proinflammatory host immune response. Described as the 'cytokine storm syndrome,' it plays an important role in the pathophysiology of COVID-19. The clinical symptoms vary among individuals from asymptomatic and non-specific to more serious, where patients need hospitalization [4]. The severity of COVID-19 disease depends on several factors imparting poor prognoses, such as high viral load, old age, and comorbidities (cancer, coronary heart disease, diabetes, and hypertension) [5, 6]. The disease pathogenesis is not clearly understood and comprises several intricate and complex physiological processes. Cytokines are released by immune cells to regulate inflammatory responses during tissue damage and infections. It has been shown that some proinflammatory cytokines, namely, tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β), interferon gamma (IFN- γ), interleukins (IL-1, IL-8, and IL-6), play a crucial role in several pathological conditions [7, 8]. The involvement of TNF-α is an intermediary in the pathogenesis of certain viral infections like influenza [9]. Some other cytokines, like IL-4 and IL-10, help reduce inflammation [10]. The role of cytokines in disease pathogenesis is still unclear, but reports suggest that their excessive outburst may be responsible for disease progression and sometimes death of the patient [11, 12]. The serum cytokine levels must be regulated, but whether anti-cytokine therapy has any benefit needs further evaluation [13]. The liver, heart, and kidneys are vital organs of the human body that perform some critical functions. Detection of specific biomarkers and their appropriate concentrations in the blood helps investigate these organs' health status [14]. A strong connection has been observed between the severity of COVID-19 and chronic liver diseases accompanied by inflammation and dysregulation of immune responses. Patients suffering from underlying conditions carry a greater risk of adverse consequences after contracting COVID-19. Moreover, when infected, healthy individuals exhibit abnormal liver function, directly implicating the virus in damaging the liver and

metabolism [15]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are biomarkers for hepatocellular damage. Bilirubin and albumin levels assess the liver's secretory and synthetic capacities, respectively [16]. Increased levels of serum creatinine can indicate renal dysfunction. Serum ferritin, creatine kinase (CK), lactate dehydrogenase (LDH), inflammatory factors, and electrolytes (sodium, potassium) can be used as indicators for COVID-19 [17]. As observed with other viral infections, increasing cytokine levels have emerged as early parameters in COVID-19 disease advancement [18]. Hence, estimating increased serum cytokine levels and abnormalities in the hepatic, renal, and cardiac biomarkers can be important in predicting poor outcomes in COVID-19 patients. The levels of IL-6, IL-10, and TNF in the serum can also predict the severity of the disease, as cytokines play an important role in host immunity and immunopathology during the infection. The present study thus attempts to correlate cytokine levels with biomarkers of vital body organs in COVID-19 patients.

2. Methods

2.1. Patients and data collection

A total of 60 COVID-19 patients were randomly selected from the Ohud Hospital, Madinah, Saudi Arabia. A healthy control group of similar age (50 subjects) was selected from Taibah University, Madinah, Saudi Arabia. The clinical and laboratory data of the patients were analyzed and documented. All the cases were assessed according to the inclusion and exclusion criteria. Individuals with a history of endocrine diseases were excluded. Patients with confirmed COVID-19 were categorized into two groups (severe and non-severe) based on clinical characteristics, symptoms, and results obtained from chest radiography [19].

Blood samples (3 ml) were collected in serum separator tubes and allowed to clot for 2 h at room temperature before centrifugation at 1000×g for 15 min. Serum was collected and stored at -80˚C. The serum concentrations of TNF-α, IL-10, and IL-6 were determined in the subjects. Biochemical assays (kidney and liver function tests) were performed to estimate the concentration of creatinine, urea, Na, K and Ca, troponin I, C-reactive protein (CRP), LDH, CK, ALT, AST, bilirubin, albumin, and ferritin using the automated ARCHITECT *c*4000, as per the manufacturer's guidelines.

2.2. Enzyme-linked immunosorbent assays (ELISA)

Cytokine concentrations were determined using ELISA development kits (CUSABIO Technology LLC, Houston, USA) in adherence with the manufacturer's instructions for TNF-α, IL-10, and IL-6. The antibody specific for each cytokine was pre-coated onto a microplate. The optical density was verified using a Mindray mr-96a/Mindray ELISA reader microplate reader (Mindray, Guangdong, China) set at 450 nm. The experiment was carried out twice, with duplicate samples within each experiment.

2.3. Statistical analysis

Quantitative data were normally distributed and expressed as mean with standard deviations (± SD). Student's t-test evaluated differences between groups. The Spearman correlation coefficient calculations were used to investigate the association between the different parameters. All statistical analyses were performed using the Statistics Package of Social Science (SPSS 20, SPSS Inc., Chicago, IL) and two-tailed p-values.

3. Results

3.1. Patient characteristics and cytokine levels

The mean age of the COVID-19 patients was comparable (50 ± 10.6 years) to that of the healthy controls (52 ± 12.8 years) and included subjects from both genders equally. The serum concentrations of liver enzymes ALT and AST in the patients were high (60.44 ± 33.07 U/L and 49.20 ± 25.17 U/L) in comparison to healthy controls, where the values were 10.8 ± 4.6 U/L and 15.7 ± 7.3 U/L, respectively. Similarly, in COVID-19 patients the concentrations of albumin and bilirubin were 45 ± 15.35 g/L and 50.34 ± 21.80 mg/dL, respectively, while healthy controls were 40.7 ± 31.9 g/L and 1.4 ± 0.8 mg/dL, respectively. Thus, the serum concentrations of liver function biomarkers in COVID-19 patients were much higher than in healthy controls.

The serum levels of creatinine, urea, Na, K, and Ca (biomarkers of renal function) in COVID-19 patients were 292.95 ± 86.73 µmol/L, 20.83 ± 10.98 mmol/L, 149.35 ± 22.96 mmol/L, 1.33 ± 1.2 mmol/L, and 2.15 ± 0.87 mmol/L, respectively. Serum concentrations for the same in healthy controls were 55.67 ± 20.93 µmol/L, 8.10 ± 5.13 mmol/L, 130.87 ± 8.77 mmol/L, 3.77 ± 0.77 mmol/L, and 2.10 ± 0.52 mmol/L, respectively. Except for Ca and K, the concentrations of renal function biomarkers were significantly higher in

COVID-19 patients compared to controls. The serum levels of LDH (391.84 ± 75.9 U/L), CK (393.88 ± 75.27 U/L), CRP (50 ± 15.35 mg/L), and ferritin (599.64 ± 51.90 ng/mL), although not troponin (0.5 ± 0.20 ng/L), were significantly increased in the patients in comparison to healthy controls where these values were respectively, 110.57 ± 27.6 U/L, 50.44 ± 40.17 U/L, 6.9 ± 3.35 mg/L, 107.69 ± 55.80 ng/mL, and 0.2 ± 0.10 ng/L (Table 1). Also, the levels of cytokines IL-6 (0.8 ± 0.75 pg/mL), IL-10 (3.2 ± 2.05 pg/mL), and TNF (6.1 ± 3.32 pg/mL) in healthy controls were found to increase to 30.27 ± 13.35 pg/mL, 17.6 ± 7.2 pg/mL and 15.4 ± 6.4 pg/mL in the diseased individuals, $p < 0.001$**.

Table 1: Clinical characteristics and cytokine levels in COVID-19 patients and the healthy population.

Parameters	**COVID-19 patients n = 60)**	**Control (n = 50)**	*P*-value
Age	50 ± 10.6	52 ± 12.8	
Sex (number)	Male = 30	Male = 25	
	Female = 30	Female = 25	
ALT (U/L)	60.44 ± 33.07	10.8 ± 4.6	0.01*
AST (U/L)	49.20 ± 25.17	15.7 ± 7.3	0.02*
Bilirubin (mg/dL)	50.34 ± 21.80	1.4 ± 0.8	<0.001**
Albumin (g/L)	45 ±15.35	40.7 ± 31.9	0.06
Creatinine (µmol/L)	292.95 ± 86.73	55.67 ± 20.93	0.01*
Urea (mmol/L)	20.83 ± 10.98	8.10 ± 5.13	0.06
Na (mmol/L)	149.35 ± 22.96	130.87 ± 8.77	0.04*
K (mmol/L)	1.33 ± 1.2	3.77 ± 0.77	0.04*
Ca (mmol/L)	2.15 ± 0.87	2.10 ± 0.52	0.072
CK (U/L)	393.88 ± 75.27	50.44 ± 40.17	<0.001**
LDH (U/L)	391.84 ±75.9	110.57 ± 27.6	0.002**
Troponin (ng/L)	0.5 ± 0.20	0.2 ± 0.10	0.08
Ferritin (ng/mL)	599.64 ± 51.90	107.69 ± 55.80	<0.001**
CRP (mg/L)	50 ± 15.35	6.9 ± 3.35	0.003*
IL-6 (pg/mL)	30.27 ± 13.35	0.8 ± 0.75	<0.001**
IL-10 (pg/mL)	17.6 ± 7.2	3.2 ± 2.05	<0.001**
TNF (pg/mL)	15.4 ± 6.4	6.1 ± 3.32	<0.001**

Data are presented as mean ± SD. *P < 0.05, **P < 0.001.

3.2. Cytokine levels and other biomarkers in severe and non-severe COVID-19 patients

Table 2 shows the comparative variations in the concentrations of hepatic, renal, and cardiac biomarkers with that of cytokines (IL-6, IL-10, and TNF) between severe and non-severe COVID-19 cases. The mean IL-6 concentration in severe COVID-19 patients was 20.17 ± 10.35 pg/mL, statistically greater than in non-severe patients (10.8 ± 5.75

pg/mL). Similarly, the mean concentrations of IL-10 (15.03 ± 5.62 pg/mL) and TNF (17.25 ± 6.47 pg/mL) in severe COVID-19 patients were again significantly higher than in non-severe patients (10.21 pg/mL and 11.21 ± 4.32, respectively). Compared to non-severe COVID-19 patients, the severe cases showed significantly higher levels of serum ALT, AST, and bilirubin, which were 65.22 ± 50.91 mmol/L and 53.95 ± 46.50 mmol/L and 50.89 ± 32.62 mg/dl, respectively. In the case of non-severe cases, the serum levels of ALT, AST, and bilirubin were 12.8 ± 8.6, 15.7± 7.3, and 20.4 ± 1.5, respectively. The serum levels of creatinine and Na were significantly higher (184.28 ± 34.89 µmol/L and 148.10 ± 20.71 mmol/L, respectively) in severe cases, while the values in non-severe cases were 78.45 ± 50.99 and 138.17 ± 78.99, respectively. The concentration of K was slightly higher (2.99 ± 2.92 mmol/L) in non-severe cases than in severe ones (1.25 ± 0.92 mmol/L). The levels of cardiac biomarkers were again significantly higher in severe cases compared to non-severe patients (Table 2). The concentrations of CK, LDH, ferritin, and CRP were 55.94 ± 100.07 U/L, 100.54 ± 25.9 U/L, 100.64 ± 45.80 ng/mL, and 15.9 ± 4.35 mg/L in non-severe cases. The concentration of the same biomarkers was considerably increased in severe COVID-19 cases. In patients with severe disease, the serum concentration values for CK, LDH, ferritin, and CRP were 350.88 ± 45.17 U/L, 362.44 ± 45.9 U/L, 750.60 ± 81.80 ng/mL, and 29 ± 9.35 mg/L, respectively.

Table 2: Alterations in cytokine levels and other biomarkers in severe and non-severe COVID-19 patients.

Biomarkers	**Non-severe cases, n = 22**	**Severe cases, n = 38**	**P-value**
	Mean ± SD	**Mean ± SD**	
IL-6	10.8 ± 5.75	20.17 ± 10.35	0.01*
IL-10	10.21 ± 4.05	15.03 ± 5.62	0.03*
TNF	11.21 ± 4.32	17.25 ± 6.47	0.01*
ALT	12.8 ± 8.6	65.22± 50.91	0.001**
AST	15.7 ± 7.3	53.95 ± 46.50	0.001**
Bilirubin (mg/dl)	20.4 ± 1.5	50.89 ± 32.62	0.002**
Creatinine(µmol/L)	78.45 ± 50.99	184.28 ± 34.89	0.003**
Na(mmol/L)	138.17 ± 78.99	148.10 ± 20.71	0.04*
K(mmol/L)	2.99 ± 2.92	1.25 ± 0.92	0.06
CK (U/L)	55.94 ± 100.07	350.88 ± 45.17	0.003**
LDH (U/L)	100.54 ± 25.9	362.44 ± 45.9	0.001**
Ferritin (ng/mL)	100.64 ± 45.80	750.60 ± 81.80	0.0001**
CRP (mg/L)	15.9 ± 4.35	29 ± 9.35	0.05*

Data were analyzed using t-test. The values indicate statistical significance ($p < 0.001$** and $p < 0.05$*)

3.3. Correlation between cytokine levels and biochemical markers in COVID-19 patients

The relationship between serum cytokines and other biochemical markers in COVID-19 patients is in Table 3. The values indicated that serum IL-6 showed significant positive correlations with ALT (r = 0.750, p = 0.02), AST (r = 0.659, p = 0.04), CK (r = 0.569, p = 0.04), Ferritin (r = 0.704, p = 0.02), and CRP (r = 0.859, p = 0.001). On the other hand, IL-10 showed significant positive correlations with only ALT (r = 0.636, p = 0.04) and AST (r = 0.672, p = 0.5). Interestingly, TNF showed a positive correlation with ALT (r = 0.726 p = 0.05), AST (r = 0.682 p = 0.03), CK (r = 0.619 p = 0.04), Ferritin (r = 0.604 p = 0.02) and CRP (r = 0.784, p = 0.03). Our results showed no significant positive correlations between serum cytokines with biological parameters like bilirubin, creatinine, Na, and LDH.

TABLE 3: Relationship between serum cytokine levels and other biochemical parameters in COVID-19 patients.

	IL-6		IL-10		TNF	
	r	*P*	**r**	*P*	**r**	P
ALT	0.750	0.02*	0.636	0.04*	0.726	0.05*
AST	0.659	0.04*	0.672	0.05*	0.682	0.03*
Bilirubin	0.210	0.060	0.329	0.07	0.043	0.08
Creatinine	0.082	0.073	0.257	0.06	0.257	0.09
Na	0.201	0.025	0.078	0.08	0.068	0.06
CK	0.569	0.04*	0.319	0.07	0.619	0.04*
LDH	0.122	0.06	0.136	0.07	0.123	0.09
Ferritin	0.704	0.02*	0.257	0.08	0.604	0.02*
CRP	0.859	0.001**	0.426	0.06	0.646	0.03*
IL-6			0.741	0.002**	0.784	0.003**
IL-10	0.741	0.002**			0.604	0.02*
TNF	0.784	0.003**	0.604	0.02*		

Correlation is statistically significant ($P < 0.05$* and $P < 0.001$**)

4. Discussion

Under normal conditions, cytokines coordinate the host's immune response against microbial infections. Insufficient cytokines are incapable of modulating inflammation and dealing with pathogenic invasion. Similarly, excessively high amounts can sometimes cause irreparable damage. COVID-19 patients discharged from the ICU seem to have more elevated levels of inflammatory cytokines than individuals who are less severely

infected. Cytokine storm generation is not a universal feature of all infections. It has been observed to be triggered by the influenza A virus and may have been the cause of death during the 1918 influenza pandemic [20]. Immunotherapy, organ transplantation, cancer therapy, AIDS, and sepsis are some conditions that can trigger the overproduction of cytokines and inflammatory molecules, affecting multiple organs and become life-threatening [21]. In the case of COVID-19, the selective behavior of the host response toward the virus needs to be investigated. Although unclear, the expression of specific genes may generate a cytokine storm besides other factors [22]. The severity of the disease depends on underlying health conditions like diabetes, heart disease, hypertension, and other comorbidities. Early diagnosis can be made by estimating the level of cytokines before the body organs are affected, specifically the heart, liver, and kidneys. Elevated cytokine levels and anomalies in the blood profiles of patients can be helpful indicators of disease progression [23]. Previous reports have implicated excessively high levels of IL-6 in disease severity and high mortality in COVID-19 patients [24]. The available clinical data have indicated that moderate and severe COVID-19 patients display abnormal liver function with increased levels of AST and ALT [25]. However, the impact of COVID-19 on liver and kidney function is yet to be completely understood. Studies have shown that cytokine storms generated in non-COVID-19 cases also lead to acute liver injury [26-27]. Early measurement of serum cytokines is thus a reliable predictor of disease outcome and, hence, is critical for early diagnosis and treatment. A model based on cytokine levels can help optimize therapeutic strategies and design clinical trials to modulate the released inflammatory molecules [28]. Increased amounts of LDH and CK enzymes in the blood indicate cellular damage. Similarly, the release of cardiac biomarkers (CK, troponin, myoglobin) in the blood indicates a damaged heart. Their levels can be estimated in diagnosing cardiac ischemia, a condition common in SARS-CoV2 infection. The protein troponin is the most commonly used biomarker, with very high sensitivity as it remains in the blood for longer periods. Although not specific, the concentration of CK often doubles when the heart is damaged [25]. In the present study, the serum concentrations of cytokines IL-6, IL-10, and TNF were all found to be elevated in COVID-19 patients. The concentration of IL-6 was found to be elevated 37-fold, which is extremely high in comparison to the other two cytokines. Ferritin and IL-10 were elevated by 5.5-fold, while TNF was only 2.5-fold higher compared to the healthy controls. TNF is a proinflammatory cytokine largely released by activated macrophages, T lymphocytes, and natural killer cells. It displays a complex network of interactions with other cytokines and further stimulates their release. IL-10, on the other hand, is an inhibitor of pro-inflammatory

cytokine production and antigen presentation in activated monocytes/macrophages [29]. Along with elevated serum cytokine levels, COVID-19 patients displayed increased levels of liver enzymes (ALT and AST). Interestingly, ALT, a more specific indicator of liver inflammation [15], was found in higher concentrations than AST. Compared to healthy individuals, the serum of COVID-19 patients showed elevated levels of AST, ALT, bilirubin, creatinine, urea, K, CK, LDH, troponin, ferritin, and CRP. Elevation of serum AST and ALT levels (3-5-fold) can be due to hepatocellular damage, but a 36-fold higher bilirubin level in COVID-19 patients suggests obstruction of bile flow (cholestasis). We also found the patients' CK and CRP levels to be 7-8-fold elevated. Although there was no change in the Na and Ca ion concentrations, the level of K was slightly lower, which may be due to its loss in urine [30]. Ferritin is a cytosolic protein that is released into the serum. Usually, it is used as a diagnostic marker for anemia, but high serum ferritin levels may indicate acute inflammatory reactions. Ferritin, CRP, and IL-6 have been considered possible immunological biomarkers for severe COVID-19 and probable tools for screening and early diagnosis [23]. As expected, the comparison of severe and non-severe COVID-19 cases revealed that the serum cytokine levels and biomarkers were slightly higher in the former cases. Severe cases showed around 7.5 times higher levels of ferritin. The liver enzyme ALT and CK concentrations were 5-6 times higher in severe cases indicating greater liver damage here.

5. Conclusion

Managing the host's immune status and other biological parameters can significantly combat the SARS-CoV-2 infection. Screening patients for hyperinflammation and managing cytokine storms in immunocompromised patients, along with other biomarkers of vital organs, can improve the mortality rate. Therapeutic strategies may include a combination of antibiotics, immunoglobulins, and inhibition of cytokine overproduction. In order to understand the mechanisms and factors involved in COVID-19 disease progression, a comparative analysis of the serum cytokine levels should be done in patients and healthy individuals. Estimating increased serum cytokine levels and abnormalities in the hepatic, renal, and cardiac biomarkers can play an important role in predicting poor outcomes in COVID-19 patients. The levels of IL-6, IL-10, and TNF in the serum can also predict the severity of the disease, as cytokines play an important role in host immunity and immunopathology during the infection. Since there is no effective treatment for COVID-19 to date, the estimation of cytokine levels and biomarker

molecules in patients may be used as indicators for early detection and appropriate treatment.

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Ethical Considerations

The study was approved by the Ethics Committee, Faculty of Applied Medical Sciences, Taibah University (SREC/AMS 2020/63/CLD) and Saudi Arabia Ministry of Health, General Administration for Research & Studies (IRB 452).

Competing Interests

The authors declare no conflict of interest.

Availability of Data and Material

The data supporting this study's findings are available in the Ministry of Health, Madinah, Saudi Arabia. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from the authors, with the permission of the Ministry of Health, Saudi Arabia.

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References

[1] Wang, C., Wang, Z., Wang, G., Lau, J. Y., Zhang, K., & Li, W. (2021). COVID-19 in early 2021: Current status and looking forward. *Signal Transduction and Targeted*

Therapy, 6, 114.

[2] Mokhtari, T., Hassani, F., Ghaffari, N., Ebrahimi, B., Yarahmadi, A., & Hassanzadeh, G. (2020). COVID-19 and multiorgan failure: A narrative review on potential mechanisms. *Journal of Molecular Histology, 51*, 613–628.

[3] Rothan, H. A., & Byrareddy, S. N. (2020). The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *Journal of Autoimmunity, 109*, 102433.

[4] Wang, C., Horby, P. W., Hayden, F. G., & Gao, G. F. (2020). A novel coronavirus outbreak of global health concern. *Lancet, 395*, 470–473.

[5] Lopes-Pacheco, M., Silva, P. L., Cruz, F. F., Battaglini, D., Robba, C., Pelosi, P., Morales, M. M., Caruso Neves, C., & Rocco, P. R. M. (2021). Pathogenesis of multiple organ injury in COVID-19 and potential therapeutic strategies. *Frontiers in Physiology, 12*, 593223.

[6] Nicholls, J. M., Poon, L. L., Lee, K. C., Ng, W. F., Lai, S. T., Leung, C. Y., Chu, C. M., Hui, P. K., Mak, K. L., Lim, W., Yan, K. W., Chan, K. H., Tsang, N. C., Guan, Y., Yuen, K. Y., & Peiris, J. S. (2003). Lung pathology of fatal severe acute respiratory syndrome. *Lancet, 361*, 1773–1778.

[7] Zhang, Y., Li, J., Zhan, Y., Wu, L., Yu, X., Zhang, W., Ye, L., Xu, S., Sun, R., Wang, Y., & Lou, J. (2004). Analysis of serum cytokines in patients with severe acute respiratory syndrome. *Infection and Immunity, 72*, 4410–4415.

[8] Velazquez-Salinas, L., Verdugo-Rodriguez, A., Rodriguez, L. L., & Borca, M. V. (2019). The role of interleukin 6 during viral infections. *Frontiers in Microbiology, 10*, 1057.

[9] Brydon, E. W., Morris, S. J., & Sweet, C. (2005). Role of apoptosis and cytokines in influenza virus morbidity. *FEMS Microbiology Reviews, 29*, 837–850.

[10] Kany, S., Vollrath, J. T., & Relja, B. (2019). Cytokines in inflammatory disease. *International Journal of Molecular Sciences, 20*, 6008.

[11] Mortaz, E., Tabarsi, P., Varahram, M., Folkerts, G., & Adcock, I. M. (2020). The immune response and immunopathology of COVID-19. *Frontiers in Immunology, 11*, 2037.

[12] Ye, Q., Wang, B., & Mao, J. (2020). The pathogenesis and treatment of the 'Cytokine Storm' in COVID-19. *The Journal of Infection, 80*, 607–613.

[13] Sinha, P., Matthay, M. A., & Calfee, C. S. (2020). Is a "cytokine storm" relevant to COVID-19? *JAMA Internal Medicine, 180*, 1152–1154.

[14] Rismanbaf, A., & Zarei, S. (2020). Liver and kidney injuries in COVID-19 and their effects on drug therapy; a Letter to Editor. *Archives of Academic Emergency Medicine, 8*, e17.

[15] Martinez, M. A., & Franco, S. (2021). Impact of COVID-19 in liver disease progression. *Hepatology Communications, 5*, 1138–1150.

[16] Clark, R., Waters, B., & Stanfill, A. G. (2021). Elevated liver function tests in COVID-19: Causes, clinical evidence, and potential treatments. *The Nurse Practitioner, 46*, 21–26.

[17] Ghahramani, S., Tabrizi, R., Lankarani, K. B., Kashani, S. M. A., Rezaei, S., Zeidi, N., Akbari, M., Heydari, S. T., Akbari, H., Nowrouzi-Sohrabi, P., & Ahmadizar, F. (2020). Laboratory features of severe vs. non-severe COVID-19 patients in Asian populations: A systematic review and meta-analysis. *European Journal of Medical Research, 25*, 30.

[18] Zhang, X., Tan, Y., Ling, Y., Lu, G., Liu, F., Yi, Z., Jia, X., Wu, M., Shi, B., Xu, S., Chen, J., Wang, W., Chen, B., Jiang, L., Yu, S., Lu, J., Wang, J., Xu, M., Yuan, Z., . . . Lu, H. (2020). Viral and host factors related to the clinical outcome of COVID-19. *Nature, 583*, 437–440.

[19] MOH-approved scientific instruction manuals and guidelines for healthcare providers on how to deal with COVID-19 patients, 2020. Available from https://www.moh.gov.sa/en/Ministry/MediaCenter/Publications/Pages/covid19.aspx; 2020 [accessed 20 June 2020]).

[20] Wang, X., Yang, K., Wei, C., Huang, Y., & Zhao, D. (2010). Coinfection with EBV/CMV and other respiratory agents in children with suspected infectious mononucleosis. *Virology Journal, 7*, 247.

[21] Ye, Q., Wang, B., & Mao, J. (2020). The pathogenesis and treatment of the 'cytokine storm' in COVID-19. *The Journal of Infection, 80*, 607–613.

[22] Forbester, J. L., & Humphreys, I. R. (2021). Genetic influences on viral-induced cytokine responses in the lung. *Mucosal Immunology, 14*, 14–25.

[23] Melo, A. K. G., Milby, K. M., Caparroz, A. L. M. A., Pinto, A. C. P. N., Santos, R. R. P., Rocha, A. P., Ferreira, G. A., Souza, V. A., Valadares, L. D. A., Vieira, R. M. R. A., Pileggi, G. S., & Trevisani, V. F. M. (2021). Biomarkers of cytokine storm as red flags for severe and fatal COVID-19 cases: A living systematic review and meta-analysis. *PLoS One, 16*, e0253894.

[24] Ghazavi, A., Ganji, A., Keshavarzian, N., Rabiemajd, S., & Mosayebi, G. (2021). Cytokine profile and disease severity in patients with COVID-19. *Cytokine, 137*, 155323.

[25] Ponti, G., Maccaferri, M., Ruini, C., Tomasi, A., & Ozben, T. (2020). Biomarkers associated with COVID-19 disease progression. *Critical Reviews in Clinical Laboratory Sciences, 57*, 389–399.

[26] Tisoncik, J. R., Korth, M. J., Simmons, C. P., Farrar, J., Martin, T. R., & Katze, M. G. (2012). Into the eye of the cytokine storm. *Microbiology and Molecular Biology Reviews, 76*,

16–32.

[27] Lee, D. W., Gardner, R., Porter, D. L., Louis, C. U., Ahmed, N., Jensen, M., Grupp, S. A., & Mackall, C. L. (2014). Current concepts in the diagnosis and management of cytokine release syndrome. *Blood, 124*, 188–195.

[28] Del Valle, D. M., Kim-Schulze, S., Huang, H. H., Beckmann, N. D., Nirenberg, S., Wang, B., Lavin, Y., Swartz, T. H., Madduri, D., Stock, A., Marron, T. U., Xie, H., Patel, M., Tuballes, K., Van Oekelen, O., Rahman, A., Kovatch, P., Aberg, J. A., Schadt, E., . . . Gnjatic, S. (2020). An inflammatory cytokine signature predicts COVID-19 severity and survival. *Nature Medicine, 26*, 1636–1643.

[29] Islam, H., Chamberlain, T. C., Mui, A. L., & Little, J. P. (2021). Elevated interleukin-10 levels in COVID-19: Potentiation of pro-inflammatory responses or impaired anti-inflammatory action? *Frontiers in Immunology, 12*, 677008.

[30] Alfano, G., Ferrari, A., Fontana, F., Perrone, R., Mori, G., Ascione, E., Magistroni, R., Venturi, G., Pederzoli, S., Margiotta, G., Romeo, M., Piccinini, F., Franceschi, G., Volpi, S., Faltoni, M., Ciusa, G., Bacca, E., Tutone, M., Raimondi, A., . . . Guaraldi, G., & the Modena Covid-19 Working Group (MoCo19). (2021). Hypokalemia in patients with COVID-19. *Clinical and Experimental Nephrology, 25*, 401–409. https://doi.org/10.1007/s10157-020-01996-4