Original Article

Profile of Interleukin-6 and Tumor Necrosis Factor-α in Hospitalized COVID-19 Patients

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Abstract

Background: In 2019's December, the new COVID-19 coronavirus induced severe lower respiratory tract syndrome in Wuhan, China. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) ultimately became responsible for the life-threatening pandemic. The magnitude and outcome of COVID-19 may be related to the overproduction of pro-inflammatory cytokine called "cytokine storm." Cytokine production besides the age of patients, and comorbidities, all influence the length and severity of SARS-CoV-2. **Objectives:** This research was performed to investigate how these factors affect patients who have severe COVID-19 in a sample of Iraqi patients. **Materials and Methods:** A total of 82 participants were enrolled in this work, confirmed hospitalized COVID-19 patients (n = 60) and healthy control (n = 22) from both sexes of age range 20-67 years. Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) levels in the serum of COVID-19 patients and HC have been evaluated using an ELISA. **Results:** According to our findings, sera from COVID-19 patients had considerably higher levels of IL-6 and TNF-α than those of the control group. Furthermore, most infections were among women (63%), followed by men (37%), the higher number of patients were within 50-59 years, while the lowest number of patients was found in the group 20-29 years. Most COVID-19 patients appeared to be overweight and obese. Additionally, those who have diabetes mellitus are also at risk of severe COVID-19. **Conclusion:** Serum levels of TNF-α and IL-6 were higher in patients than in the control group, suggesting that they could be used as indicators of the severity of COVID-19 illness. Also, their combined detection provided highest specificity and sensitivity for early prediction of COVID-19 severity, which has significant clinical values.

Keywords: Age, comorbidity, COVID-19, IL-6, severity of disease, TNF-α

Introduction

Coronavirus disease 2019 (COVID-19), a highly contagious disease caused by the SARS-CoV-2 coronavirus, was detected in China, Wuhan (Hubei Province), and soon spread to other nearby cities and countries.[1] Coronaviruses (CoVs) are single-stranded, positive-strand RNA viruses that infect the respiratory tracts of domestic and companion animals, as well as humans, causing mild to serious respiratory diseases.[2] Fever, cough, myalgia, and fatigue have been identified as common confirmed symptoms in cases. These symptoms, however, are not specific to COVID-19; they are similar to those of other virus-infected diseases, such as influenza.[3]

It has been depicted that the acute respiratory distress in COVID-19 and the development of severe illness were related to up-regulated levels of pro-inflammatory cytokines, and accordingly, this condition was termed cytokine release syndrome or cytokine storm. The most important cytokines in this context are Interleukin-1β, Interleukin-6, Interleukin-18, Tumor Necrosis Factor-α (TNF-α) and Interferon-γ (IFN-γ).[4] However, IL-6 a major inflammatory mediator is the most encountered cytokine, and it has been found that IL-6 is a key cytokine linked with a higher risk of severity and death in patients with COVID-19.[5] The anti-inflammatory and the

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pro-inflammatory cytokines have an important role in many diseases, IL-10 was meaningfully raised in colorectal cancer patients, signifying its worth for the diagnosis. Moreover, IL-31, which belongs to the IL-6 family, proved to have a remarkable role in some conditions like smoking.[6,7]

Furthermore, the IL-12 family has important roles in autoimmunity disorder and serum IL-12 may be a more critical and predictive marker in rheumatoid arthritis development.[8] Similarly, the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) levels were shown to be highest in individuals with severe COVID-19 symptoms and pneumonia.[9]

To identify the cellular process behind the pathogenesis of coronavirus, it is crucial to compare the levels of these cytokines in COVID-19 patient's sera to those of healthy individuals. In this research, TNF-α and IL-6 levels have been measured using an ELISA in sera from COVID-19 severe patients compared to a control group of healthy volunteers. As well as explained their potential correlation with some demographic parameters. The novelty of this work is the first that assesses these pro-inflammatory factors in a sample of Iraqi hospitalized patients in Erbil city, Kurdistan-Iraq.

Materials and Methods

Study design and subjects

Confirmed COVID-19 hospitalized patients aged from 20 to 67 years of both sexes are eligible for this study in comparison to age- and sex-matched healthy subjects randomly selected from healthy Soran city residents to serve as the control group. Hospitalized patients from both West Erbil Emergency Hospital and the COVID-19 control center in Soran/Erbil from September 2021 to October 2021 served as the subjects for this study. All patients in this study m*et al*l inclusion criteria including age, sex, body mass index (BMI), presence of chronic conditions or comorbidity (e.g., high blood pressure, diabetic mellitus, and asthma), serostatus, and ethnicity (Kurdish people). And no exclusion criteria except for pregnant women due to no case was found.

Sample collection and serum preparation

Blood samples were taken from sixty hospitalized COVID-19 patients from both West Erbil Emergency Hospital and the COVID-19 control center in Soran/Kurdistan from September to October 2021. Before obtaining the samples, authorization was acquired from hospital staff, doctors, and patients. In addition, peripheral blood samples from age- and gender-matched healthy controls were also collected. Blood samples in the amount of 3 mL were taken from the study participants and placed in a gel tube for serum separation.

The blood samples were centrifuged at 3000 rpm for 15 min. Two aliquots of serum (0.7 mL) were kept in Eppendorf tubes from each sample at −20°C for further examination. Sera from COVID-19 patients and healthy controls were used to perform an (ELISA) to measure the concentrations of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α).

Estimation of serum interleukin-6 and tumor necrosis factor-α levels by ELISA

Interleukin-6 and TNF-α levels in the serum samples were assessed as described previously.[6] The Human IL-6 and TNF-α ELISA Kits based on the biotin double antibody sandwich technique (Wuhan Hi-tech Medical Devices Park, CUSABIO/China, Catalog Number. CSB E04593h) were used in this study. Standard solutions and diluted horseradish peroxidase solution (HRP-avidin) were made and processed following the kit's manufacturer's instructions. The optical density of each well was determined utilizing a microplate reader that scans at 450 nm. The ELISA standard curve was created by plotting the calibration concentrations (IU/mL) on the x-axis and the optical density values on the y-axis to determine the concentration of the tested materials. And the slope equation was used to calculate the IL-6 and TNF-α concentrations in the research subjects.

Statistical analysis

A normality test was used to first statistically analyze the data, and then a non-parametric test (Mann-Whitney Confidence Interval) was used. All data were created with the GraphPad Prism version 8.0 (GraphPad Software Inc., San Diego, CA). The test was considered statistically significant at <0.05. Pearson correlation was utilized to analyze the relationships between IL-6 and TNF-α blood levels and various research individuals' characteristics such as age and BMI. Receiver operating characteristic (ROC) curves were used to assess the predictive discriminatory capability.

Ethical approval

This work was achieved following the Declaration of Helsinki. It was carried out with the patient's relatives' verbal and written approval before the sample was taken. A scientific ethics committee reviewed and approved the study proposal in the Faculty of Science, and the official form numbered 1/1/178 with the date of November 15, 2021.

Results

Study subject demography

The demographic data of the hospitalized COVID-19 patients and the healthy control including gender, age, and BMI kg/m^2 are presented in Table 1. Regarding the distribution of sex among COVID-19 patients, the majority of patients with COVID-19 were women in all age groups (63%), followed by men (37%). The control group involves (55%) of males and (45%) were females, as a normal distribution of the population. The findings revealed that the mean ages of the

Table 1: Distribution of the research subjects by gender, age, and body mass index (BMI) (Kg/m²)

Age groups years	COVID-19 group				Healthy control group			
	Number		Percentage		Number		Percentage	
	F	M	%	%	F	M	%	%
G 1 (20–29)	1	0	2	0	0	1	0	5
G2 (30–39)	9	3	15	5	2	0	9	0
G3 (40–49)	11	3	18	5	1	2	5	9
G4 (50–59)	8	9	13	15	4	3	18	13
G5 (≥60)	9	7	15	12	3	6	13	28
Total	38	22	63	37	10	12	45	55
BMI Categories kg/m²	No.		%		No.		%	
Normal weight (18.5–24.9)	10		17		12		55	
Overweight (25–29.9)	28		47		9		40	
Obesity (≥30)	22		36		1		5	
Total	60		100		22		100	

F: represents females and M: signifies males

COVID-19 patients and control group were (52.8 ± 2.47) years and (50.7 ± 1.82) years, respectively. The COVID-19 patients' age distribution showed that group 4 (50-59) had the highest percentage of patients, with 17 (28%) patients, while group 1 (20-29) had the lowest percentage of patients, with just 1 (2%). Regarding the BMI, most COVID-19 patients appeared to be overweight and obese, 10 patients were within the normal range of BMI, whereas 28 were overweight and 22 were obese as shown in Table 1.

Regarding the comorbidity associated with COVID-19 in hospitalized patients, our present results detected that patients with comorbidities represented 55% of all cases. The highest incidence rate was noticed among diabetic patients 12 and 10 of them had hypertension, whereas 5 patients had cardiovascular disease, 4 patients with asthma, and 2 patients were suffering from more than one type of disease. Moreover, 45% of the COVID-19 patients included in this study seemed without comorbidities, as shown in Figure 1.

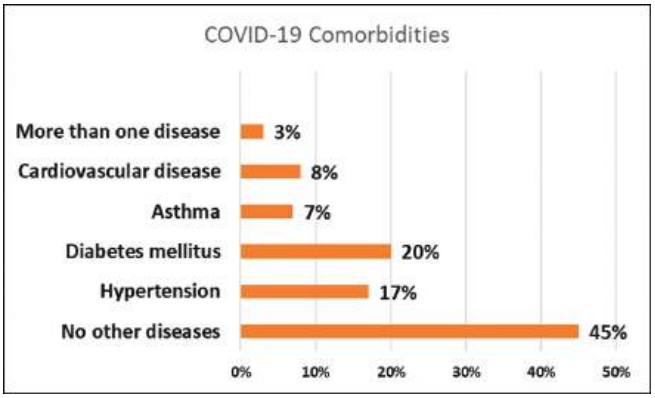

Figure 1: Comorbidities in hospitalized patients with COVID-19

Serological analysis

Serum IL-6 level estimation

According to the present findings, the COVID-19 patient group's IL-6 levels were significantly higher than those of the healthy control group ($P < 0.05$), the means were (59.11 ± 6.94) and (17.43 ± 1.74) pg/mL, respectively, as illustrated in Figure 2A.

Serum TNF-α level assessment

The present results showed that levels of TNF-α in the serum were significantly higher in patients with COVID-19 than in healthy controls ($P < 0.05$) with means of (103.84 ± 7.84) and (22.98 ± 5.17) pg/mL, respectively, as shown in Figure 2B.

Serum IL-6 and TNF-α levels according to gender

According to gender, the current outcome revealed a statistically significant increase in serum IL-6 levels of the COVID-19 group in comparison to the healthy control group ($P < 0.05$). The IL-6 levels were higher in males than females in the patients group, as shown in Figure 2C. Looking at the TNF-α concentrations, the results of the current research revealed no significant differences in TNF-α serum levels among COVID-19 patients according to their gender, as shown in Figure 2D.

Serum IL-6 and TNF-α levels according to age groups

The current research found that IL-6 levels were significantly higher in COVID-19 patients than healthy controls across all age categories ($P < 0.05$). The G5 group's sera showed a high value of IL-6 that corresponded to an age of about 60 years, followed by the G4 group, which had an age range of 50 to 59 years, as in Figure 3A. Regarding the serum TNF-α levels according to age groups, the present study's findings revealed a significant increase in TNF-α levels in the sera of COVID-19 patients of G1 and G4 compared to the healthy control ($P < 0.05$) [Figure 3B].

Serum IL-6 and TNF-α levels according to BMI

The results of this investigation demonstrated that the BMI and serum IL-6 levels of COVID-19 patients had not

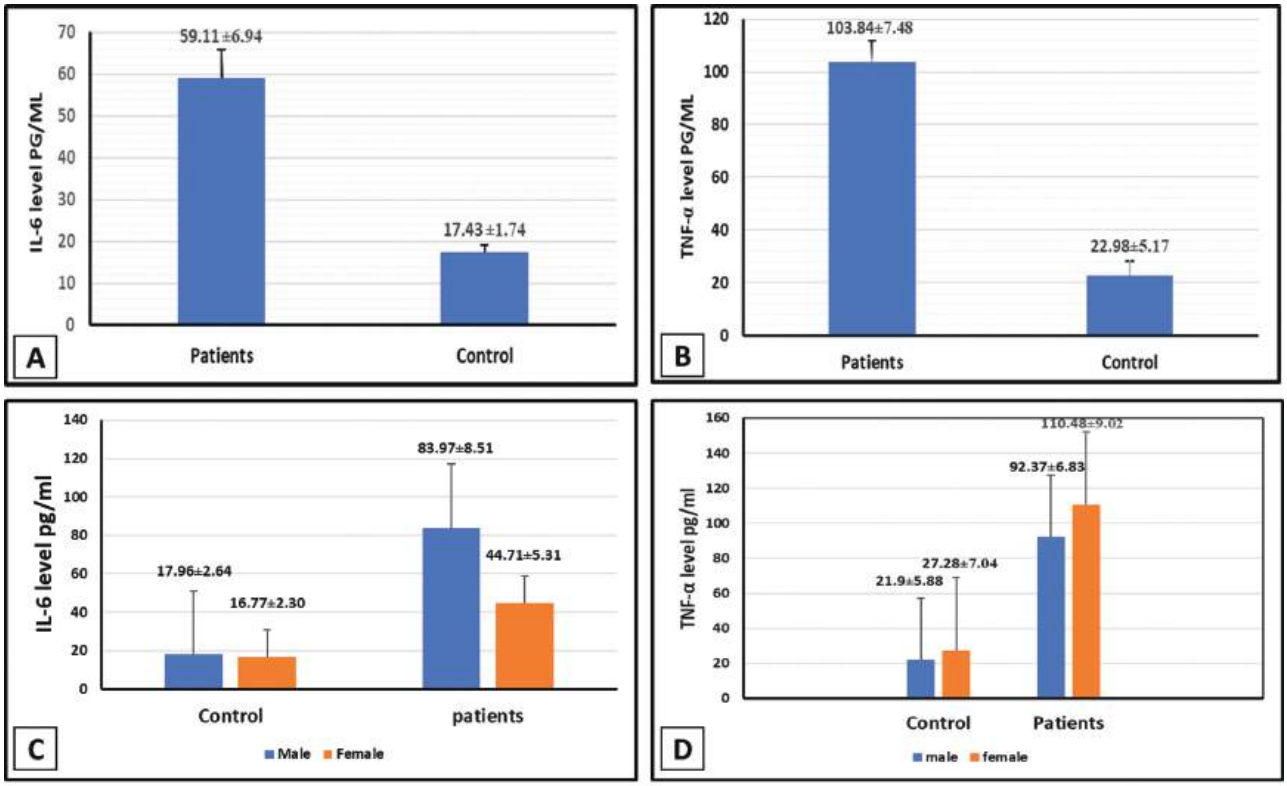

Figure 2: (a) Depicts the IL-6 level in COVID-19 patients as contrasted to the healthy control group; The level of IL-6 was considerably elevated in COVID-19 patients in comparison to healthy control (59.11 ± 6.94 and 17.43 ± 1.74 pg/mL, respectively) evaluated by ELISA ($P < 0.05$). (b) The TNF-α level in COVID-19 patients as contrasted to the control group; TNF-α levels were significantly higher in COVID-19 patients compared to healthy control (103.84 ± 7.84 and 22.98 ± 5.17 pg/mL, respectively) evaluated by ELISA ($P < 0.05$). (c) The mean of IL-6 levels according to the gender of COVID-19 patients and control. And, (d) Shows the mean serum level of TNF-α according to the gender of COVID-19 patients and control

significantly changed ($P < 0.05$) [Figure 4A]. Similarly, there were no significant variations among the COVID-19 patients' sera TNF-α levels according to their BMI ($P < 0.05$) [Figure 4B].

Serum level of IL-6 and TNF-α according to infection with other diseases

The current study revealed a significant rise in IL-6 levels in COVID-19 patients suffering from other diseases ($P < 0.05$). High levels of IL-6 were detected in the sera of patients with diabetes mellitus, followed by patients with multiple diseases [Figure 4C]. Our research detected a significant increase in serum TNF-α levels among COVID-19 patients infected with other diseases ($P < 0.05$). The TNF-α levels were shown to be elevated in diabetes mellitus patients' sera, followed by patients suffering from more than one disease [Figure 4D].

Pearson's correlation between IL-6 levels and certain study subject parameters

Figure 5A and B illustrate the evaluation of the correlation between IL-6 levels and some parameters. Between serum levels of IL-6 and the COVID-19 patient's ages, a non-significant weak negative connection was found, with Pearson's correlation = −0.02. In addition, in COVID-19 patients, a non-significant weak negative correlation between IL-6 serum levels and BMI was detected, with Pearson's correlation = −0.04.

Pearson's correlation between TNF-α levels and some study subjects' parameters

The correlations between TNF-α levels, age and BMI of the COVID-19 patients were analyzed as shown in Figure 6. TNF-α serum levels and COVID-19 patient ages showed a non-significant weak positive association with Pearson's correlation = -0.02. Additionally, in COVID-19 patients, a non-significant weak negative relationship between TNF-α serum levels and BMI was found with Pearson's correlation = −0.003.

The diagnostic performance of IL-6 and TNF-α in COVID-19 patients

The diagnostic performance of the cytokines markers in COVID-19 patients was evaluated using a receiver operator characteristic (ROC) curve analysis. The best serum IL-6 cutoff value that can accurately predict COVID-19 diagnosis was 24.38 pg/mL, the sensitivity was 90%, the specificity was 86.67%, and the area under the curve (AUC) was 0.937 as illustrated in Figure 7. Regarding TNF-α, the cutoff value was >48.76 pg/mL with 85% sensitivity, and

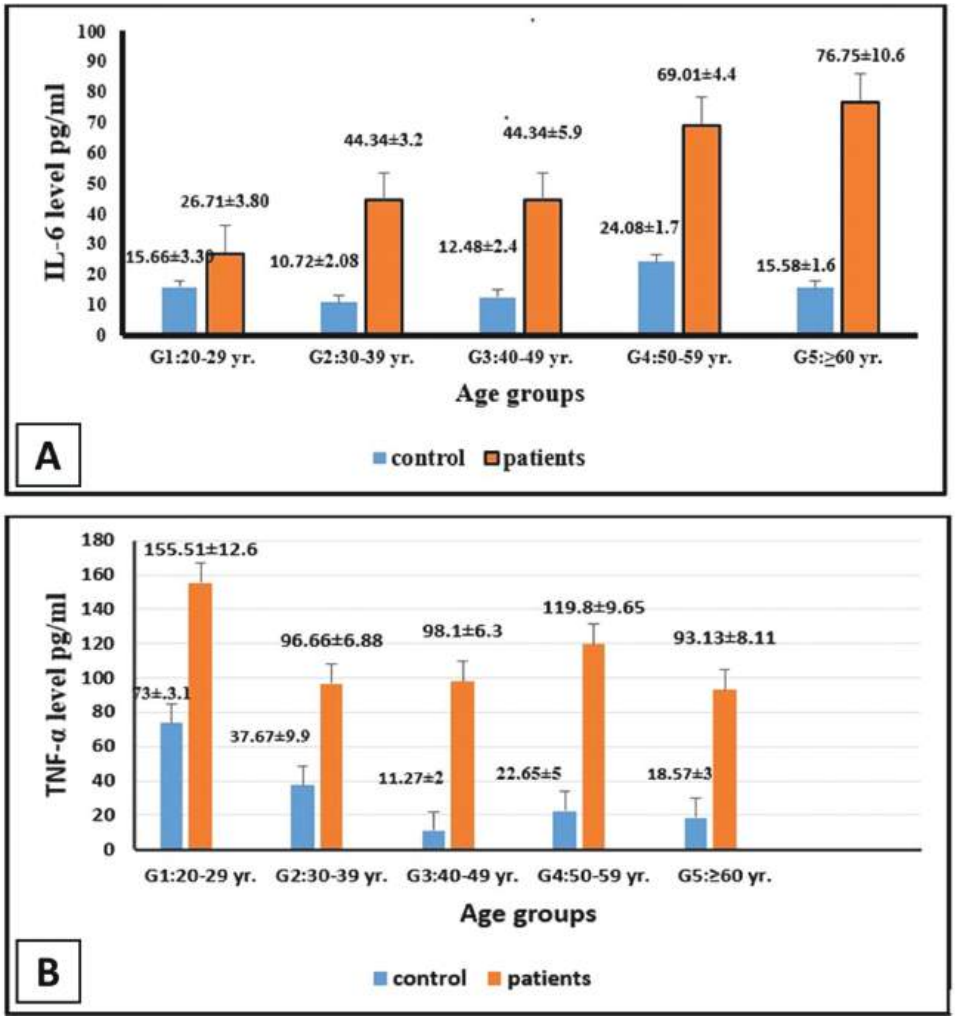

Figure 3: (a) Levels of IL-6 in COVID-19 patients' serum and healthy control according to age groups. (b) Levels of TNF-α in COVID-19 patients' serum and healthy control according to age groups

86.36% specificity, and the (AUC) was 0.9106 as shown in Figure 8.

Discussion

According to the present study's findings, 63% of females were infected more frequently than men as 37% and these differences were statistically significant. This outcome is similar to the observation of Saeed *et al*.[10] who showed a higher prevalence of COVID-19 infection in females (62%) than in males (38%) in Mosul. This differs from previous studies performed outside of Iraq, such as a study conducted in Latin America that indicated a greater male-to-female ratio of disease.[11] A study conducted in Diyala, South Iraq, and Kurdistan revealed that women have less knowledge about COVID-19 and are less equipped to combat the disease than males. Coronavirus has a greater impact on women's income and livelihoods than men; 15%–30% of women interviewed were economically active before the pandemic, and most were unable to stop the epidemic.[12]

Numerous studies show that there are no or very little differences in the range of infected with COVID-19 between males and females, Hussein *et al*.[13] showed that the prevalence of SARS-COV-2 infection among males (49.11%) and females (50.89%) was equal in Kurdistan.

The current study's findings revealed that the group (50-59 years) of age was the most impacted. This is consistent with Ad'hiah *et al*.[14] findings which showed that the majority of cases (48.3%) of patients in Iraq were over the age of 50. These results also agreed with the findings of a study done by Ma *et al*.[15] who established that older individuals are more susceptible to severe disease symptoms. The high infection rate of SARS-COV-2 in old age may be due to several comorbidities among them, in addition to poor clinical outcomes.[16]

Although a statistically significant variation was recorded in our study regarding the normal weight, overweight and obese individuals in COVID-19 cases compared to HC, more than 80% of patients were overweight and obese. This may indicate the risk potential of obesity

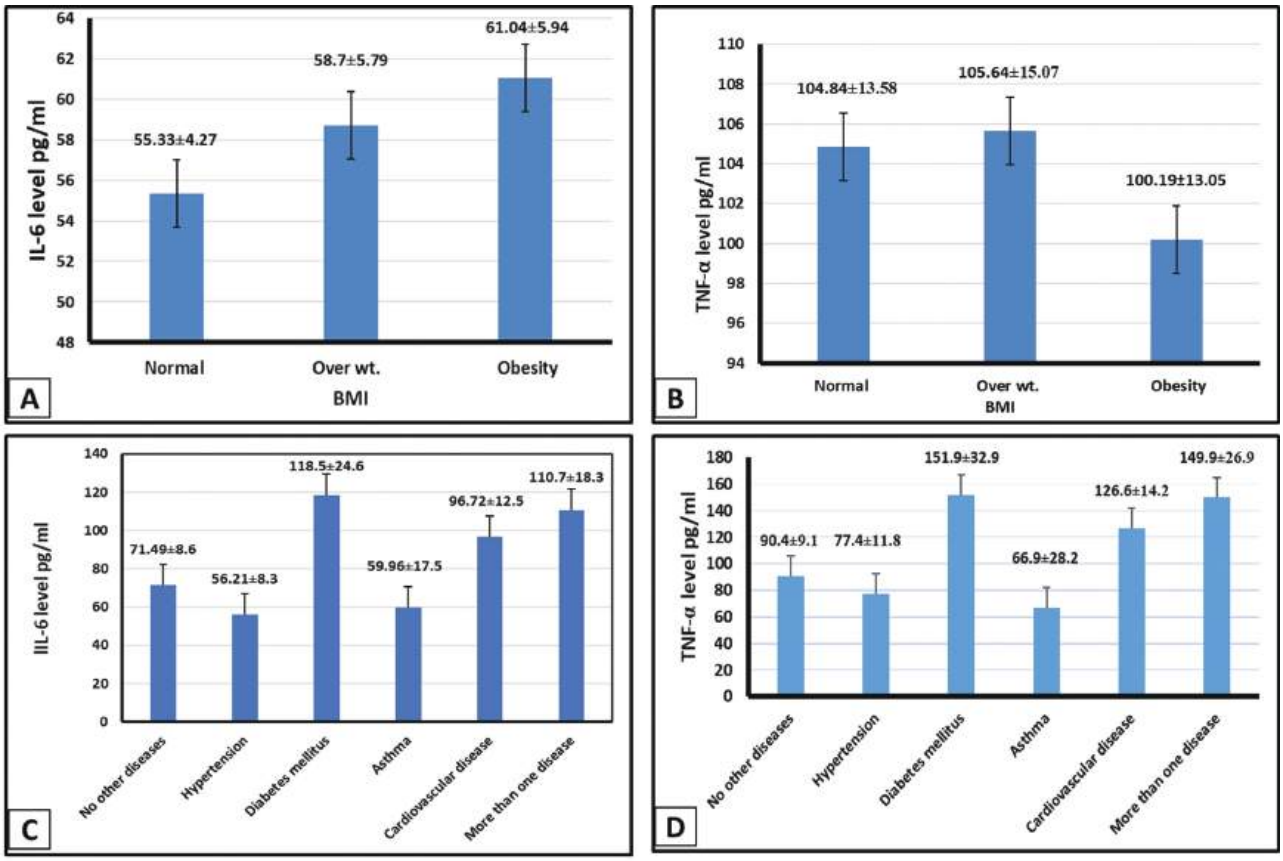

Figure 4: (a) IL-6 levels in COVID-19 patients' serum according to their body mass index. (b) TNF-α levels in COVID-19 patients' serum according to their body mass index. (c) Serum IL-6 levels in COVID-19 patients according to infection with other diseases. (d) Serum TNF-α levels in COVID-19 patients according to infection with other diseases

as a factor included in COVID-19 susceptibility. In a Chinese study, it has been described that 88% of deceased COVID-19 patients were overweight and obese, while among survivors, the frequency was 18%.[17] The reasons behind the association of overweight and obesity with the progression severity of COVID-19 have been subjected to some speculations. It has been depicted that adipose tissue in obese patients shows an up-regulated expression of ACE2 and this may facilitate the virus entry into host cells.[18] According to current research, the ratios of SARS-COV-2 patients who also had diabetes mellitus, hypertension, asthma, cardiovascular disease, and more than one disease were (20%, 17%, 7%, 8%, and 3%, respectively). This finding is consistent with the outcomes of Huang *et al.*,[3] study, which found that less than half of the (41) SARS-COV-2 patients in Wuhan City had comorbidities such as diabetes mellitus (20%), hypertension (15%), and cardiovascular disease (15%).

Some research investigations on the occurrence of comorbidities found that (20%–51%) of COVID-19 patients at the time of admission had at least one condition, including (10%–15%) hypertension, (10%–20%) diabetes mellitus, and (7%–40%) cardiovascular disease.[19] This might be caused by immunological function, thymus gland atrophies, and aging-related decreases in thymus gland functioning, which is a primary lymphoid organ responsible for producing immunocompetent T-cells.[20]

In comparison to the control, IL-6 level was significantly higher in the patients. This finding is consistent with Chen *et al.*,[21] who observed an increase in IL-6 levels among SARS-COV-2 patients in Wuhan, China. There have been multiple reports of an increased IL-6 level in severe cases. More recently, Bello *et al.*,[22] determined that the severity and mortality of COVID-19 patients were correlated with the presence of TNF-α, IL-6, IL-8, and G-CSF. The clear progression of IL-6 levels throughout the illness, particularly on days 10–16, was a good indicator of the development of critical status and mortality and could guide IL-6 blockade.

According to several studies, there is a clear association between elevated IL-6 levels and the development of respiratory failure in SARS-COV-2 patients.[23] In addition, TNF-α levels were significantly elevated in COVID-19 patients compared to healthy controls in the current study. Previous research by Halim *et al.*,[24] found that the mean levels of TNF-α were considerably higher in patients with severe COVID-19 compared with non-severe COVID-19

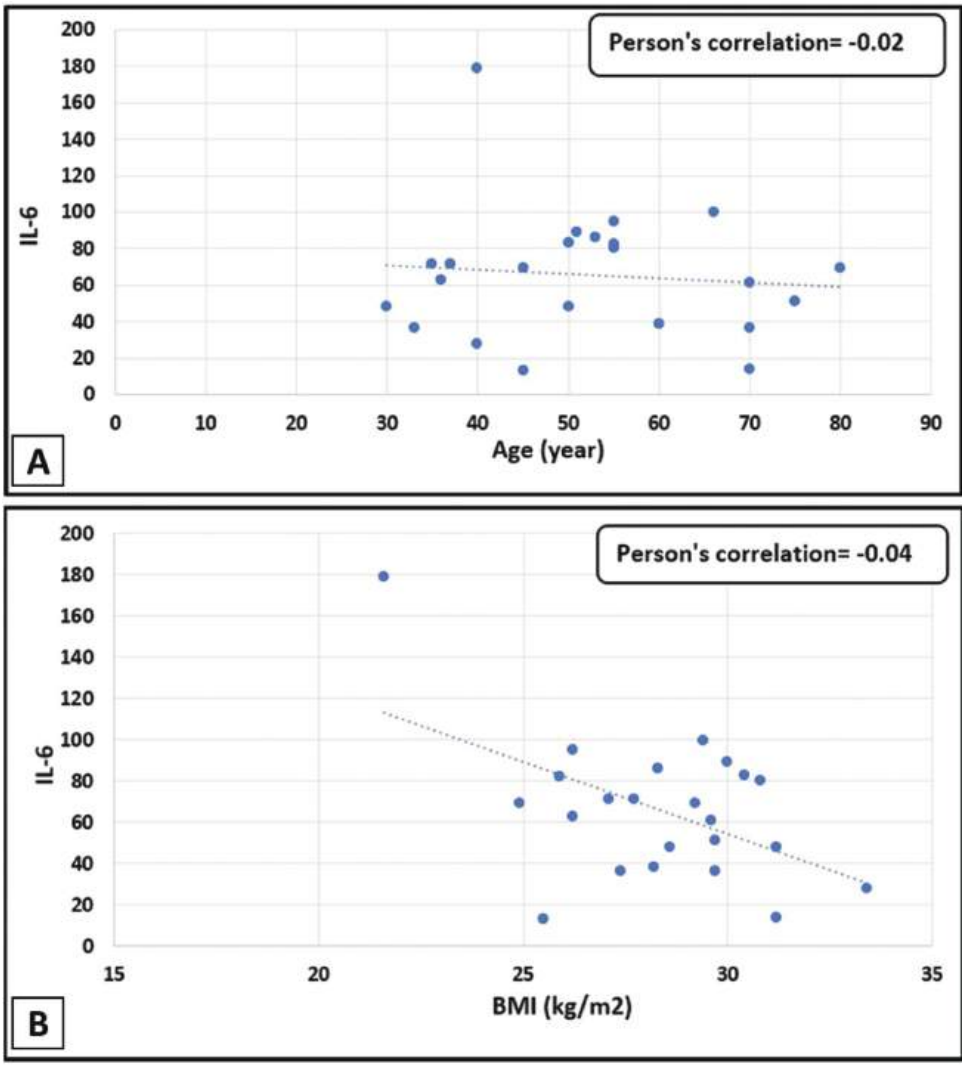

Figure 5: The correlation between COVID-19 patients' age and the level of IL-6 was weakly negative and non-significant. The correlation between COVID-19 patients' BMI and the level of IL-6 was weakly negative and non-significant

patients, providing support to the present study's findings. The TNF-α levels rise early in the infection and stay increased throughout the infection, TNF-α stimulates HA-synthase-2 (HAS2) in EpCAM+ lung alveolar epithelium, CD31+ lung alveolar endothelium, and fibroblasts in COVID-19 patients' lungs. The fluid influx in the lung alveoli caused by HA (hyaluronan) is a major cause of deoxygenation and ventilator admission.[25]

The current findings revealed a statistically significant rise in the IL-6 serum level of the COVID-19 patient group as compared to the healthy control group based on gender. The elevated levels of total serum IL-6 in COVID-19 patients, particularly in men, are consistent with earlier studies, including those by Mi *et al.*,[26] So at this point, the mechanisms behind the observed gender differences remain unknown. Some assumptions can be made based on current knowledge of gender differences in respiratory viral infections. Some lifestyle choices, such as smoking, are most likely linked to the unfavorable progression and outcomes of COVID-19 in men.[27] While there were no significant differences in TNF-α serum levels among COVID-19 patients based on gender, this observation was in accordance with the results of other studies conducted by Petrey *et al.,*[28] Also, the current research found that COVID-19 patients had significantly higher serum levels of IL-6 at age (≥60) years, followed by an age range of 50 to 59 years. Another study showed that serum concentration of IL-6 rises with age and is unaffected by ethnicity, and a high level of this cytokine can even indicate higher mortality in the elderly.[29] In contrast, the TNF-α serum levels were significantly increased in the COVID-19 patients in age 20 to 29 years, followed by the age range 50 to 59 years. Younger patients had higher levels of TNF-α.[30]

The results of this investigation demonstrated that the COVID-19 patients' serum IL-6 levels had not

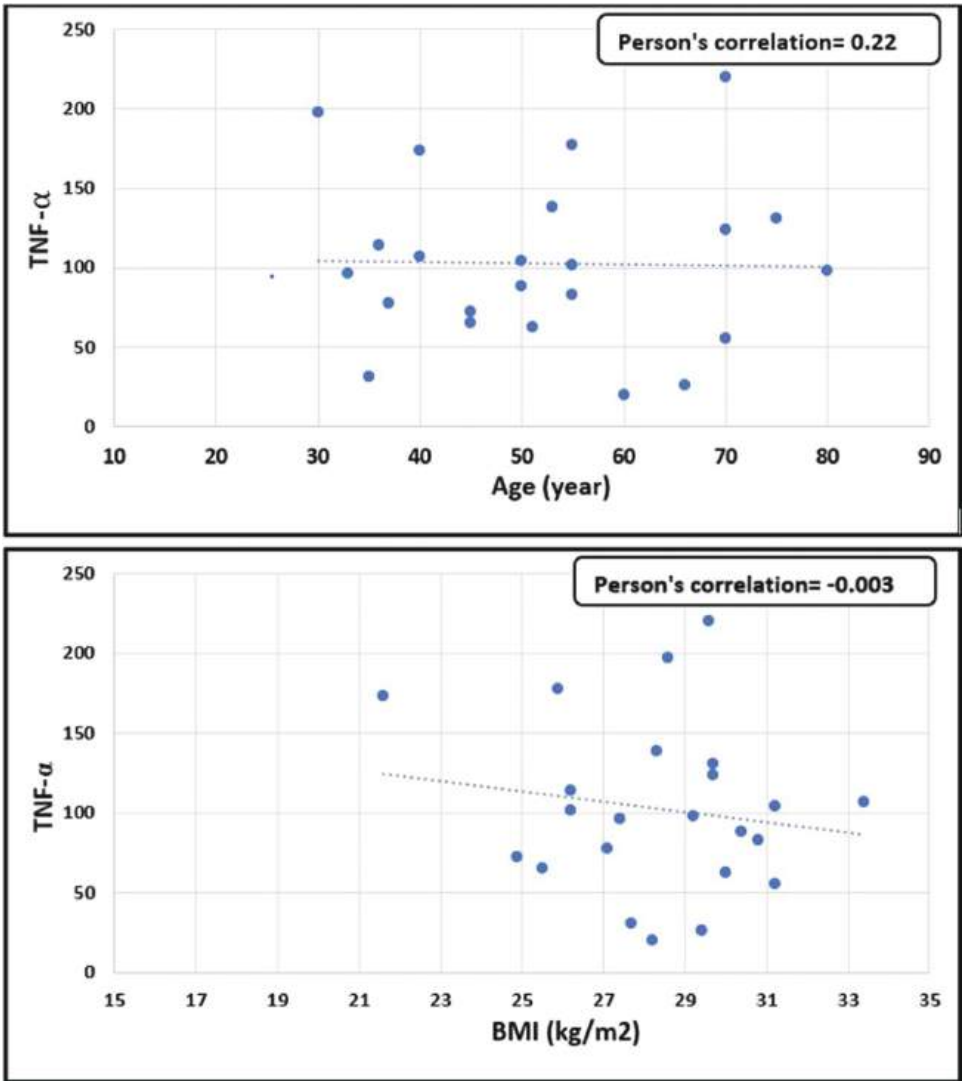

Figure 6: (A) Correlation between the age of COVID-19 patients and TNF-α level was weakly positive and non-significant. (B) Correlation between BMI of COVID-19 patients and the level of TNF-α was a weakly negative non-significant

significantly changed concerning their BMI. These results are in agreement with other studies which confirmed no association between obesity and IL-6 levels.[31] As Guerson-Gil *et al*.,[32] recorded there was no clear association between BMI and IL-6 level due to the number of patients in this group was too small to draw definite conclusions. The same results for TNF-α levels in the COVID-19 patient's serum showed no significant variations based on their BMI which consistent with another study demonstrated that (BMI) did not correlate with TNF-α concentrations.[33]

According to infection with other diseases, The current data revealed a significant increase in IL-6 levels in COVID-19 patients, a high level of IL-6 was detected in the sera of patients with Diabetes Mellituse. This finding is consistent with a previous study by Kozakova *et al*.,[34] which found that Diabetes Mellitus patients had significantly higher levels of inflammatory cytokines. Serum levels of biomarkers indicate inflammation, such as IL-6, were considerably higher in patients compared to controls, suggesting that people with diabetes are more susceptible to an inflammatory storm that finally causes COVID-19 to deteriorate rapidly.[35] Additionally, the current results demonstrated a statistically significant increase in TNF-α levels in the sera of the diabetes mellitus patients infected with COVID-19. The findings of our study are similar to a study done by Randeria *et al.*[36] The increased secretion of IL-1, IL-6, IL-8, and TNF-indicates that diabetes mellitus increases the pro-inflammatory cytokine response. The presence of a cytokine storm, which is caused by persistent low-grade inflammation in individuals, is highly correlated with the severity of COVID-19 pneumonia and eventual mortality.[37] Along the same line, a recent study in Iraq found that patients with comorbidities like hypertension, diabetes, cardiac diseases, kidney diseases, malignancy,

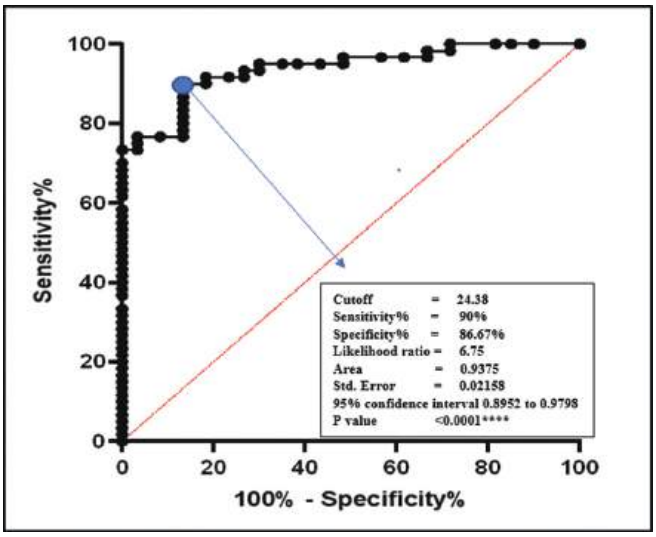

Figure 7: The receiver operating characteristic (ROC) curve of IL-6 for critical illness of COVID-19. The area under the curve (AUC) was 0.9375. Standard error of AUC = 0.02158, *P* value < 0.0001, 95% Confidence interval = 0.8952%–0.9798%. Cutoff value = 24.38 pg/mL. Sensitivity was 90%, and specificity was 86.67%

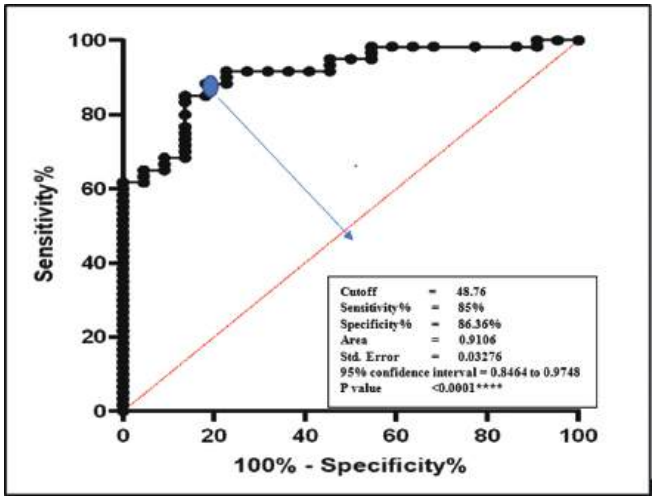

Figure 8: The receiver operating characteristic (ROC) curve of TNF-α for critical illness of COVID-19. The area under the curve (AUC) was 0.9106. Standard Error of AUC = 0.03276, *P* value < 0.0001, 95% confidence interval = 0.8464%–0.9748%. Cutoff value= 48.76 pg/mL. Sensitivity was 85%, and specificity was 86.36%

and hepatic diseases are at significant risk for severe COVID-19.[38]

In the COVID-19 patients, the correlation between levels of IL-6 and gender, age, BMI, and infection with other diseases was analyzed. A weakly negative non-significant association between serum levels of IL-6 and the ages of the COVID-19 patients. In addition, it was discovered that there was a weakly negative non-significant correlation between IL-6 and BMI in COVID-19 patients. In disagreement with Xu *et al.*,[39] who showed that IL-6 was positively correlated with age and BMI.

Besides, the Correlations between TNF-α levels and age, gender, BMI, and infection with other diseases of the COVID-19 patients were evaluated. There was a weakly positive non-significant association between the serum levels of TNF-α and COVID-19 patients' ages. Which in contrast to other studies, confirmed that serum levels of all cytokines, particularly TNF-α, were significantly positively correlated with age and that the poor prognosis of the elderly may be related to this; some of them have relationships with gender or antibody, the former may be related to the high level of ACE2 in the male reproductive system, and which may further elucidate that the severe inflammatory response is more likely to occur among the elderly population.[40]

A weakly negative non-significant association between the serum levels of TNF-α and BMI was also found in COVID-19 patients. This result disagreed with Xu *et al.*,[39] results who found a positive correlation between BMI and TNF-α. The ROC analysis specified 90% sensitivity and 86.67% specificity for IL-6 while for TNF-α 85% sensitivity, and 86.36% specificity, indicating that IL-6 was predictive of increasing COVID-19.

Conclusion

Both IL-6 and TNF-α serum levels in patients were elevated compared to those from the control group, indicating them to be dependent predictors in the COVID-19 disease. Moreover, serum concentration of IL-6 and TNF-α may be proposed as an indicator of COVID-19 severity. In addition, these findings indicate distinct immunoregulatory events throughout the severity of SARS-CoV-2, which may aid in gaining an understanding of the pathogenesis of this disease. Further investigations need to be conducted to validate these findings.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, *et al*; China Novel Coronavirus Investigating and Research Team. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020;382:727-33.
2. Su S, Wong G, Shi W, Liu J, Lai A, Zhou J, *et al*. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol 2016;24:490-502.
3. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506.
4. Olbei M, Hautefort I, Modos D, Treveil A, Poletti M, Gul L, *et al*. SARS-CoV-2 causes a different cytokine response compared to other cytokine storm-causing respiratory viruses in severely ill patients. Front Immunol 2021;12:629193.

5. Santa Cruz A, Mendes-Frias A, Oliveira AI, Dias L, Matos AR, Carvalho A, *et al*. Interleukin-6 is a biomarker for the development of fatal severe acute respiratory syndrome coronavirus 2 pneumonia. Front Immunol 2021;12:613422.
6. Hamad RS, Shnawa BH, Al-Ali SJ. Correlation between IL-10 as cancer biomarker and demographic characteristics of colorectal cancer patients. J Pharm Res Int 2021;33:1-10.
7. Shnawa BH, Al-Ali SJ, Ahmad HF, Essa SA. An investigative study of smoking effects on IL-31 levels and leukocyte differential counts in humans. Ann Trop Med Public Heal 2020;23:1-7.
8. Amjadipoor NM, Shnawa BH. Investigating the potential correlation between vitamin D with Rheumatoid factor, anticyclic citrullinated peptides antibody, and interleukin-12 levels in rheumatoid arthritis women in Erbil, Iraq. Med J Babylon 2023;20:517-24.
9. Shakoor H, Feehan J, Al Dhaheri AS, Ali HI, Platat C, Ismail LC, *et al*. Immune-boosting role of vitamins D, C, E, zinc, selenium and omega-3 fatty acids: Could they help against COVID-19? Maturitas 2021;143:1-9.
10. Saeed BQ, Al-Shahrabi R, Bolarinwa OA. Socio-demographic correlate of knowledge and practice toward COVID-19 among people living in Mosul-Iraq: A cross-sectional study. PLoS One 2021;16:e0249310.
11. Undurraga EA, Chowell G, Mizumoto K. COVID-19 case fatality risk by age and gender in a high testing setting in Latin America: Chile, March–August 2020. Infect Dis Poverty 2021;10:1-11.
12. Oxfam. Gender Analysis of the COVID-19 Pandemic in Iraq. Oxfam Research Report 2020. DOI: 10.21201/2020.6218. Available from: https://policy-practice.oxfam.org › Publication.
13. Hussein NR, Naqid IA, Saleem ZSM. A retrospective descriptive study characterizing coronavirus disease epidemiology among people in the Kurdistan Region, Iraq. Mediter J Hematol Infect Dis 2020;12:e2020061.
14. Ad'hiah AH, Abdullah MH, Alsudani MY, Shnawa RMS, Al-Sa'ady AJR, Allami RH, *et al*. Association between ABO blood groups and susceptibility to COVID-19: Profile of age and gender in Iraqi patients. Egypt J Med Hum Genet 2020;21:1-10.
15. Ma Y, Hou L, Yang X, Huang Z, Yang X, Zhao N, *et al*. The association between frailty and severe disease among COVID-19 patients aged over 60 years in China: A prospective cohort study. BMC Med 2020;18:1-8.
16. Vaduganathan M, Vardeny O, Michel T, Mcmurray JJV, Pfeffer MA, Solomon SD. Renin-angiotensin-aldosterone system inhibitors in patients with Covid-19. N Engl J Med 2020;382:1653-9.
17. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, *et al*. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020;323:1061-9.
18. Al-Benna S. Association of high level gene expression of ACE2 in adipose tissue with mortality of COVID-19 infection in obese patients. Obes Med 2020;19:100283.
19. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, *et al*. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. Lancet 2020;395:507-13.
20. Odegaard JI, Chawla A. Connecting type 1 and type 2 diabetes through innate immunity. Cold Spring Harb Perspect Med 2012;2:a007724.
21. Mojtabavi H, Saghazadeh A, Rezaei N. Interleukin-6 and severe COVID-19: A systematic review and meta-analysis. Eur Cytokine Netw 2020;31:44-9.
22. Badawi A, Ryoo SG. Prevalence of comorbidities in the middle east respiratory syndrome coronavirus (MERS-CoV): A systematic review and meta-analysis. Int J Infect Dis 2016;49:129-33.
23. Chen X, Zhao B, Qu Y, Chen Y, Xiong J, Feng Y, *et al*. Detectable serum severe acute respiratory syndrome coronavirus 2 viral load (RNAemia) is closely correlated with drastically elevated interleukin 6 level in critically ill patients with coronavirus disease 2019. Clin Infect Dis 2020;71:1937-42.
24. Halim C, Mirza AF, Sari MI. The Association between TNF-α, IL-6, and vitamin D levels and COVID-19 severity and mortality: A systematic review and meta-analysis. Pathogens 2022;11:195.
25. Fara A, Mitrev Z, Rosalia RA, Assas BM. Cytokine storm and COVID-19: a chronicle of pro-inflammatory cytokines: Cytokine storm: The elements of rage! Open Biol 2020;10:200160.
26. Qin L, Li X, Shi J, Yu M, Wang K, Tao Y, *et al*. Gendered effects on inflammation reaction and outcome of COVID-19 patients in Wuhan. J Med Virol 2020;92:2684-92.
27. Vardavas CI, Nikitara K. COVID-19 and smoking: A systematic review of the evidence. Tob Induc Dis 2020;18:1-4.
28. Petrey AC, Qeadan F, Middleton EA, Pinchuk IV, Campbell RA, Beswick EJ. Cytokine release syndrome in COVID-19: Innate immune, vascular, and platelet pathogenic factors differ in severity of disease and sex. J Leukoc Biol 2021;109:55-66.
29. Valiathan R, Ashman M, Asthana D. Effects of Ageing on the Immune System: Infants to Elderly. Scand J Immunol 2016;83:255-66.
30. Queiroz MAF, Neves PFM, Lima SS, Lopes JC, Torres MKS, Vallinoto IMVC, *et al*. Cytokine profiles associated with acute COVID-19 and long COVID-19 syndrome. Front Cell Infect Microbiol 2022;12:1-11.
31. Chiappetta S, Sharma AM, Bottino V, Stier C. COVID-19 and the role of chronic inflammation in patients with obesity. Int J Obes 2020;44:1790-2.
32. Guerson-Gil A, Palaiodimos L, Assa A, Karamanis D, Kokkinidis D, Chamorro-Pareja N, *et al*. Sex-specific impact of severe obesity in the outcomes of hospitalized patients with COVID-19: A large retrospective study from the Bronx, New York. Eur J Clin Microbiol Infect Dis 2021;40:1963-74.
33. Kooistra EJ, de Nooijer AH, Claassen WJ, Grondman I, Janssen NAF, Netea MG, *et al*; RCI-COVID-19 study group. A higher BMI is not associated with a different immune response and disease course in critically ill COVID-19 patients. Int J Obes 2021;45:687-94.
34. Kozakova M, Morizzo C, Goncalves I, Natali A, Nilsson J, Palombo C. Cardiovascular organ damage in type 2 diabetes mellitus: The role of lipids and inflammation. Cardiovasc Diabetol 2019;18:1-11.
35. Guo W, Li M, Dong Y, Zhou H, Zhang Z, Tian C, *et al*. Diabetes is a risk factor for the progression and prognosis of COVID-19. Diabetes Metab Res Rev 2020;36:e3319:1-9.
36. Randeria SN, Thomson GJA, Nell TA, Roberts T, Pretorius E. Inflammatory cytokines in type 2 diabetes mellitus as facilitators of hypercoagulation and abnormal clot formation. Cardiovasc Diabetol 2019;18:1-15.
37. Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, *et al*. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. J Allergy Clin Immunol 2020;146:110-8.
38. Hussein G, Al-Saadi A. Association between clinical characteristics and laboratory findings of 102 patients with COVID-19 in Iraq. Med J Babylon 2023;20:332337.
39. Xu X, Yu MQ, Shen Q, Wang LZ, Yan RD, Zhang MY, *et al*. Analysis of inflammatory parameters and disease severity for 88 hospitalized COVID-19 patients in Wuhan, China. Int J Med Sci 2020;17:2052-62.
40. Dey G, Majumdar A, Sinha A. Altered hematological profile: The forerunner of fatalities caused by COVID-19. Med J Babylon 2022;19:379-82.