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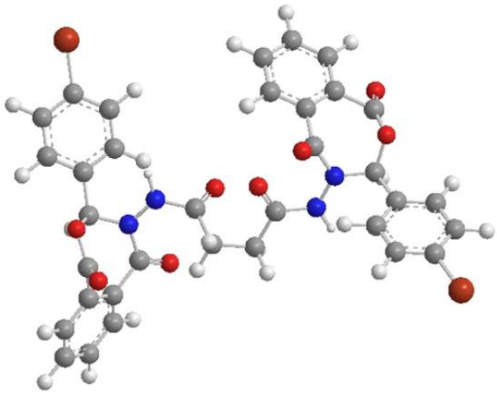

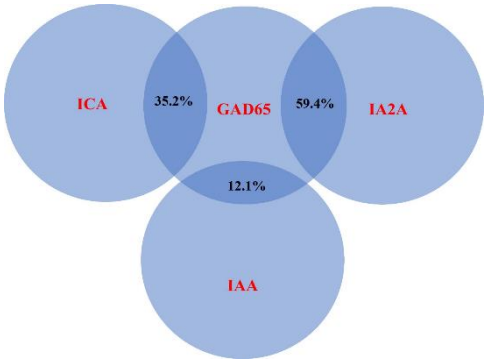

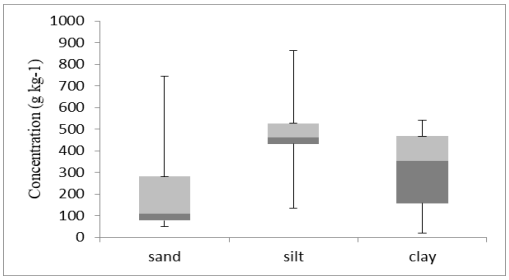

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Deciphering COVID-19 Severity: Assessing FGF-18, WNT-5A, IL-17, and IL-33 Levels in the Infected Patients

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Abstract

Background: Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) is a novel coronavirus that causes an infectious disease named coronavirus disease-2019 (COVID-19). **Objectives:** To test some biomarkers (Fibroblastic growth factor (FGF)-18, wingless-related integration site (WNT-5A), interleukin (IL)-17, and IL-33 to predict the severity of COVID-19. **Patients and Methods:** A cross-sectional study was done on 180 participants (90 healthy control and 90 COVID-19-positive patients) at the emergency units of the hospitals in four cities of Northern Iraq from July 01 to December 01, 2021. The obtained serum from collected blood was tested to determine IL-17, IL-33, FGF-18, and WNT-5 levels using the ELISA technique. Additionally, CRP concentration was measured using immunoturbidimetry on Cobas c311. **Results:** Regarding the sociodemographic data, most moderately infected patients were aged 46-64 years and were males (35.6%, 64.4%, respectively). The most severely infected patients were older than 65 years and were females (60%). The highest mean serum levels of C-reactive protein (CRP) (59.53±40.06 mg/dl), IL-17 (36.90±22.61 pg/ml), FGF-18 (179.8±291.37 pg/mL), IL-33 (248.11±506.71 pg/ml) were reported among the severely infected patients, while the highest mean serum level of WNT-5A was observed among moderately infected patients (5.03±5.73 ng/ml). Regarding the correlations between biomarkers, in moderately infected patients, a negative correlation between CRP and IL-33; CRP and IL-17 was observed, with a positive correlation between IL-33 and IL-17. Conversely, no correlations were seen between IL-33 and WNT-5 whereas correlations between IL-33 and IL-17 were seen in severely infected individuals. Simultaneously, a correlation between the patient's body mass index (BMI) and CRP was detected. **Conclusions:** The disease was more severe among elderly, females, and overweight patients. All biomarkers, except WNT-5A, were significantly associated with the severity of COVID-19.

Introduction

World Health Organization (WHO) declared Coronavirus disease-2019 (COVID-19) to have a significant impact worldwide due to the high mortality rate among critically ill individuals, rapid transmission, and contagious nature. Severe acute respiratory syndrome- coronavirus-2 (SARS-CoV-2) mainly creates pulmonary damage such as acute respiratory distress syndrome (ARDS) and pneumonia that in certain groups of patients may result in disseminated intravascular coagulation (DIC) and serious organ failure [1,2]. Early signs of COVID-19 infection are pyrexia, cough, and shortness of breath. In some patients, fatigue, muscle aches, and loss of smell (anosmia)/taste were seen, and up to 10% of people showed gastrointestinal symptoms such as diarrhea [3].

Although most cases of COVID-19 are mild, they can cause more severe symptoms in some people, especially those who are older than 65 years old, significantly overweight, and immunocompromised

individuals. People with a history of underlying diseases such as diabetes, heart disease, lung disease, and pregnant women are also at risk [4,5]. While most patients shows mild to moderate symptoms, the disease has significant mortality in infected individuals [6]. Sometimes, severe illness is diagnosed with pneumonia, a management challenge for physicians [7,8]. In patients with severe COVID-19, ARDS is a vital complication that with a mortality effect [9,10]. Other complications have been reported, including acute cardiac injury, liver injury, renal failure, and heart failure [11]. Severe cases of COVID-19 lead to a mysterious condition that disrupts the immune system. The immune system also attacks healthy cells instead of attacking only infected cells (cytokine storm), which can be fatal and is an abnormal systemic inflammatory response that is followed by fever, tiredness, anorexia, joint/headache, nausea, vomiting, diarrhea, dermal complications, difficulty breathing, palpitations, hypotension, seizures, and tremors [12].

Cytokines are small signaling proteins that enter the body by the cells of the immune system including macrophages, lymphocytes, natural killer. They are vital for the immune response as they can limit the spread of the virus, as well as stimulate inflammation which some time may trigger some autoimmune disorders [11].

During viral infection, cytokines play a crucial role in immune pathology, and the innate immune response is the first line of defense against viral infection [13,14]. Pattern recognition receptors (PRRs) at the innate immune response to viral infection identify several molecular structures named pathogen-related molecular patterns (PAMPs) that are specific to the virus. PAMP to PRR binding triggers the beginning of the inflammatory response which activates various signaling pathways, and then transcription factors that trigger the expression of genes responsible for producing multiple products participate in the immune response of the host to the virus, like genes that encode various pro-inflammatory cytokines [12,15].

Interleukin-17 (IL-17) is a common biomarker of respiratory inflammation that is involved in the cytokine storm. Dysfunction of T helper 17 (Th17) cells and improved appearance of IL-17 in the lungs stimulates the creation of downstream pro-inflammatory substances such as IL-1β, tumor necrosis factor-alpha (TNF-α), IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1/CCL2) [16]. IL-33 is enrolled in innate/adaptive immune responses to enhance airway inflammation, mucus secretion, and Th2 production in the lungs after respiratory infections. Thus, contact with SARS-CoV-2 leads to the expression of IL-33, T-cell activation, and worse respiratory infections [17]. Moreover, the fibroblastic growth factor (FGF) plays a vital role in many physiological processes such as inflammation, angiogenesis, and skeletal development, especially FGF-18 that have a potential impact when incorporated with hydrogel and scaffolds showing implicit bone regeneration. However, it plays a role in COVID-19 infection, although it's role is not declared, in stimulating hepatic and intestinal proliferation [18].

Regarding the wingless-related integration site (WNT-5A), previous reports have demonstrated that it has a pro-inflammatory effect; its concentration should be explored to find its potential relevance to COVID-19 infection [19]. Thus, biomarkers significantly target therapeutic strategy and drug delivery systems. The current study is designed to find the association of several biomarkers (FGF-18, WNT-5A, IL-17, and IL-33) with the severity of COVID-19 patients.

Patients and Methods

Study design and setting

This cross-sectional study was carried out from July,1 to December,1 2021. One hundred eighty (180) individuals were included (90) healthy -controls- and (90) COVID-19-positive patients who were attended the Emergency Departments of the Hospitals in four cities of the Kurdistan region, Iraq. The COVID-19-positive patients were further subdivided into two groups based on the clinical manifestations; moderately infected COVID-19 patients (n=45) and severely infected COVID-19 patients (n=45).

Inclusion criteria

Adult COVID-19-positive patients who were non-vaccinated and were not involved in any therapeutic intervention were enrolled in this study.

Exclusion criteria

Asymptomatic or mildly infected patients with COVID-19 were excluded from the study.

Questionnaire

A validated questionnaire form collected participant's sociodemographic data, including their age, gender, and BMI.

Ethical considerations

The Scientific and Ethics Committees approved the study protocol at the College of Medicine, University of Sulaimani, Sulaimaniyah, Iraq, with approval number (No. 197 on September 28, 2021). All parameters were done according to the Declaration of Helsinki. Participants' written consent was obtained before starting the study.

Sampling

About 10 ml of fresh venous blood samples were collected from all participants (the volunteers on admission to the hospital), then the clotted blood samples were centrifuged at 1500 rpm for 10 minutes, the sera samples were preserved at -80 °C until use.

Estimation of serum parameters

Serum levels of IL-17 (Cat. No. E-EL-H0105), IL-33 (Cat. No. E-EL-H2402), and FGF-18 (Cat. No. E-EL-H5434) were measured using Elabscience ELISA kit from USA, whereas the level of WNT-5 (Code E6012Hu) was measured using Biotechnology Laboratory (BT LAB) kit, from UK. Additionally, immunoturbidimetry was done to measure C-creative protein (CRP4, Roche, Germany) using Cobas c311 (Cobas, Roche Diagnostic, Mannheim, Germany).

Statistical analysis

The data were analyzed using Statistical Package for the Social Sciences (SPSS, IBM, USA, version 26). The Shapiro-Wilk and Kolmogorov–Smirnov tests were used to determine the normal distribution of the data. The Chi-square test was used for categorical variables. Data were expressed as numbers and frequencies for categorical data and mean± standard deviation (SD) for numerical data. Analysis of variance (ANOVA) and Kruskal-Wallis tests were used for parametric and non-parametric data, respectively. The Spearman correlation test determines a correlation between the numerical variables. P-value ≤0.05 was considered a significant difference.

Results

Sociodemographic characteristics of the participants

In the current study, the mean age of healthy controls was 41±15.55, while for moderately infected COVID-19 patients was 38±16.44, and for severely infected patients was 63.42±11.61. Most healthy individuals (44.4%) were aged 26-45 years, while most moderately infected patients (35.6%) were aged 46-64 years, and most severely infected patients were older than 65 years (51.1%), with a highly significant difference between the groups (p<0.001). Considering the gender, most of the healthy individuals (72.2%) and moderately infected patients (64.4%) were males, while most of the severely infected patients (60%) were females, with significant differences between groups (p=0.001). Regarding the BMI, most normal controls, moderately and severely infected patients, were overweight (43.3%, 48.9%, and 40%) respectively without significant differences (p=0.13) (Table 1).

Table 1: Distribution of essential characteristics of the participants.

Variable		Healthy	Moderate	Severe	p-value
		Number (%)			
Age (Years)	15 - 25	14 (15.6)	11 (24.4)	0.0 (0.0)	<0.001**
	26 - 45	40 (44.4)	15 (33.3)	3.0 (6.7)	
	46 - 64	26 (28.9)	16 (35.6)	19 (42.2)	
	≥ 65	10 (11.1)	3.0 (6.7)	23 (51.1)	
Gender	Female	25 (27.8)	16 (35.6)	27 (60.0)	0.001*
	Male	65 (72.2)	29 (64.4)	18 (40)	
BMI (Kg/m^2)	Healthy weight	33 (36.7)	19 (42.2)	13 (28.9)	
	Overweight	39 (43.3)	22 (48.9)	18 (40)	0.13
	Obese	18 (20.0)	4.0 (8.9)	14 (31.1)	
Total		90 (100)	45 (100)	45 (100)	

BMI: Body mass index; *: Significant difference; **: Highly significant difference

As shown in Figure 1, there was a highly significant difference in the CRP level between the groups (p<0.001) please write the exact p-value; the highest mean CRP serum level was among the severe groups (59.53±40.06 mg/dl), followed by the moderate (11.75±28.79 mg/dl), while the lowest mean level was recorded among the healthy individuals (0.83±1.57 mg/dl). Regarding the IL-17 level, the highest mean value was among the severe group (36.90±22.61 pg/ml), then the moderate (31.64±7.12 pg/ml), and healthy

individuals (23.89±15.89 pg/ml). Significant differences were found among the groups considering IL-17 serum levels (p=0.04).

Notably, the serum level of FGF-18 was sharply increased from the healthy individuals (84.83±65.40 pg/mL) to the moderately infected patients (172.49±151.44 pg/mL), then sluggishly raised in the severely infected patients (179.8±291.37 pg/mL) with highly significant difference between them (p<0.001). Regarding IL-33, the highest mean serum concentration was among severely infected individuals (248.11±506.71 pg/ml), followed by the moderate cases (162.37±373.19 pg/ml), then healthy controls (43.77±132.92 pg/ml). Statistical analysis showed significant differences between groups considering IL-33 (p=0.019). It was concluded that there were no significant differences between the studied groups considering the serum levels of WNT-5A (p=0.065) (Figure 1).

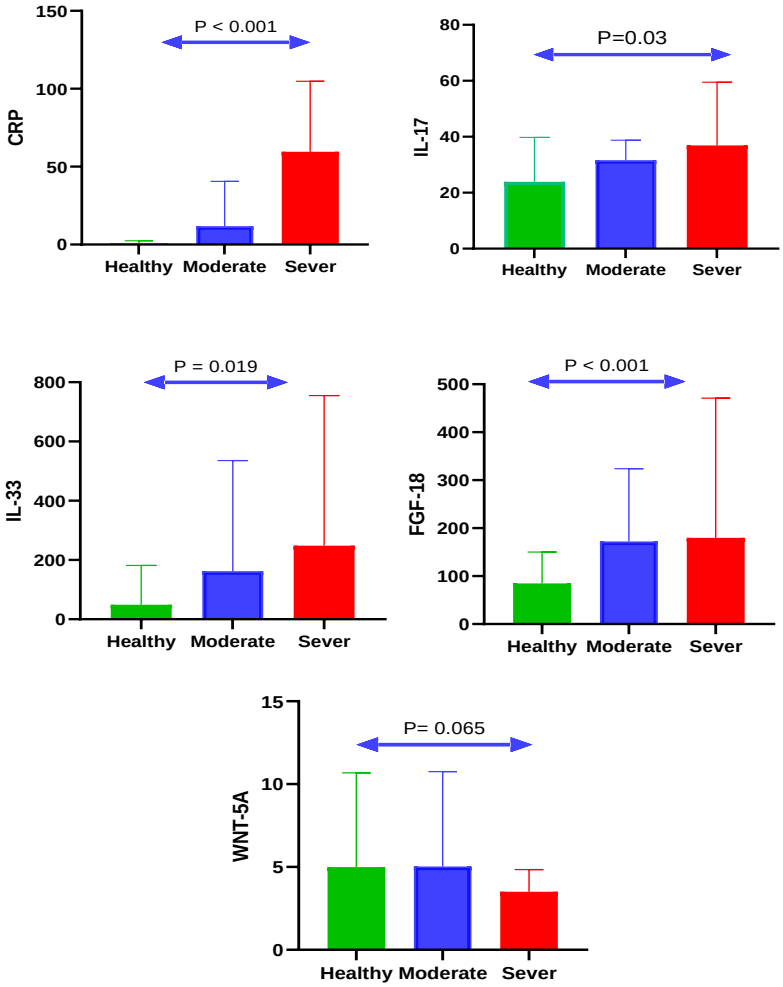

Figure 1: Comparison of the serum level of the biomarkers among studied groups.

Correlation between the biomarkers in the infected patients

It was appeared that there was a negative correlation between CRP and IL-33 (p=0.005, Rho= -0.345), as well as between CRP and IL-17 (p=0.047, Rho= -0.249) among moderately infected patients, while a positive correlation was noted between IL-33 and IL-17 (p=0.001, Rho=0.427). Otherwise, there was no correlation between other biomarkers. Moreover, negative correlation between IL-33 and WNT-5A was found among severely infected patients (p=0.008, Rho=-0.310) whereas positive correlation between IL-33 and IL-17 (p=0.009, Rho= 0.307) was recorded, while no other correlation was documented (Table 2).

Table 2: The correlation between blood parameters among the infected patients.

Variable	Biomarker	Correlation	Biomarker			
			IL-33	FGF18	IL-17	WNT5A
Moderately infected patients	CRP	Spearman's rho	-0.345**	0.012	-0.249*	-0.213
		p-value	0.005	0.927	0.047	0.091
	IL-33	Spearman's rho		-0.099	0.427**	0.203
		p-value		0.439	0.001	0.107
	FGF18	Spearman's rho			-0.099	-0.167
		p-value			0.439	0.187
	IL-17	Spearman's rho				0.148
		p-value				0.243
Severely infected patients	CRP	Spearman's rho	-0.003	-0.169	-0.104	0.131
		p-value	0.988	0.157	0.385	0.274
	IL-33	Spearman's rho		0.007	0.307**	-0.310**
		p-value		0.951	0.009	0.008
	FGF18	Spearman's rho			0.007	-0.016
		p-value			0.951	0.891
	IL-17	Spearman's rho				-0.118
		p-value				0.322

CRP: C-reactive protein; IL: Interleukin; FGF: Fibroblast Growth Factor; WNT: Wingless-related integration site; *: Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed).

Correlation between BMI and biomarkers of the infected patients

The current results revealed a positive correlation between the patient's BMI and CRP serum level (p=0.023, Rho = 0.195), whereas no correlation between BMI and other biomarkers was found (Table 3 and Figure 2).

Table 3: Correlation between BMI and the biomarkers.

	Correlation	Biomarker				
		CRP	IL-33	FGF-18	IL-17	WNT-5A
BMI	Spearman's rho	0.195	-0.035	-0.065	0.099	-0.016
	p-value	0.023*	0.684	0.455	0.25	0.855

BMI: Body Mass Index; CRP: C-Reactive Protein; IL: Interleukin; FGF: Fibroblast Growth Factor; WNT: Wingless-related integration site; *: Correlation is significant at the 0.05 level (2-tailed).

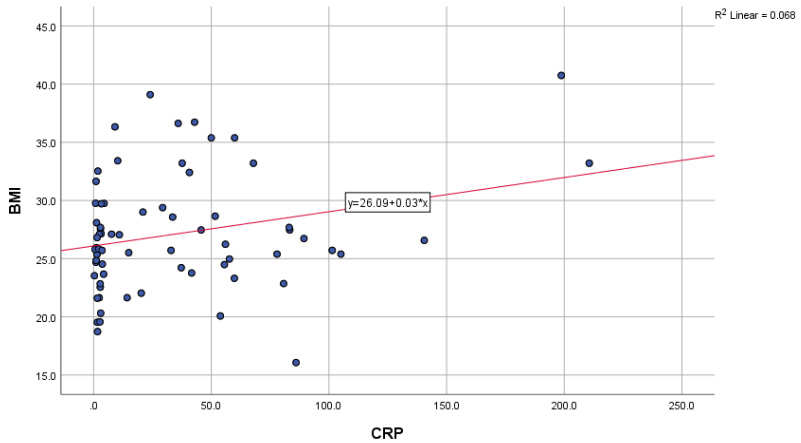

Figure 2: Correlation between body mass index and C-creative protein levels.

Discussion

The novel COVID-19 was identified first in Wuhan City, China, in December 2019, and the WHO declared the outbreak of this pandemic respiratory illness as a Public Health Emergency of International Concern [20]. In the Kurdistan region of Iraq, the first case was detected on March 1, 2020. After that date, effort was intensified by The Health teams/Governmental agencies to quarantine citizens, block , and travel bans to prevent the virus spreading in the region [21]. There is a limited number of studies on the biomarker identification and their specific levels for the patients infected with COVID-19 in Iraq, including the Kurdistan region. Some studies have examined the role of biomarkers for the general risk assessment without referring to the position of biomarkers in the pathogenesis of the disease [22].

The current study was designed to determine the levels of some biomarkers, including FGF-18, WNT-5, IL-17, and IL-33, in patients with COVID-19 infection and their role in the pathogenesis/progression of the disease. Regarding the sociodemographic characteristics of participants, this study investigated their age, gender, and BMI. In concern to age, most moderately infected patients (35.6%) with COVID-19 infection aged 46-64 years, while most severely infected patients (51.5%) were aged ≥65 years. These results indicated that the disease intensity is directly related to the patient's age, which agrees with many international studies worldwide [23-25]. A study in the United Arab Emirates (UAE) showed that only 6.85% of young adults were severely infected with COVID-19 [must correlate with your results]. In comparison, midlife (21.61%) and older adults (37.5%) had severe cases, while older adults had critical cases (25%), then midlife adults (13.14%), and young adults (2.4%) [25]. Moreover, it was found that the risk of in-hospital and case mortality by COVID-19 increased per age year by 5.7% and 7.4%, respectively, while the risk of hospitalization increased by 3.4% per age year, and no risk was observed for intensive care unit (ICU) admission and intubation by age year [26]. So, investigating age-related infection severity for COVID-19 may be helpful for public health policies and communications to help protect vulnerable age groups and minimize fatality rates. The reasons behind this event that the elderly present with more severe cases are that generally, older adults suffer from immunosuppression and have comorbidities with other metabolic diseases such as diabetes, hypertension, hypercholesteremia, heart/kidney diseases, and so on. Thus, they are more vulnerable to the infection and get it rather severely than younger people.

Considering the patient's gender, most of the moderately infected patients were males (64.4%), while most severely infected patients (60%) were females. In general, most studies sustained that COVID-19 is more predominant in males than females, and among the factors that have been endorsed to the gender variances in the predisposition to COVID-19 is more expression of angiotensin-converting enzyme 2 (ACE2) receptors [where? Among males or females? What is the relation of that with your observations?], poor immune response, and lack of preserving responsible COVID-19 prevention lifestyle may explain the higher rates of infection among males than females [27]. Additionally, raised estrogen levels among female COVID-19 patients may minimize the severity/mortality through promotion in the innate/humoral responses. Also, pre-clinical studies showed that ACE2 manifestation may enhance the vulnerability to COVID-19 among pregnant women [28]. Another study stated that men and women have the same prevalence of COVID-19, although, males have higher mortality rates than females due to the severity of the disease, regardless of age [29]. Moreover, in European studies, it was revealed that the relative disadvantage of women infected with COVID-19 peaks at ages 20-29, whereas the male disadvantage in infection rates peaks at ages 70-79 [30]. These observation variations may have referred to the sampling process, sample size, the gender prevalence in the studied area, age mismatch of study samples, and gender mismatch of study samples in different research and countries.

Obesity is a risk factor for intensive care unit (ICU) admission and COVID-19 intubation among males/females [31], since it was found from the current study that most of the moderately and severely infected patients with COVID-19 were overweight (48.9% and 40%, respectively). These observations were close to the results reported by another hospital cohort study in UAE, who found that patients of both sexes aged >65 years had a BMI >31 kg/m^2 (obese people). Furthermore, it was found that obesity was correlated independently with COVID-19 patients' mortality [where?]. Also, a BMI >25 kg/m^2 is a risk of increasing severe pneumonia; however, mortality was higher among underweight and morbidly obese patients [32,33]. The variations may be related to the patient's lifestyle, including physical activity, healthy diet, stress, education level, and awareness.

Furthermore, the inflammation enzyme, CRP, was estimated in the studied participants. The highest mean serum CRP level was found in patients with severe infection (59.53±40.06 mg/dL), followed by the moderate disease (11.75±28.79 mg/dL); however, the CRP level was average in healthy controls (0.83±1.57

mg/dL). These results were parallel with the outcomes reported in other studies [34-39] who indicated that higher CRP level was observed among severe COVID-19 pneumonia and longer inpatients duration.

Inflammatory biomarkers have several potential applications in managing COVID-19, including risk assessment, monitoring of disease progression, determining prognosis, selecting therapy, and predicting response to treatment. The highest mean level for IL-7 and IL-33 was found among patients with severe infection, whereas, lower levels for these two biomarkers were seen in patients with moderate disease. These results indicated that the expression level of these two biomarkers is directly related to the age and severity of the infection [Reference]; however, interpretation of the multiplex cytokine data for COVID-19 patients is challenging. It was reported by others that the serum levels of pro-inflammatory cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, and IL-17) among severe COVID-19 cases were behind the cytokine storm-like syndromes which may develop among these patients [22].

From the current study, it was found that the serum levels of FGF-18 were sharply elevated among moderately infected patients comparing to the normal controls whereas, sluggishly raised among the severely infected patients with a highly significant difference ($p<0.001$) between studied groups. At the same time, normal serum levels were reported for WNT-5A in normal controls and moderately/severely infected patients without significant differences. These observations indicated that the expression level of FGF-18 is directly correlated to age and severity of the infection, while it had no effect in the case of WNT-5A. To our knowledge, this is the first study that estimates the serum level of these biomarkers in Iraq with crucial results**.** It was mentioned that the WNT-5A levels in COVID-19 patients may indicate poor prognosis [Who mentioned?]. In contrast, WNT -11 levels may be a good indicator of the ability to survive the disease [Reference?]. Consequently, WNT-11 efficiently inhibits inflammatory responses and cytokine production and could be exploited as a therapeutic target for the treatment of patients [19]. Collectively, an initial study in January 2020 in China reported elevated concentrations of plasma IL-1B, IL-1RA, IL-7, IL-8, IL-9, IL-10, FGF, MCP1, and TNF-α, in COVID-19 infected patients [40]. Also, another study indicated that FGF-2 significantly increased with the severity of the COVID-19 infection [41]. Moreover, Smail et al. [42] have documented that IL-17 was a predictor of severity and mortality in COVID-19.

No correlation was observed between CRP and IL-33, CRP and IL-17, with a significant correlation between IL-33 and IL-17 moderately infected COVID-19 patients. Also, no correlation between IL-33 and WNT-5A and a correlation between IL-33 and IL-17 were found in severely infected patients. Concerning correlations between biomarkers and patient's sociodemographic characteristics, only a positive correlation was found between the patient's BMI and CRP levels [Also this is mentioned in results]. To our knowledge, this is the first research that found these correlations with potential outcomes; however, there has yet to be a study on this topic in the literature to compare our results. Hence, the identification of potential biomarkers for the accurate prediction of other similar dangerous viral diseases may enhance patient management inform clinical trials, and indicate novel pathogenetic and therapeutic targets that further improve clinical practice among populations in the community.

Conclusions

COVID-19 infection predominantly and severely affects the elderly, females and overweighted patients. IL-17, IL-33, CRP, and FGF-18 were significantly associated with the severity of SARS-CoV-2 infection, except WNT-5A. Positive correlations between IL-33 and IL-17, as well as BMI and CRP, were reported among all patients. For future studies, it is recommended to use platform and laboratory tests when monitoring differences in cytokine levels between groups of individuals or for the same individual over time. It may be important to correlate cytokine profiling data with the SARS-CoV-2 nucleic acid amplification testing and imaging observations to accurately interpret the inflammatory status and disease progression in COVID-19 patients. Also, targeting of therapeutic strategy, targeting drug delivery systems, and more biomarker identification in patients with pandemic viral diseases using proteomic tools such as mass spectrometric analysis and microarray analysis are strongly recommended.

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Conflict of interest

The authors confirm that they are not affiliated with or involved in any organization or entity with financial interests.

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