https://doi.org/10.34883/PI.2024.13.1.004

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Immunopathy of COVID-19 Patients without Chronic Disease: Proinflammatory and Anti-Inflammatory Cytokines Attributable to Disease Severity

Conflict of interest: nothing to declare.
Authors' contribution: Hayder Fadhil Okab – conceptualization, methodology, formal analysis, investigation, resources, data curation, writing – original draft, soft wire; Manal Badi Salih – supervision, conceptualization, methodology, formal analysis, investigation, writing – original draft; Bassim Abdulhussein Jarulla – methodology, resources, data curation, writing – original draft.
Ethical approval. The thesis project has been approved according to the decision of the Research Committee in the Thi-Qar Health Directorate [issue No. 208/2022 on 15/8/2022] and facilitation task issued by the Thi-Qar Health Directorate, Department of Training and Development [issue No. 617 on 17/8/2022], which are attached to the document of University of Thi-Qar / College of Science [issue No. 3/11/982 on 17/7/2022], where the patient's consent is taken verbally when reviewing hospitals designated for COVID-19 patients.
Statement of data availability. All data obtained in this study are available at main text, and the original data are available upon request.
Acknowledgements. The authors thank the COVID-19 patients for allowing sample collection despite their illness, and the laboratory staff at Imam Hussein Teaching Hospital for providing assistance during the sample collection period.
The article is published in the author's edition.

Submitted: 25.08.2023
Accepted: 12.02.2024
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Abstract

Introduction. Immune system dysregulation is characteristic of the severe stages of COVID-19 patients.
Purpose. To highlight the defective immune regulation of COVID-19 patients without chronic diseases.
Materials and methods. The study included 180 individuals, 60 as a control group, and 120 patients with COVID-19, including 67 males and 53 females, whose ages ranged from 27 to 70 years, at Imam Al-Hussein Teaching Hospital in Thi-Qar Province, South of Iraq. The proinflammatory and anti-inflammatory cytokines were evaluated by enzyme-linked immunosorbent assay (ELISA).
Results. The current study recorded that IL-6 and IL-17 were significantly increased <0.001 in patients compared to controls, while IL-4 and IL-13 decreased significantly <0.001 in patients compared to controls. Within the severity of disease, both IL-6 and IL-13 increased significantly in severe patients, while IL-4 and IL-17 did not show a significant difference. With regard to BMI, IL-4 increased significantly in normal-weight patients, while IL-6 and IL-17 increased in obese patients. Also, the IL-13 had no significant difference. The ROC test gave reliable predictive values for interleukin-6 and interleukin-17 in determining disease progression.
Conclusion. Increased IL-6 was related to both hyperglycemia and insulin resistance, making it useful in predicting the progression of GDM. Where's an increase in interleukin-17

is associated with an increase in the production of IL-6, IL-1, and TNF and thus inhibits apoptosis, while IL-4 and IL-13 are mostly associated with fibrotic remodeling.
Keywords: immunopathy, COVID-19, IL-4, IL-6, IL-13, IL-17

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Иммунопатия у пациентов с COVID-19, не имеющих хронических заболеваний: уровень провоспалительных и противовоспалительных цитокинов в зависимости от степени тяжести течения коронавирусной инфекции

Конфликт интересов: не заявлен.
Вклад авторов: Хейдер Фадиль Окаб – концепция исследования, методология, формальный анализ, проведение исследования, подбор материала, обработка данных, написание текста, программное обеспечение; Маналь Бади Салих – руководство, концепция исследования, формальный анализ, проведение исследования, написание текста (черновой вариант); Бассим Абдулхуссейн Джарулла – методология, подбор материала, обработка данных, написание текста.
Этическое заявление. Диссертационный проект утвержден решением исследовательского комитета Управления здравоохранения Ти-Кара (протокол № 208/2022 от 15.08.2022) и распоряжением об оказании содействия, выданным Управлением здравоохранения Ти-Кара, Департаментом обучения и развития (протокол № 617 от 17.08.2022), приложенными к документу Университета Ти-Кара / Научного колледжа (протокол № 3/11/982 от 17.07.2022), на основании которого согласие пациента подтверждается в устной форме при посещении медицинских учреждений, предназначенных для лечения пациентов с COVID-19.
Доступность данных. Все данные, полученные в ходе этого исследования, отражены в тексте; оригинальные данные доступны по запросу.
Благодарности. Авторы благодарят пациентов с COVID-19 за то, что они позволили собрать образцы, несмотря на их болезнь, а также персонал лаборатории Учебного медицинского центра Имама Хусейна за оказание помощи в период сбора образцов.
Статья опубликована в авторской редакции.

Подана: 25.08.2023
Принята: 12.02.2024
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Резюме

Введение. Нарушение состояния иммунной системы, характерное для пациентов с тяжелым течением COVID-19, связано с изменениями в системе механизмов гуморальной регуляции иммунного статуса.
Цель. Выделить дефектную иммунную регуляцию у пациентов с COVID-19 без хронических заболеваний.
Материалы и методы. В исследовании приняли участие 180 испытуемых; 60 участников контрольной группы и 120 пациентов с COVID-19, включая 67 мужчин и 53 женщины, возраст которых варьировался от 27 до 70 лет, из Учебного медицинского центра Имама Аль-Хусейна в провинции Ти-Кар (Южный Ирак). Содержание провоспалительных и противовоспалительных цитокинов определяли с использованием иммуноферментного анализа (ИФА).

Результаты. В ходе настоящего исследования было зарегистрировано значимое повышение уровня провоспалительных цитокинов IL-6 и IL-17 (p<0,001) у пациентов по сравнению с лицами контрольной группы, а также значимое снижение содержания противовоспалительных цитокинов IL-4 и IL-13 (p<0,001) по сравнению с лицами контрольной группы. Относительно зависимости между степенью тяжести заболевания и уровнем цитокинов, то у пациентов с тяжелым течением заболевания наблюдалось значимое повышение уровня IL-6 и IL-13, в то время как в отношении IL-4 и IL-17 существенных различий не наблюдалось. Что касается влияния индекса массы тела на уровень цитокинов, то у пациентов с нормальной массой тела было отмечено значимое повышение уровня IL-4, а у пациентов с ожирением – IL-6 и IL-17. Статистически достоверных различий в показателях IL-13 не наблюдалось. С помощью ROC-анализа были получены достоверные для IL-6 и IL-17 критерии оценки прогрессирования заболевания.
Заключение. Повышение уровня IL-6 ассоциировано как с гипергликемией, так и с инсулинорезистентностью, что делает данный тест пригодным для использования в прогнозировании прогрессирования гестационного сахарного диабета (GDM). Повышение уровня IL-17 сопровождается увеличением продукции IL-6, IL-1 и фактора некроза опухолей (TNF) и, соответственно, подавлением апоптоза; сдвиги в содержании IL-4 и IL-13 в основном ассоциировались с развитием фиброза.
Ключевые слова: иммунопатия, COVID-19, IL-4, IL-6, IL-13, IL-17

■ INTRODUCTION

Following Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) emergence in 2002 to 2003 that infected 8,000 people, with a fatality rate ~10%, and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in 2012 that infected more than 1,700 people, with a fatality rate ~36%, a new pathogenic human coronavirus (HCoV) emerged in 2019 that soon spread worldwide, resulting the COVID-19 pandemic named by ICTV on 11th of February 2020, unlike SARS and MERS, the outbreak has spread aggressively in almost all regions, resulting in a global health care crisis, and is considered the seventh virus of the family that can cause serious illness in humans [1–3]. Although the fact that the pathophysiology of human COVID-19, SARS-CoV-2, and MERS is not fully understood, they are all related to an imbalanced and exaggerated immune response, particularly in the generation of cytokines. SARS-CoV-2 caused a deficiency in both adaptive and innate immune responses, which was the primary pathophysiology of COVID-19. Multiorgan failure results from this dysregulation or uncontrolled adaptive and/or innate immune response, resulting in delayed viral clearance, inflammation, and tissue damage that's not only restricted to the lung but also systemically in additional organs [4]. Lymphopenia is one of the hallmarks of COVID-19, a condition in which there are fewer than normal numbers of lymphocytes such as B cells, T cells, and innate lymphocytes [5]. On the other hand, myeloid cells undergo increased aberrant activation and recruitment in COVID-19, which may contribute to immune pathology [6]. Moreover, increased serum inflammatory cytokines are one of the most important characteristics of patients with severe COVID-19 and are significantly associated with acute lung injury in COVID-19 [7]. Moreover, inflammatory cytokines and chemokines are significantly more expressed

in bronchoalveolar lavage fluid [BAL] than in blood in patients with severe COVID-19, suggesting continued vulnerability to viral stimulation in the lung microenvironment leading to a locally increased inflammatory state [8]. One of the most important of these are the cytokines IL-6, IL-8, IL-1, IL-17, and chemokines.

Cytokines are glycoproteins that are membrane-bound or screened and have the charge of regulating a wide range of biological processes, including the balance between the immune system's innate and adaptive responses, which play a key role in the controller of infections or chronic diseases. In order to prevent and treat coronavirus infections, it is believed that the cytokines are essential during COVID-19 infection. However, uncontrolled and excess cytokine production may lead to tissue damage throughout the entire human body, which may result in immunopathogenesis. The majority of COVID-19 infected individuals may experience mild to moderate symptoms; however, certain individuals may experience cytokine release syndrome [CRS], a hyper-inflammation brought on by large cytokine/chemokine production that can result in deadly pneumonia and acute respiratory distress syndrome [9, 10]. The respiratory mucosa is first invaded by viruses, which then spread to other cells, causing a series of immune reactions and the development of a systemic CRS. CRS is characterized by the release of pro-inflammatory cytokines such as IL-6, IL-1, IL-17, TNF, and others at higher levels than normal, typically resulting in a systemic inflammation with high inflammation parameters and multiple organ failure [11].

The severity of COVID-19 was positively correlated with greater serum IL-6 concentrations in various trials [10]. A study of Xiong et al. [12], showed that in two out of three COVID-19 patients, the serum levels of IL-6 was elevated. However, peripheral blood mononuclear cell specimens (PBMC) from COVID-19 patients showed no appreciable change in the transcription levels of IL-6, suggesting that the production IL-6 protein in serum might originate from lung epithelial cells. Statistical analysis showed a negative correlation between T cells number and the serum concentration of IL-6 [13]. Clinical trials targeting antibodies that block the IL-6 receptor as a potential treatment for COVID-19 are presently underway in light of this rise in IL-6 [14]. In response to viruses, TH17 cells in the lung release IL-17, which causes the production of multiple chemokines and the recruitment of immune cells to the site of inflammation [15]. Combining IL-17 and TNF causes pro-coagulation factors to be expressed, which encourages thrombosis and blocks the endothelium anticoagulatory pathway. In addition to the local effects of IL-17, these effects also contribute to the increase in cardiovascular risk associated with inflammatory disease [10]. In addition to CRS mediation, IL-17 exhibits enhanced viral multiplication and promotes viral persistence by suppressing the apoptotic process in infected cells in conjunction with IL-6 [16].

The two main cytokines in type 2 immune responses, L-4 and IL-13, are comparable in both structure and function. T-helper type 2 [Th2] cells and some innate immune cells, such as basophils, mast cells, eosinophils, and macrophages, are the main producers of both cytokine [17]. On the other hand, fibrosis and Th2 immune responses are tightly related. Although IL-13 and IL-4 have been widely researched for their effects on fibrosis development, their function in WAT fibrosis has not yet been understood [18]. The development and progression of ARDS appear to be closely correlated with the inflammatory cytokine storm observed in COVID-19 patients. COVID-19 would significantly increase the release of cytokines due to macrophage activation and a delay in the recruitment of TCD8+ cells, leading to an insufficient T1 response [19].

Therefore, the presents designated to study the relation between pro-inflammatory and anti-inflammatory cytokines and their relation with severity of disease.

■ PURPOSE OF THE STUDY

To highlight the defective immune regulation of COVID-19 patients without chronic diseases.

■ MATERIALS AND METHODS

Sample Collection

A total of 180 blood samples were collected for this investigation by using disposable syringes, arterial blood samples were taken from COVID-19 patients while control group venous samples were drawn as follows. About five ml were collected from each sample and placed in a gel tube and left at room temperature for about 30 minutes. The gel tubes containing samples were centrifuge at 4000 RPM for 5 minutes and the serum sample was divided into three replicates in Eppendorf tubes, each part contains approximately 500 µl and stored in a deep freeze at -20 C°. The serum samples were used for evaluation cytokines (IL-4, IL-6, IL-13, and IL-17) by Enzyme-Linked-Immuno-Sorbent-Assay Technique (ELISA).

Inclusion and Exclusion Criteria

The current study included patients with COVID-19 hospitalized in Thi-Qar Health Directorate of both sexes up to the age of 70 years, with the exception of patients with chronic diseases and pregnant women, as well as those over 70 years old.

Statistical Analysis

The data of this study were statistically analysis by using the statistical package for social sciences version 26 SPSS, based on the use of an independent sample t-test to compare rates between patients and the control group, and ANOVA was used for mean comparison according to disease severity and body mass index. Also, person correlation was used to determine the relationship between cytokines, as well as the ROC test to provide predictive values for the level of cytokines according to body mass and disease severity.

■ RESULTS

Level of Cytokines in COVID-19 Patients and Control Group

The present study noted a high significant increase of level IL-6 and IL17 in COVID-19 patients than control group, in contrast the level of IL-4 and IL-13 were decreased

Table 1
Cytokines level in COVID-19 patients and control group

	Patients No. 120	Control No. 60	p. value
	Mean±SD		
IL-4, ng/l	9.92±3.65	11.7±3.29	<0.01**
IL-6, ng/l	75.3±25.4	29.3±7.90	<0.01**
IL-13, ng/l	16.2±71.7	32.7±10.9	<0.01**
IL-17, ng/l	23.2±3.76	15.1±4.66	<0.01**

significantly in COVID-19 patients than control group at p. value <0.05 as show in the Table 1.

Level of Cytokines in COVID-19 Patients According to Severity of Disease

The level of IL-4 was non-significantly increased in both sever and critical COVID-19 patients, also, the IL-17 was non-significantly increased both moderate and critical COVID-19 patients in the other hand the level of IL-6 was increase significant in sever COVID-19 patients than other severity categories, IL-13 increased significantly in sever COVID-19 patients, while decreased in critical patients at p. value <0.05 as Table 2.

Table 2
Cytokines level in COVID-19 patients according to severity of disease

	IL-4	IL-6	IL-13	IL-17
	Mean±SD			
Moderate No. 50	9.25±3.08	72.7±24.6[b]	16.4±5.20[b]	23.5±3.83
Sever No. 31	10.6±3.27	86.8±28.1[a]	19.9±5.37[a]	21.9±3.50
Critical No.39	10.6±3.62	71.7±22.9[b]	13.3±3.89[c]	23.5±3.72
P. value	0.109 Non-Sig	0.021[1, 2] 0.851[1, 3] 0.026[2, 3]	0.004[1, 2] 0.004[1, 3] < 0.001[2, 3]	0.181 Non-Sig

Level of Cytokines in COVID-19 Patients According to BMI

The level of IL-4 was significantly increased in normal weight of COVID-19 patients, while decreased significantly in overweight patients, the IL-6 was significantly increased in both overweight and obesity patients than normal weight, the IL-17 was increased significantly with increasing BMI, in the other hand the level of IL-13 was non-significant increase in overweight patients at p. value <0.05 as Table 3.

Table 3
Cytokines level in COVID-19 patients according to BMI

	IL-4	IL-6	IL-13	IL-17
	Mean±SD			
Normal Weight No. 50	13.5±2.17[a]	66.0±22.0[b]	15.0±4.98	21.7±2.88[b]
Overweight No. 31	6.42±1.62[c]	81.9±22.8[a]	17.3±5.77	23.9±3.52[a]
Obesity No.39	8.14±1.91[b]	81.9±28.0[a]	16.9±5.31	24.5±4.34[a]
P. value	<0.01[1, 2] <0.01[1, 3] <0.01[2, 3]	0.005[1, 2] 0.003[1, 3] 0.997[2, 3]	0.1 Non-Sig	0.008[1, 2] <0.001[1, 3] 0.008[2, 3]

Association between Cytokines in COVID-19 Patients

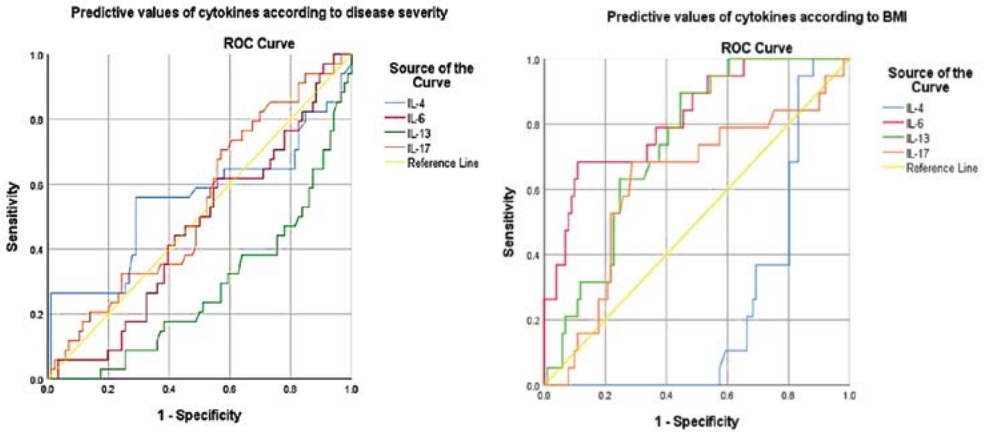
The IL-4 sored negative weak relationship with both IL-6 and IL-17, and very weak positive with IL-13, the IL-6 scored weak positive relationship with IL-13, IL-13 scored weak relationship with IL-17, as in Table 4.

Predictive value of Cytokines in COVID-19 Patients according to Disease Severity and BMI

The IL-6, IL-13 and IL-17 were scored a significant positive value for diagnosis the severity according to BMI, while depending on the severity of the disease, the values of IL-6 and IL-17 can be relied upon to predict the severity of disease as in Figure.

Table 4
Person correlation between cytokines

Person Correlation		IL-6	IL-13	IL-17
IL-4	r	–0.326**	0.195*	–0.353**
	p. value	0.000	0.033	0.000
IL-6	r		0.255**	0.081
	p. value		0.005	0.380
IL-13	r			–0.212*
	p. value			0.020



Cytokines	AUC by BMI	AUC by Severity
IL-4	0.242	0.550
IL-6	0.820	0.463
IL-13	0.738	0.275
IL-17	0.623	0.536

Figure. ROC Predictive value of cytokines according to severity of disease and BMI

■ DISCUSSION

The present study recorded the level of IL-4 decrease significantly in patients than control group, IL-4 not effected by disease severity, while according to BMI showed increase significantly in normal weight patients, furthermore noted the IL-4 has a negative correlation with IL-6, IL-17, while positive with IL-13 and IL-10.

IL-4 is a key interleukin in the Th2 immune response, and plays an important role as effector and inducer in the Th2 immunity. IL-4 and IL-13 are mostly related with inflammatory-fibrotic remodeling, in contras the Th1 cells exhibit anti-fibrotic activity by producing IFN-γ and IL-2 [20]. The Yuan et al. [21], results with same line with current result was showed a non-significant difference between regular group and both sever and critical groups. Also, the study of [22] Chang et al., showed the Level of IL-4 not effected by severity of disease, while elevated in patients in progress stage of disease

than control. In addition, the Queiroz et al., 2022, showed the IL-4 increased in mild and moderate patients than sever patient. On other hand this current study was opposed with study of Lu et al. [23], in China recorded the level was higher in patients than control, also this study with line of this study with regard correlation between IL-4 and IL-6. The reason for the difference between the two results may be due to the fact that the interleukin level is low at the beginning of infection within the first week of infection, but after two weeks of infection it rises by a large percentage to induce programmed cell death. It was also noted that intensive care patients have a high concentration compared to non-ICU patients. Also, the study of Motta Junior et al. [24], demonstrated the mast cells expressed high concentration of IL-4 in postmortem and alveolar septal-biopsies in patients with COVID-19. It is proposed that its high presence is strongly complicated in the pathophysiology of COVID-19 disease [25]. The high level of interleukin-4 in COVID-19 patients in the previous study compared to a decrease in the current study may be due to the time of sampling, as its decrease was observed at the beginning of the disease and its rise as the disease progressed, and the strength and period of mechanical ventilation and immobility for a long time cannot be ruled out and its effect on the immune level. The study of Luo et al.[26], they reached the same conclusions as the current study, where they found that there is no relationship between the level of interleukin-4 with the severity of the disease. The patients with COVID-19 have numerous clinical independent characteristics of circulating levels of inflammatory cytokines in peripheral blood including IL-2, IL-4, CRP, TNF-α and FN-γ [27]. Both Grifoni et al. [28] and Braun et al. [29], identified COVID-19 specific CD4+ memory T cells may reach 100% while CD8 + T cells reach about 70% of patients who recover from disease. Although acute disease is characterized by an elevated viral titer and dysregulated of prolonged innate inflammatory cytokine and lymphocyte chemokine responses, antibody-based therapies for dominant CD4+ TH2 cytokines such as IL-4, IL-5, and IL-13 do not appear to contribute to severity of COVID-19.

The patients had high IL-6 level than control, within patients' severity of disease increase in sever than moderate and critical, and scored high level in overweight and obesity patients than normal wight, in addition the IL-6 has negative correlation with IL-4, and positive with IL-13. In this study, evidence was providing that inflammation reflected by cytokine (IL-6 and IL-17) in patients who have exacerbated the disease due to the high concentrations of cytokines induced by the disease, the treatment for reduce lung damage associated with inflammation is critical. Importantly, our analysis of the predictive values of cytokines and other immunological markers is a proof of concept that these markers should be assessed preferentially for early diagnosis of patients, especially in patients with more severe disease, and to reduce the burden of care centers and hospitals.

Study of Han et al. [30], showed the IL-6 serum level was increased in COVID-19 patients than control, but disagree with regard severity was noted increased in critical than sever and moderate patients. Also, Lu et al. [23], showed the IL-6 considered the most accurate interleukin in expressing the severity and progression of the disease, was increased significantly in all patients. The Lau study was designed to study the immune response within 28 days of infection for the purpose of morbidity and mortality, while in the current study the samples collected during first week of infection. L-6 has a critical role in inducing the immune response during the acute illness phase, resulting in a variety of local and systemic alterations, including the activation and recruitment of leukocytes, an increase in body temperature, and hemodynamic consequences [31].

For reasons that are not clear, some individuals, particularly the elderly and diseased, may had an irregular immune system that fails to save the response to pathogens in check. This can cause an uncontrolled immune response, resulting in a high-production of immune cells and their signaling molecules, this can cause a cytokine storm often associated with a recruitment the immune cells to the lungs. SARS-CoV-1, and SARS-CoV-2 induce excessive, abnormal, and ineffective host immune responses associated with severe lung disease, leading to death [32]. Such as patients infected with SARS-CoV and MERS-CoV, some patients with COVID-19 progress to acute respiratory distress with characteristic vitreous and pulmonary changes on imaging. In greatest dying patients, the infection is also related with a cytokine-storm, which is characterized by highest level of variety of cytokines [33].

The current study agreed with the study of Lazar et al. [34] and Hashizume [35] where it was observed that obese COVID-19 patients had an elevated level of IL-6 compared to other patients and the control group, and their disease severity was higher than their peers. Adipose tissue facilitates macrophage infiltration and secretion of leptin as well as other inflammatory adipokines, including IL-6 and TNF-α [36]. The hyperglycemia and insulin resistance are associated with elevated IL-6 and CRP, IL-6 has been proposed as a marker capable of predicting the development of DM2. IL-6 is an important molecule produced from adipocyte and associated with obesity related cytokines Fedullo et al. [37]. It is known that 30% of the level of IL-6 in the body is secreted from adipocyte, and this explains that obese people mainly have high levels of C-reactive protein and a naturally high body temperature.

The study of Kesmez Can et al. [38], in line with current study, involved 60 patients and recorded the IL-6 increased in sever patients than both mild and moderate patients. Numerous studies have shown that in severe cases of SARS-CoV-2 infection activate the adaptive and innate immunity in the alveolar tissue, leading to release cytokine and then a syndrome associated with cytokines, indicating highest concentration of pro-inflammatory cytokines (IL-6, IL-1, IL-8, TNF-α and IFN-γ) as an important cause of death [39, 40]. It is possible to explain the negative

The level of IL-13 decreases significantly in patients than control group, within patients' severity of disease increase in sever and moderate than critical patients, while non-significant was scored according to BMI categories, also, IL-13 has positive correlation with IL-4 and IL-6. IL-13 is implicated in numerous adverse processes, including eosinophils and M2 macrophages recruitment to the lung, goblet cell metaplasia and mucus secretion in the airways, smooth muscle cells proliferation, fibrosis induce by activation of fibroblast and collagen deposition [41]. The study of Vaz de Paula et al. [42], were showed non-significant difference between COVID-19 patients than control, while decreased significantly when compared with Influenza infected patients. Like IL-4, the T2 pathway was activated by IL-13, because both interleukins share the same receptor (IL4Ra). Both interleukins induce activation of macrophage M2 [Sphingosine-1] by alternative pathway, which enhances the production of platelet-derived factor and TGF-β. This stage is characterized by transient expansion of resident fibroblasts, temporary matrix formation, proliferation of airway progenitor cells, and type II pulmonary cells [43, 44]. Donlan et al. [45], disagreed with present study was reported the IL-13 increase in patients than control group, in other hand was noted the level increase in patients with mechanical ventilation, also noted the level increased after week of symptoms onset. Also, Carapito et al. [46],

was revealed that the level of IL-13 was elevated in ICU COVID-19-patients than non-ICU patients when analyzing large-scale serogroups of young less than 50 of patients without comorbidities of varying severity of COVID-19 disease. The difference in the level of IL-13 and other interleukins between the current study and previous studies may be due to the fact that COVID-19 is more morbidity and mortality in the age 65 and older after long periods of mechanical ventilation, secondary infection during the recumbent period, so it is difficult to ignore the effect of age, mechanical ventilation duration, strength, and secondary infection on the levels of parameters involved in this study.

On other hand the study of Gibellini et al. [47], agrees with the current study to some extent, was noted the IL-13 was reduced in patients who with mechanical ventilation or died patients, also noted IL-13 driven positive regulation of profibrotic actions of TGF-b. This may be explaining the low level of anti-inflammatory cytokines in patients who are in a critical state of the disease. The TH2 cytokines, especially IL-13 and IL-4, also has a role in M2 macrophages differentiation, which in turn had a role in the developed of fibrosis of pulmonary through TGF-β secretion [42]. the study of Bonser et al. [48], were noted the patients suffer from type 2 asthma had protection against COVID-19 infection due to an elevated level of IL-13 which regulates the expression of a programed genes in mucus-producing goblet cell specially SPDEF, which in turn produce mucus like film, depending on the level of viral-RNA and RNA staining, their study showed that both mucosal gel and IL-13 stimulation reduced viral infectivity. Even when the mucosal layer had been removed before the infection, IL-13 effects could still be visible, while increasing their level during the second week of infection causes opposite effects such as M2 activation, goblet cell metaplasia, and mucus secretion in the airways [49]. Several mechanisms may explain who the IL-13 inhibition of COVID-19 infection. A study showed the IFN-γ-stimulates genes that mediate restriction infection of COVID-19, demonstrating how a single-cytokine induce a wide variety of antiviral pathways. and found that the changing gene expression induced by IL-13 was very different from those induced by IFN-γ, which suggests that these cytokines activate different antiviral pathways [50].

The level of IL-17 increases significantly in patients than control group, while non-significant difference was noted according disease-severity, according to BMI the level increased in both overweight and obesity than normal, furthermore the IL-17 has negative correlation with all anti-inflammatory IL-4, IL-13. Th17 cells were the chief source of interleukin 17, it stimulates IL-8 secretion of, which is a strong chemoattractant for neutrophils, Consequently, IL-17 plays a significant role in neutrophil-regulating. Neutrophil is significant in the host-defense against variety of pathogens. Importantly, neutrophils had been showed to play a main-role in several immune system conditions and acute lung damage [51]. The present study agreed with study of Ghazavi et al. [52], was noted a significant increase in patients than control, while noted the level increased in mild than sever patients, this result may be due to the small size of the sample, which may affect the statistical results, as explained in the same research. Also, Avdeev et al. [53], reported the COVID-19 patients had a high level of IL-17, also, use of anti-IL-17 therapy and noted improvement in clinical and laboratory parameters, although there was non-significant effect on clinical outcomes and mortality. In theory, IL-17 blockade appears to be a promising therapeutic area, as it can also reduce the production of pro-inflammatory mediators such as IL-6, IL-1β, and TNF, recruit neutrophils and prevent apoptosis, which ultimately leads to increased lung parenchyma damage. These events are a major factor

in the development of ARDS in COVID-19 [54,55]. Study of Pacha et al. [56], investigated inhibition IL-17 was approved as a common and positive strategy for dropping incidence related with autoimmune diseases such as psoriatic arthritis and psoriasis. Several studies reported high IL-17 production as a result of TH17 cells dysregulation in the synovial space, skin, and endothelium and other organs the enhances secretion of pro-inflammatory mediators such as IL-6, IL-1β, TNF and neutrophil and chemo-attractants such as IL-8, CCL2, CXCL10, and CCL20. The recruited neutrophils and production high level of interleukin-6 and reactive oxygen species, resulting in characteristic skin lesions and joint destruction [57, 58]. Based on the above findings, it may be the same IL-17 mechanism that causes diabetes, other chronic diseases and autoimmune diseases in COVID-19 patients during or after recovery from the disease. The imbalance of the cytokines examined was noted in the current study such as ratio of IL4/IL-17, was decreased in patients than control, supports the hypothesis that a shift of the immune system towards Th1 or Th17 than Tregs and Th2 may be responsible for the development of autoimmune diseases in future. IL-17 specifically enhances pro-inflammatory mediators, but not-antiviral, expression gene in cells infected by viruses by stimulating non-immune cells such as epithelial cells and fibroblasts to produce high levels of pro-inflammatory cytokines and chemokines in response to viral-infection attracting other of immune cells such as neutrophils, which can lead to increase morbidity, while at the same time remaining ineffective in preventing the spread of the pathogen [59].

■ CONCLUSION

Increased IL-6 was related to both hyperglycemia and insulin resistance, making it useful in predicting the progression of GDM. Where's an increase in interleukin-17 is associated with an increase in the production of IL-6, IL-1, and TNF and thus inhibits apoptosis, while IL-4 and IL-13 are mostly associated with fibrotic remodeling.

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