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The measurement of some cytokines can predict the severity of COVID-19 infection among different age groups

Karrar Sobhi Rahim

College of Medicine, University of AL-Qadisiya, Iraq

Corresponding author email: med.post50@qu.edu.iq

Buthainah Abbas Farhan

Professor. College of Medicine, University of AL-Qadisiya, Iraq

Email: buthainah.alazzawi@qu.edu.iq

Abstract---Coronavirus disease 2019 (COVID-19. Outbreak of severe acute respiratory syndrome (SARS-CoV-2)) started in Wuhan, the capital of Hubei Province, Republic of China, and has spread worldwide. Pandemic 11 March 2020 is closely related to the pangolin and the bat virus and is also believed to be of zoonotic origin. Symptoms include cough, muscle aches, sputum production, headache, hemoptysis, diarrhea, shortness of breath, and in some cases, acute respiratory distress syndrome (ARDS). Acute respiratory distress syndrome (ARDS), sudden heart damage, or secondary infection can happen to affected people. This study provides evidence that inflammation reflected by cytokine storms in COVID-19 patients could have contributed to disease exacerbations, so we analyzed the gene expression predictive values of (IL-6 and INF α). A cytokine storm can occur due to an infection, autoimmune condition, or other diseases. It may also occur after treatment with some types of immunotherapy. Symptoms include high fever, inflammation (redness and swelling), severe fatigue, and nausea. Sometimes, a cytokine storm may be severe or life-threatening and lead to multiple organ failures. Also called hyper cytokines. Recent studies on COVID-19 infected patients have shown that these individuals exhibit high levels of both pro-inflammatory cytokines, which include IFN- γ , IL-1 β , IL-6, IL-2, and chemokine's Results: Measurement of serum TNF- α and IL-6 concentrations in (pg/ml) on the 5th, 10th, and 15th day after diagnosis showed a significantly higher level ($P < 0.0001$) for the five COVID-related age subgroups without the chronic disease group compared to the incident control group. all the time. Compared with the control group, the increase in TNF- α was observed from the fifth day after diagnosis for both main groups (COVID with and without

chronic disease). This increase continued for the 10th day, after which the gradual decline in chronic non-pathogenic COVID began. In contrast to COVID with chronic diseases, the serum level of TNF- α continues to rise. For COVID without chronic disease group, the increase in IL-6 concentration was observed since the 5th day after diagnosis and the increase continued through the 10th and 15th days for all age groups, but with chronic diseases, the IL-6 level seemed to decrease on the 15th post-diagnosis day in All age groups. Conclusions: In conclusion, SARS-CoV-2 infection elicited an inflammatory response. It led to organ failures in COVID-19 patients even after relief from clinical symptoms and negative results from RT-PCR testing for virus RNA extracted from or pharyngeal swabs. Elevated TNF- α , and IL-6 have been positively associated with increased damage magnitude in COVID-19 patients. These data suggest that early infection intervention by controlling elevated IFN- γ and IL-1 β levels could be essential in preventing damage and comorbidities caused by this virus and improving functional recovery.

Keywords---cytokines, COVID-19 infection, different age.

Introductions

The outbreak of severe acute respiratory syndrome (SARS-CoV-2) began in Wuhan, the capital of Hubei Province in the Republic of China and spread to the whole world(1,2); the WHO Emergency Committee declared a global health emergency on 30 January 2020 and then it was declared a pandemic on the eleventh of March 2020(3). The virus is closely related to pangolin and bat corona virus; it is also thought to be of zoonotic origin(4). They were named coronaviruses (Latin: corona = crown) because of their shape as spherical viruses with a core-shell and surface projections resembling a solar corona (5). Coronaviruses are divided into four subfamilies: alpha, beta, gamma, and delta (6). While alpha and beta coronaviruses are thought to have originated in mammals, particularly bats, gamma and delta viruses are thought to have originated in pigs and birds (7). The genome size varies between 26 kb and 32 kb(8).

Symptoms of COVID-19 disease include fever, high temperature ($> 37.3^{\circ}\text{C}$), cough, muscle aches, sputum production, headache, hemoptysis, diarrhea, shortness of breath, and in some cases acute respiratory distress syndrome(9). sudden heart damage, or secondary infection can occur in affected people. These provide objective measurements as the disease progresses. Patients will be classified as moderate, severe, or critical in the future, allowing early intervention (7)(8), Severe acute respiratory syndrome (SARS) is caused by an airborne virus that spreads through small droplets of saliva, similar to the common cold or influenza virus. In addition, it can also be transmitted indirectly through contact with virus-infected surfaces (9).

SARS-CoV-2, like other coronaviruses, contains four structural proteins: S (spike), E (envelope), M (membrane), and N (nucleocapsid); the N protein encases

the RNA genome, while the S, E, and M proteins comprise the viral envelope(10). Coronavirus S proteins contain glycoproteins and type I membrane proteins (membranes containing a single trans membrane domain-oriented on the extracellular side). They are divided into two distinct portions that serve distinct purposes (S1 and S2)(11).

The mechanism by which COVID-19 infection is transmitted, the molecular mechanism through which the virus acts must be revealed. So far, researchers believe that coronaviruses can enter host cells in three ways: by receptor-mediated plasma membrane fusion, receptor-mediated cellular endocytosis, or antibody-dependent viral entry Both fusion and endothelium require the presence of receptor proteins on the surface of the host cell. The interactions between SARS-CoV-2 and its receptors have shed new light on how viruses spread, and set a framework for creating new approaches to prevention and clinical treatment. (12)

SARS-CoV-2 infection requires the presence of the host receptor ACE2, which is a type I integral protein that catalyzes the conversion of angiotensin I to nitric oxide. ACE2 is a membrane protein containing an amino acid chain consisting of 805 amino acids in humans(13). They migrate to the cell surface and successfully attach after transcription to their N-terminal signal peptide, effectively anchoring across the C-terminal trans membrane domain (13). When coronaviruses come into contact with the surface of host cells, the receptor-binding domain (RBD) binds to the ends of one of the lobes, initiating viral entry.(12)

The interaction between RBD and ACE2 is complex. Each ACE2 peptidase domain contains one RBD, with the magnifying loop component of the RBD crossing a single helix bridge-shaped to the ACE2 peptidase domain (14). There are three sets of connections between these two molecules. The RBD protein residues Q498, T500, and N501 create a hydrogen bond network with amino acid residues Y41, Q42, K353, and R357 of the ACE2 peptidase domain at the N terminus of this bridge-shaped structure(15). At the C-terminal of the single bridge-shaped helix, RBD Q474 forms a hydrogen bond with ACE2 Q24, while RBD F486 connects to ACE2 M82 via van der Waals forces. In addition, RBD protein residues, K417 and Y453 interact with residues D30 and H34 of the ACE2 protein, respectively(15).

Material and Method

Ninety patients participated in this study. They were selected from Al-Amal Center for the Corona epidemic, Al-Sadr Hospital and Al-Hakim General Hospital in Najaf from February 1, 2021 until August 2021. They were selected according to specific criteria for each group as shown below: - The first group included thirty healthy people who were not infected with COVID-19 and had no history of any chronic disease; Within this group, five other subgroups were formed based on their ages, including the first subgroup with 20-29 years of age, another subgroup with 30-39 years of age, 40-49, and 50-59, And in 60-69 posts.

The second main group included 30 participants with confirmed COVID-19 infection (diagnosed by a specialist based on PCR results), and again, no history

of any chronic disease. The age range was similar to the first group (i.e., five subgroups). The third main group included 30 patients with confirmed COVID-19 infection with a history of hypertension and/or type 2 diabetes (T2DM). Again, this group was divided into five subgroups based on age. where 5 ml of blood was taken from each participant on days 5, 10, and 15 after COVID-19 was confirmed; As for the control examination, only one blood sample was taken. Each sample was divided into two parts, 3 ml was centrifuged, the serum was isolated and stored at -20°C for further analysis. The second part, 2 ml, was kept inside an EDTA tube and stored at -80°C for use in genetic analysis. Exclusion criteria include cancerous disease, heart failure, abnormal liver, dialysis patients, patients aged less than 20 years or more than 70 years, pregnancy, and alcoholics. Then, enzyme-linked immunosorbent assay (ELISA) was measured. Serum cytokine levels (TNF- α and IL-6) next, quantitative real-time polymerase chain reaction (qRT-PCR) was used. The relative gene expression of cytokines (TNF- α and IL-6) was measured.

Statistical analysis

The results were analyzed using graph pad Prism 9.2.1 and presented as mean $\pm$ standard . deviation. T-test, ANOVA, correlation and ROC analysis were used to measure.

Results

Measurement of serum TNF- α concentration in (pg/ml) on the 5th, 10th, and 15th day after diagnosis showed a significantly higher level ($P < 0.0001$) for the five COVID-related age subgroups without the chronic disease group compared to the incident control throughout. A similar trend was found for COVID in the chronic disease group. The serum concentration of TNF- α in (pg/ml) was significantly higher in the patients than in the incident control group throughout the time

Then, COVID with and without chronic disease groups had a significantly higher ($P < 0.05$ - $P < 0.0001$) level of TNF- α concentration in the serum of COVID with chronic disease group compared to COVID without chronic disease group for all age groups and at all-time incident The following figure illustrates this (Figure 1).

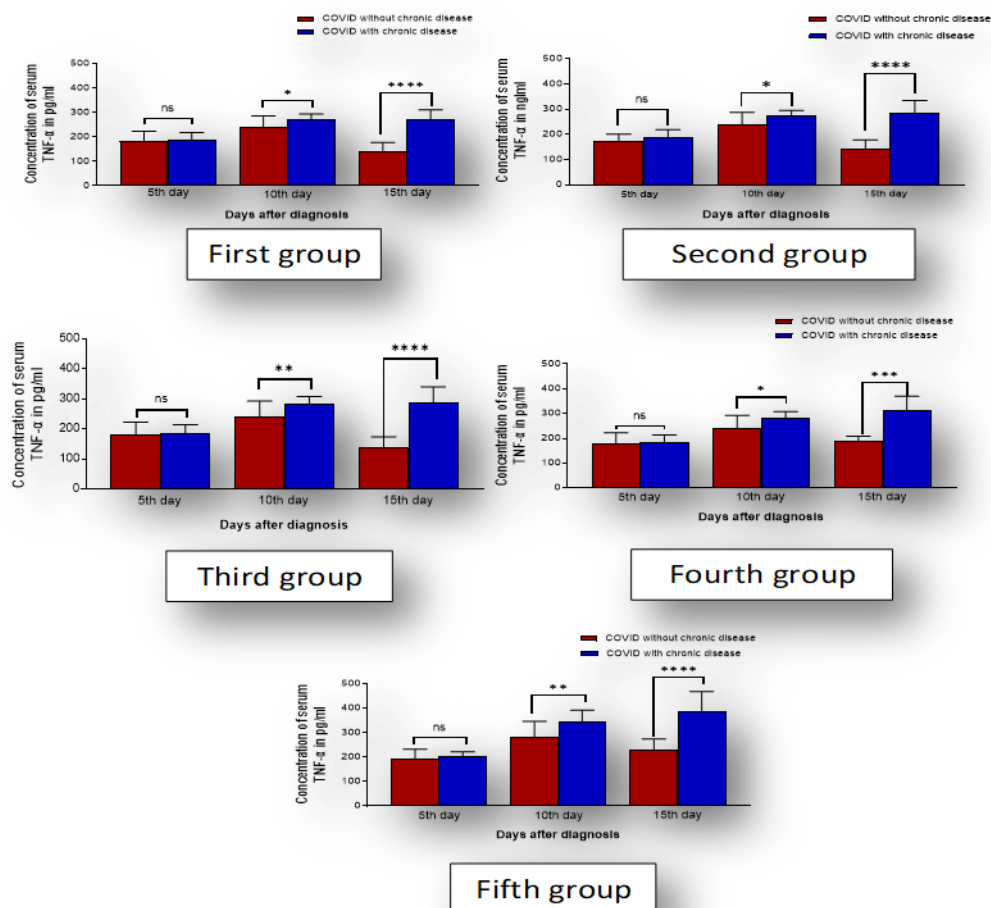


Figure 1 The measurement of TNF-α concentration (pg/ml) at the 5th, 10th, and 15th days after COVID-19 diagnosis. A significantly higher concentration of TNF-α ($P < 0.05$ - $P < 0.0001$) was found for COVID with chronic disease group as compared to COVID without chronic disease

Compared with the control group, the increase in TNF-α was observed from the fifth day after diagnosis for both main groups (COVID with and without chronic disease). This increase continued for the 10th day, after which a gradual decline of the chronic non-pathogenic COVID began. In contrast to COVID with chronic diseases, the serum level of TNF-α continues to rise, as shown in (Fig. 2).

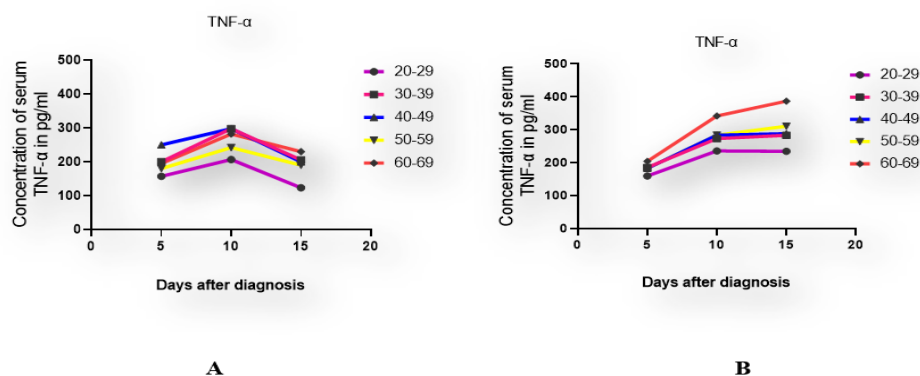


Figure 2 The pattern of serum TNF- α concentration increment through the 5th, 10th, and 15th day after diagnosis, (A) for COVID without chronic disease group, (B) for COVID with chronic disease group

TNF- α concentration was evaluated for all age subgroups on the 5th, 10th, and 15th days after diagnosis; the results showed no significant difference among the different age subgroups on the 5th and 10th days for COVID without chronic disease. While on the 15th day, a significant ($P < 0.01$ - $P < 0.001$) difference was found between the (60-69) age subgroup and the (40-49), (30-39), (20-29) age subgroup as shown in (Figure -8-). For COVID with chronic disease in the (60-69) age subgroups, the TNF- α concentration was significantly ($P < 0.05$ - $P < 0.0001$) higher than in other age subgroups.

Evaluation of serum IL-6 concentration

The measurement of serum IL-6 concentration (pg/ml) for both main groups follows a similar trend; it was significantly ($P < 0.0001$) higher than the control group at the all-time incident. The comparison between the two main groups (COVID without and with chronic disease) revealed that for the first (20-29) and second (30-39) age subgroups, no significant difference was found on the 5th and 15th day after diagnosis, the significance was found only on the 10th day, it was significantly ($P < 0.05$ - $P < 0.01$) higher in COVID with the chronic disease more than COVID without the chronic disease (Figure 3.14), while for the third (40-49) and the fourth (50-59) age subgroup the significance was found in the 10th and 15th day after diagnosis. However, a significant increase was found in the COVID without chronic disease on the 15th day after diagnosis, indicating that the increase observed on the 10th day in COVID with chronic disease group did not persist (Figure 3).

Subgroup; the IL-6 serum concentration was significantly ($P < 0.01$) higher in COVID with chronic disease on the 10th day after diagnosis. However, on the 15th day, the opposite was found; it was significantly ($P < 0.0001$) higher in COVID without than in COVID with chronic disease, as demonstrated in (Figure 3).

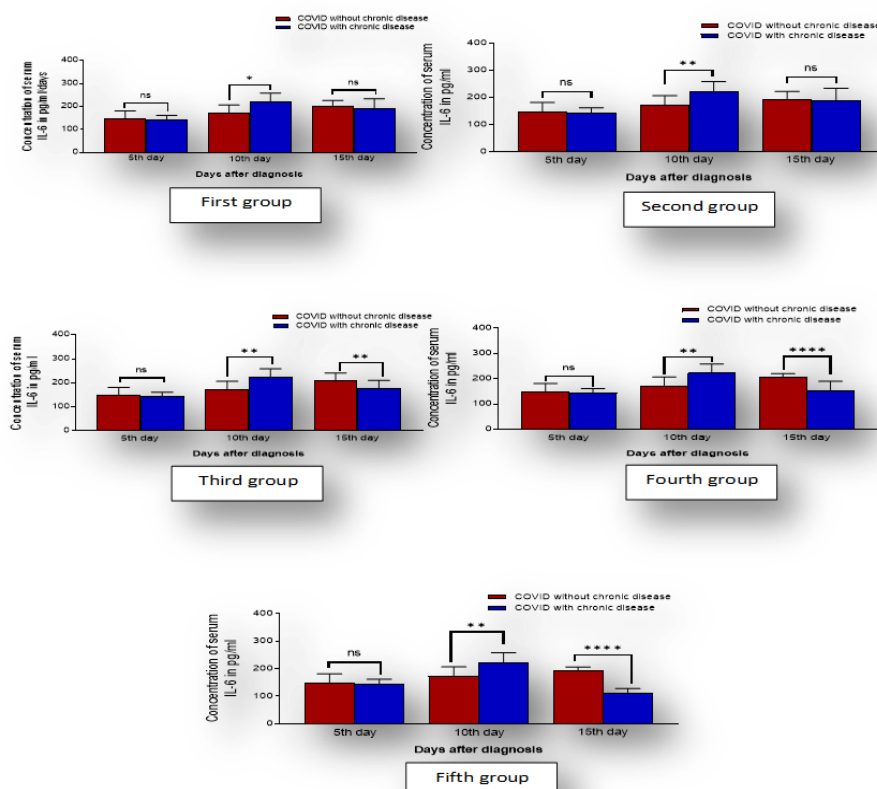


Figure 3 The measurement of IL-6 concentration (pg/ml) at the 5th, 10th, and 15th days after COVID-19 diagnosis showed a significant variation between the 10th and 15th days. There was a significant ($P<0.05$ - $P<0.01$) increase on the 10th day in COVID with the chronic disease more than in COVID without chronic disease, while on the 15th day, the increase ($P<0.0001$) was noticed in COVID without more than COVID with chronic disease

For COVID without chronic disease group, the increment in IL-6 concentration was observed since the 5th day after diagnosis and continued to increase through the 10th and 15th day for all age subgroups as shown in (Figure 4). A similar trend was found in the COVID with chronic disease. However, the level of IL-6 appeared to decline on the 15th day after diagnosis in all age subgroups, as shown in (Figure 4).

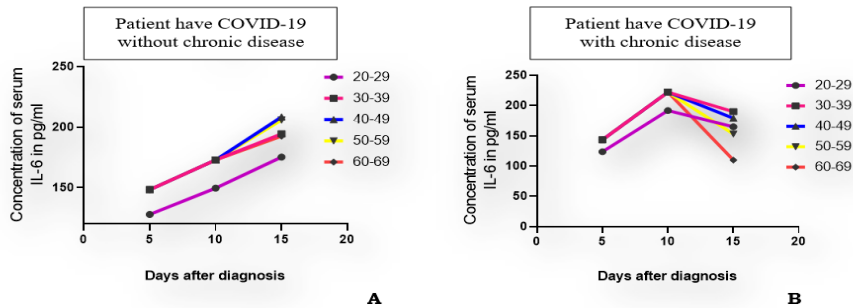


Figure 4 The pattern of serum IL-6 concentration increment through the 5th, 10th, and 15th day after diagnosis, (A) for COVID without chronic disease group, (B) for COVID with chronic disease group.

Looking closer at the five different age subgroups based on days' after diagnosis showed that there was no significant difference in the level of IL-6 in both main groups (COVID without and with chronic disease) at all-time incidents except for the (60-69) age subgroup, a significantly ($P<0.05$ - $P<0.01$) lower concentration was found on the 15th day after diagnosis as shown in (Figure 5).

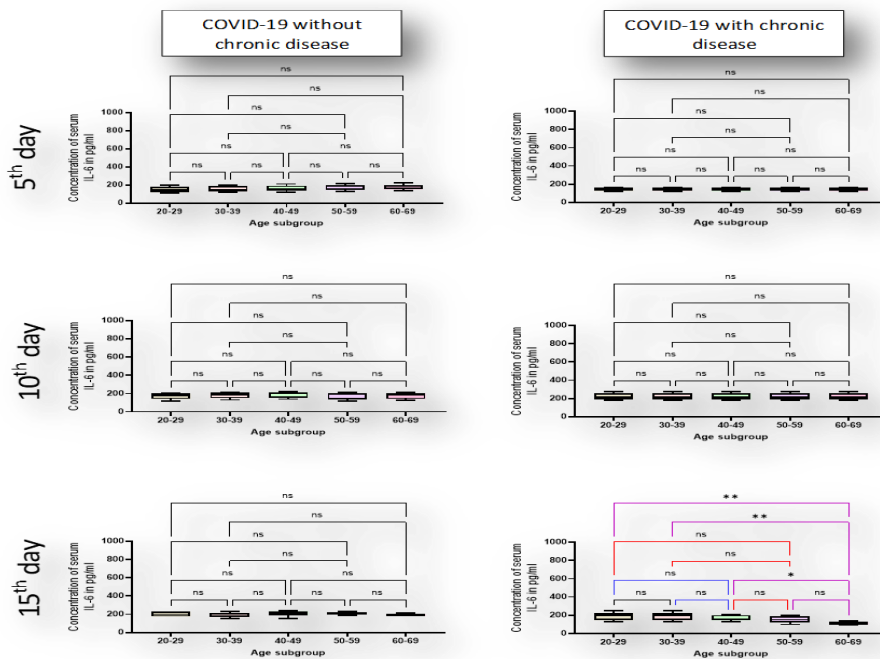


Figure 5. The measurement of IL-6 concentration (pg/ml) at the 5th, 10th, and 15th days after diagnosis. On the 15th day, a significant ($P<0.05$ - $P<0.01$) decrease was observed for the (60-69) age subgroups as compared to other subgroups for COVID with chronic disease.

Simple linear regression was done to evaluate the association of cytokine levels with the patient's age concerning the presence or absence of chronic disease. The results showed a positive correlation between age and (TNF- α) ($P < 0.05$ - $P < 0.001$) for both groups (COVID without and with chronic disease). For IL-6, a positive ($P < 0.001$) correlation was found in the COVID without chronic disease, while a negative correlation ($P < 0.05$) was found in COVID with chronic disease

Discussion

The COVID-19 pandemic has prompted worldwide investigations to describe the molecular mechanisms of cellular infection and inflammation by SARS-CoV-2(16). The release of TNF- α is one of the pro-inflammatory proteins resulting from the interaction between angiotensin-converting enzyme 2 (ACE2) and SARS-CoV-2(17). There is a hypothesis that TNF- α blockers play an essential role in preventing severe symptoms of COVID-19(18).

TNF- α mediates many biological activities by binding to its receptors TNFR1 or TNFR2. The level of TNF- α increases in response to pathogens, such as bacteria and viruses, and this pro-inflammatory cytokine is responsible for inflammation. (19) As mentioned previously, the release of TNF- α occurs during SARS-CoV-2 infection, which, together with the over-release of other pro-inflammatory cytokines, leads to a cellular storm (hyper cellularity)(20). According to previous studies, the rapid development of COVID-19, multiple organ failure and death will occur after a cellular storm in COVID-19 patients(21). An effective strategy to treat and rescue COVID-19 patients is to control the cytokine storm(22). It has also been reported that treating patients with immune-mediated inflammatory diseases with cytokine inhibitors can reduce the risk of SARS-CoV-2 infection(23). Therefore, if we can control the elevation of TNF- α , it may be a potential candidate for this treatment strategy(24). For example, it was reported that the severity of COVID-19 symptoms and the need for hospitalization were lower in patients with inflammatory bowel disease (IBD) who were receiving anti-TNF treatments(25).

In another study, no association was observed between TNF- α and increased severity of COVID-19 in patients with chronic diseases(26)(27) Furthermore, results obtained from the COVID-19 Global Rheumatology Alliance registry data indicated that TNF- α antagonists reduced the chance of COVID-19-induced hospitalization and that TNF inhibitors are not associated with COVID-19-induced death(28)(29). This contrasts with the results we obtained through our research, which showed a significant difference in TNF- α concentration between people with and without COVID-19. The 5th, 10th, and 15th days after diagnosis showed a significantly higher level ($P < 0.0001$) for the five COVID-related age subgroups compared to the incident control for all time. Because of that, they found that TNF- α production by circulating monocytes was sustained. All of their SARS-CoV-2-induced pneumonia patients who develop pneumonia show severe inflammatory responses, overproduction of pro-inflammatory cytokines by monocytes and lymphatic arrhythmia characterized by CD4 lymphocytopenia and thus B-cell lymphopenia.(30) Elevation of TNF- α can facilitate viral infection and organ damage ,(31) A similar trend was found for COVID in the chronic disease group. The serum TNF- α concentration in (pg/ml) was significantly higher in patients than in the incident control group.

Although TNF- α contributes to antibody production, the overexpression of TNF- α and the resulting cytokinesis, in addition to inducing sepsis, damage the lymphoid germinal centers and disrupt the production of antibodies produced by B cells. (32) As a result, it may be the main reason for the weak and transient antibody response in COVID-19(33). Moreover, suppose scientists can control the overexpression of TNF- α . It can reduce the level of other pro-inflammatory cytokines, including IL-6, in patients with COVID-19, where the effect of anti-inflammatory therapy is TNF- α on autosome regulation(34).

The results of previous studies demonstrated the positive effects of anti-TNF drugs on reducing COVID-19 severity and hospitalization rate(35). Moreover, our results indicated the effect of TNF- α in increasing the severity of COVID-19, especially in the elderly, as evaluation of TNF- α concentration for all age groups on the fifth, tenth and fifteenth day after diagnosis showed that there is no significant difference between different age groups on the 5th and 10th days of COVID without chronic disease. At the same time, there was a significant difference on the fifteenth day ($P < 0.01$ - $P < 0.001$) between the age group (60-69) and the age group (40-49), (30-39), (20-29). the group. For chronically ill COVID patients in age groups (60-69), the concentration of TNF- α was significantly higher ($P < 0.05$ - $P < 0.0001$) compared to other age groups, This is because immunosuppressive cytokines such as TNF and IL-1 β can reduce immune function.(36) Moreover, TNF- α stimulates the proliferation and differentiation of myeloid precursors into myeloid-derived inhibitory cells (MDSCs) after pSTAT3 activation(37) and the numbers of Myeloid-derived suppressor cells (MDSCs) are elevated in the blood of COVID-19 patients . (38)This provides a mechanism by which increased levels of TNF in serum of ICU patients may lead to more serious disease.

IL-6 is the first proof of concept that those markers should be preferentially evaluated for the early diagnosis of patients with more severe diseases, especially under the heavy burden of medical care(39,40). When conducting this study, we made a comparison between the control group and COVID-19 patient for both groups, where the measurement of serum IL-6 concentration (pg/ml) for both main groups (chronic and non-chronic) showed a similar trend, which was significantly higher ($p < 0.0001$) than the control group at all times, which is remarkable evidence of this, the IL-6 level increased in COVID-19 patients. We believe that the reason for elevated IL-6 among patients with COVID-19 and among healthy subjects is that IL-6 levels are associated with respiratory failure (PaO₂ and SpO₂), which is in line with recent studies, which show that SARS-CoV-2 activates innate immune responses. and adaptive, leading to the release of IL-6 and other cytokines, and increased permeability. Vascular and respiratory failure.(41) The fact that the affected lungs are the main source of IL-6 may explain the observed association between cytokine levels and oxygen need .(41)

IL-6, a secreted 25 kDa glycopeptide, consists of 184 amino acids (42)(43) IL-6 is a multidirectional cytokine that is part of the pro inflammatory cytokine family; it affects several immune and physiological processes (44), including the production of acute-phase proteins, such as C-reactive protein and ferritin, antigen-specific immune responses, inflammation, apoptosis, differentiation, hematopoiesis, and cellular metabolism,(45) IL-6 is mainly synthesized and secreted by monocytes

and macrophages; however, T and B cells, hepatocytes, fibroblasts, endothelial cells, mesangial cells, adipocytes and keratinocytes, in addition to several tumor cells(46). Several studies have reported elevated sera IL-6 levels in patients diagnosed with COVID-19 infection and determined that the sera IL-6 circulating levels were positively correlated with severity and mortality in COVID-19(47).

IL-6 also contributes to host defense against tissue damage and infection. However, exaggerated and excessive IL-6 synthesis in response to SARS-CoV-2 infection results in acute and severe systemic inflammatory responses(39)(43). After that, we made another comparison in terms of days, as the comparison between the two main groups (COVID not associated with chronic diseases) showed that no significant difference was found for the first age group (20-29) and the second (30-39) on the fifth and fifteenth day after diagnosis. Found on the tenth day only, it was significantly higher ($P < 0.05$ - $P < 0.01$) in COVID with chronic disease than in COVID without chronic disease, while on the third (40-49) and fourth (50-59) days, The age subgroup found significance on the tenth and fifteenth day after diagnosis. However, a significant increase in COVID without chronic disease was found on the 15th day after diagnosis, indicating that the increase observed on the 10th day in COVID with the chronic disease group did not persist. A similar trend was also found for the fifth age group (60-69), the serum IL-6 concentration was significantly higher ($p < 0.01$) in COVID with chronic disease on the 10th day after diagnosis. However, on the 15th day, the opposite was found; it was significantly higher ($P < 0.0001$) in COVID without COVID with chronic diseases.

After that, we made a comparison between days, noting the decrease of IL-6 in the age group (60-69), for COVID without chronic disease group, the increase in IL-6 concentration was observed from the fifth day after diagnosis, and the increase continued during the tenth and fifteenth days for all groups age. A similar trend is found in COVID with chronic diseases. However, the IL-6 level appears to decrease on the 15th day after diagnosis in all age subgroups. Another transmission showing the five different age groups based on days after diagnosis showed no significant difference in IL-6 level in both major groups (without COVID and with chronic disease) except for the (60-69) age subgroup. A significantly lower concentration ($P < 0.05$ - $P < 0.01$) was found on the fifteenth day after diagnosis, Despite this, IL-6 is not a distinct diagnostic marker for its elevation in covid-19 patients, and IL-6 cannot be considered as a prognostic marker for the disease

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