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SARS-CoV-2 Viral Load Has an Impact on Cytokine Storm in Severe COVID-19 Patients

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Abstract

Viral load is a topic of interest among researchers, especially to estimate COVID-19's severity. The current research is performed to demonstrate the correlation between the viral load of SARS-CoV-2 in hospitalized patients and cytokine storm progression. A case-control study has been conducted from October 2022 to June 2023. Nasopharyngeal samples were taken from 89 patients for the assessment of the SARS-CoV-2 viral load. The virus was detected and quantified by molecular tests employing real-time polymerase chain reaction (qPCR). Serological assays were also done for the determination of inflammatory cytokine levels (IL-6, IL-8, and IL-1β) in the blood and the estimation of some biomarkers of inflammation (C-reactive protein, D-dimer, and ferritin). Fifty random samples of severe COVID-19 and forty mild cases of COVID-19 and apparently healthy individuals as a control group were selected for this purpose. The multiplexed RT-PCR confirmed the positive samples by detecting the *ORF1ab* gene of the virus. Viral load results reveal a range from one thousand (10^3) to one million (10^6) copies/ml of the virus in each sample depending on Ct values (a final dynamic range of 3.00 to 6.289 log10 copies/ml and an average of 4.425 log10 copies/ml). The inflammatory cytokines (IL-6, IL-8, and IL-1β) and inflammatory biomarkers (CRP, D-dimer, and ferritin) were increased during the disease. The correlation between log10 viral load and cytokine storm markers was positive. The number of viral particles present may impact the cytokine storm progression and extend the duration hospital stay.

Keywords: SARS-CoV-2, Viral load, Covid-19, Cytokine storm, Interleukin, CRP.

الحمل الفيروسي لـ SARS-CoV-2 له تأثير على عاصفة السيتوكين في الاصابات الشديدة لمرض كوفيد-19

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الخلاصة

الحمل الفيروسي هو موضوع مثير للاهتمام بين الباحثين، وخاصة لتقدير شدة مرض كوفيد-19. يتم إجراء البحث الحالي لإثبات العلاقة بين الحمل الفيروسي لفيروس كورونا لدى المرضى في المستشفيات وتطور عاصفة السيتوكين. أجريت دراسة في الفترة ما بين أكتوبر 2022 و يونيو 2023. تم أخذ عينات من الانف والبلعوم من 89 مريضًا لتقييم الحمل الفيروسي لفيروس كورونا. تم الكشف عن الفيروس وقياسه

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من خلال الاختبارات الجزيئية باستخدام تفاعل البوليميراز المتسلسل في الوقت الفعلي (qPCR). كما تم إجراء اختبارات مصلية لتحديد مستويات السيتوكين الالتهابي IL−6 وIL−8 وIL−1β) في الدم وتقدير بعض المؤشرات الحيوية للالتهاب (اختبار البروتين المتفاعل واختباردي دايمر واختبار مخزون الحديد). تم اختيار خمسين عينة عشوائية من مرضى كوفيد−19 الشديد وأربعين فردًا سليمًا على ما يبدو كمجموعة تحكم لهذا الغرض.

أكد اختبار تفاعل البوليميراز المتسلسل العكسي المتعدد العينات الإيجابية من خلال الكشف عن جين *ORF1ab* للفيروس. تكشف نتائج الحمل الفيروسي عن نطاق من ألف (10^3) إلى مليون (10^6) نسخة / مل من الفيروس في كل عينة اعتمادًا على قيم Ct (نطاق ديناميكي نهائي من 3.00 إلى 6.289 نسخة / مل ومتوسط 4.425 نسخة / مل). زادت السيتوكينات الالتهابية (IL−6 و IL−8 و IL−1β) والعلامات الحيوية الالتهابية (اختبار البروتين المتفاعل واختباردي دايمر واختبار مخزون الحديد) أثناء المرض. كان الارتباط بين الحمل الفيروسي log10 وعلامات عاصفة السيتوكين إيجابيًا. قد يؤثر عدد الجسيمات الفيروسية الموجودة على بدء عاصفة السيتوكين، مما يزيد من احتمالية دخول المستشفى لدى مرضى كوفيد−19.

1. Introduction

In December 2019, a new type of coronavirus appeared in China. The virus causes pneumonia-like symptoms. In viral pneumonia patients, Chinese researchers discovered a new coronavirus type called SARS-CoV-2, formerly known as 2019-nCoV. In February 2020, WHO specified the disease as coronavirus disease 2019 (COVID-19) [1–3].

Some SARS-CoV-2 infections are severe and may progress into acute respiratory distress syndrome (ARDS), which eventually leads to death. The main reason for increasing the morbidity of COVID-19 due to organ failure and ARDS is the cytokine storm [4,5]. The cytokine storm was marked by an uncontrolled, massive yield of several pro-inflammatory markers, both locally and systemically. These markers, which include IL6, IL-1β, IL-17, and IL-8, have a prominent role in increasing the severity and the risk of morbidity [6–8].

Laboratory indicators and comorbidities have been suggested for risk categorization of the disease [9–12]. Growing research indicates that hyperinflammation traits, such as higher blood records of D-dimer, hyperferritinemia, and C-reactive protein (CRP), are associated with critically ill individuals. These results point to a potential critical role for a cytokine storm in the pathology of coronavirus disease 2019 [13].

For the purpose of directing antiviral therapy, infectious particles, and immunization, there is a need for accurate data for profiles of viral load. Viral load became a topic of interest among researchers, especially to estimate the severity of the disease [14].

The purpose of the current study is to demonstrate the interplay between SARS-CoV-2 viral load from hospitalized severe patients and cytokine storm progression. The presence of cytokine storm phenomena was determined by estimating the records of the main inflammatory interleukines (IL6, Il-8, and IL-1β) and the levels of biomarkers of inflammation (CRP, D-dimer, and ferritin) that lead to cytokine storms during severe COVID-19.

2. Materials and Methods

2.1 Patients and Sampling

A case-control study has been performed from October 2022 to June 2023. Severe SARS-CoV-2 cases were collected from Ibn Al-Khatteeb Hospital in Baghdad, Iraq. The study protocol and proposal were approved by the Iraqi Ministry of Health and Environment and the University of Baghdad's Ethical Committee (reference: CSEC/0922/0083). For every participant, written informed permission was acquired. The World Medical Association's (Declaration of Helsinki) Code of Ethics was followed during conducting the study. The current study included 89 severe cases of COVID-19 taken from the hospital for the assessment of viral load. According to the WHO classification, severe cases are patients who

require oxygen therapy but are not in need of mechanical ventilation and suffering from hypoxemia. Nasopharyngeal swabs were collected for this purpose. Swabs were repeated and agitated to confirm the presence of epithelial cells in the sample. Then, a centrifuge was applied to the samples at 5000 g/min for five minutes for virus detection and, eventually, viral load estimation. Fifty random cases of severe COVID-19 were selected to perform serological tests, and 40 apparently healthy individuals as well as mild cases of COVID-19 were also included as a control group. Five milliliters of blood were drawn from severe coronavirus patients and controls to determine the records of IL-6, IL-8, and IL-1β by ELISA. The inflammatory biomarkers (CRP, D-dimer, and ferritin) were also determined in the blood by routine laboratory methods**.**

2.2 Molecular Assay

After sample collection, a purification kit of nucleic acid called ReliaPrep by Promega was used to extract and purify RNA from nasopharyngeal swab specimens. Proteinase K solution (20 µl) and cell lysis buffer (200 µl) were added to the samples, respectively. Then, 250µl of 100% isopropanol was added and placed in a vortex for ten seconds. Then, it was transferred to the ReliaPrep binding column. The elution step required adding to the column 60 µl of nuclease-free water, then centrifugation for 1 minute at 1200 rpm. After the extraction of RNA, the concentration of RNA was determined using a quantum fluorometer device. The Sacace SARS-COV-2 Real-TM kit was used to detect the *ORF1ab* gene of the virus by reverse transcription in one step and PCR amplification in the JOE/HEX/YELLOW channel. Internal Control-IC (RNA extraction control) detected on the FAM/GREEN channel. For each sample, 15 µl of RT-PCR buffer and 0.5 µl of enzymes Taq/RT were multiplied by the number of samples. After vortexing for 3-5 seconds, 15 µl of reaction mix was added to each PCR tube along with 10 µl of the extracted RNA. The plate was put in the SaCycler-96TM real-time PCR thermal cycler with the following temperature profile: RT step activation for five minutes at 50 ˚C. Five minutes of initial denaturation at 95 ˚C were followed by 50 cycles of ten seconds at 95 ˚C, ten seconds at 58 ˚C, and twenty seconds at 72 ˚C for the hold stage.

2.3 SARS-Cov-2 Viral Load Determination

When the duration period of the real-time PCR thermal cycler is achieved, the cycle threshold (Ct) records of all the severe positive cases are obtained. The direct conversion of a cycle threshold (Ct) value to an exact viral load (in terms of viral RNA copies per millilitre) is performed by making serial dilutions of positive control RNA concentrations. The *Ct* values of all positive controls were collected and plotted with the log10 viral load provided in order to determine the standard curve for estimation of the log10 viral load of all the samples tested in the current study (Figure 1). The present determination method of viral load (copies/ml) was calculated according to Hill *et al.* [15].

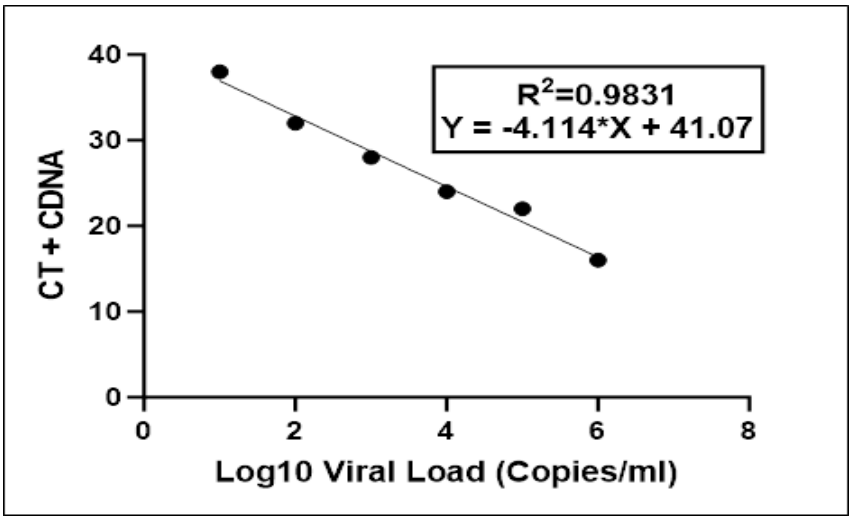

Figure 1: The standard curve plotted between Ct values of positive control of SARS-CoV-2 and Log10 viral load (copies/ml)

2.4 Serological Assays

Five ml of blood was drawn from 50 out of 89 patients with severe COVID-19 and 40 control healthy individuals as well as mild cases of COVID-19. The serum was obtained from the samples after centrifugation (1200 rpm) to perform serological tests. The serology part of the current study includes the determination of IL-6, IL-8, and IL-1β levels in the severe cases group and control group. Tests were performed using the Human Interleukin-6 ELISA kit called BT LAB Bioassay Technology Laboratory, China, the Human Interleukin-8 (IL-8), and the Human Interleukin-1β (IL-1β) ELISA kit (YL Biont, Shanghai). A sandwich ELISA kit was used for the accurate quantitative detection of human interleukins in serum. Obligations listed by the manufacturers were applied, and the ELISA test was performed.

CRP records in blood were determined using the hs-CRP+CRP Fast Test Kit, China which is dependent on the immunofluorescence assay. This test can be done with the aid of the Getein 1100 immunofluorescence quantitative analyzer. It is used for the estimation of CRP in serum, plasma, and blood [16]. The D-dimer level is also calculated. D-dimer Exclusion II (DEX2) is an automated quantitative test designed to be used in the VIDAS family of instruments for the immune-enzymatic detection of products resulting from fibrin degradation in human plasma [53, 54]. By using Roche/Hitachi Cobas C 311 equipment, the Tina-quant ferritin Gen. 4 kit, ROCHE, Switzerland is used for quantitative measurements of ferritin in human serum [17].

2.5 Statistical analysis

Raw data sheets from the present study were input into the computer and analyzed using the SPSS program, version 23. It contains the T-test, mean, standard deviation, standard error, and other well-known statistical characteristics. The significance of the differences depends on whether they are equal or less than 0.05. Less than 0.01 was considered highly significant. Also, the correlation coefficient (r) was determined among the studied parameters.

3. Results

3.1 Viral Load Estimation

After getting Ct values from the real-time PCR thermal cycler device, the viral load was estimated for 89 severe COVID-19 samples according to the standard curve (Figure 2). Results of the real-time PCR quantitative assay demonstrated a SARS-CoV-2 final dynamic range of 3.00 to 6.289 log10 copies/ml and an average of 4.425 log10 copies/ml. of 60 µl of

eluted RNA. The multiplexed RT-PCR confirmed the positive samples, depending on the detection of the *ORF1ab* gene. The Ct was plotted against the viral load to create the correlation matrix. Pearson (r) of the positive *ORF1ab* gene were significantly (p = 0.0001) different compared to viral load, and there was a negative correlation (r = - 0.8390) detected significantly between the Ct of the *ORF1ab* gene of the virus and viral load. Log 10 viral load results are divided into three categories (< 4 copies/ml, 4–5 copies/ml, and > 5 copies/ml). In terms of sex, non-significant differences between males and females viral load results (P > 0.05) were observed, as shown in Table 1.

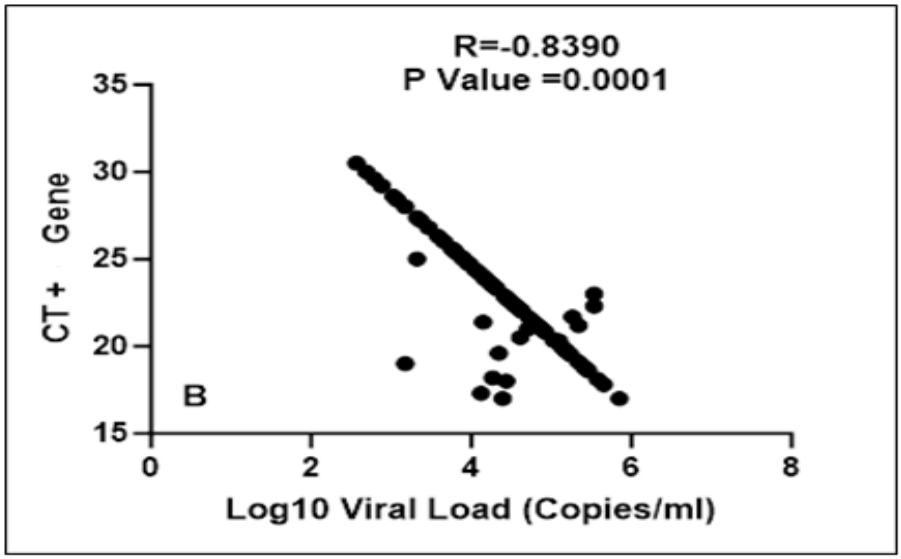

Figure 2: Standard curve plotted between Ct values of positive *ORF1ab* gene and Log10 viral load of SARS-CoV-2

Table 1: Distribution of males and females (sex) of the studied patients

Log10 Viral Load (Copies/ml)		Sex		P - value
		Males	Females	
< 4	N	17	6	P = 0.215
	%	32.7%	16.2%	
4 - 5	N	23	20	
	%	44.2%	54.1%	
> 5	N	12	11	
	%	23.1%	29.7%	
Total	N	52	37	
	%	100%	100%	

The studied sample information included the age of severely hospitalized patients with COVID-19. Samples categorized the ages into three groups (20–40 years old group, 41–60 years old group, and >60 years old group). The P value was determined for the age groups, and the result was no significant differences between the age groups included in the current study (P > 0.05) (Table 2).

Table 2: Distribution of COVID-19 patients age groups according to viral load calculations presented as Log 10 (copies/ml)

Log10 Viral Load (Copies/ml)		Age groups / Year			P - value
		20 – 40	41 - 60	> 60	
< 4	N	15	5	3	P = 0.083
	%	29.4%	14.7%	75.0%	
4 - 5	N	24	19	0	
	%	47.1%	55.9%	0.0%	
> 5	N	12	10	1	
	%	23.5%	29.4%	25.0%	
Total	N	51	34	4	
	%	100%	100%	100%	

3.2 Determination of pro-inflammatory cytokines

An enzyme-linked immunosorbent assay (ELISA) was applied to 90 randomly selected serum samples present in our study. These included fifty severe COVID-19 group and forty individuals from the control group of mild COVID-19 and healthy individuals. Results of serum levels show that interleukin-6 was expressed in severe COVID-19 patients higher than the control group. The mean of interleukin-6 serum levels in severe COVID-19 patients was 34.6 (ng/L), while the mean of the control group was 4.6 (ng/L). Results from the ELISA technique showed highly significant differences between the interleukine-6 levels of severe COVID-19 patients and the control group ($P<0.01$). Results of serum levels show that IL-1β was expressed in severe COVID-19 patients higher than the control group. The mean of severe patients was 65.8 (pg/ml), while the mean of control serum levels was 20.2 (Pg/ml). Results from the ELISA technique showed highly significant differences between the interleukin-1β levels of severe COVID-19 patients and the control group ($P<0.01$). Also, results of serum levels show that interleukin-8 was expressed in severe COVID-19 patients higher than in the control group. The mean of severe patients' levels was 1.7 Pg/ml, while the mean of control serum levels was 0.5 Pg/ml. Results from the ELISA technique showed highly significant differences between the interleukin-8 levels of severe COVID-19 patients and the control group ($P<0.01$) (Figure 3).

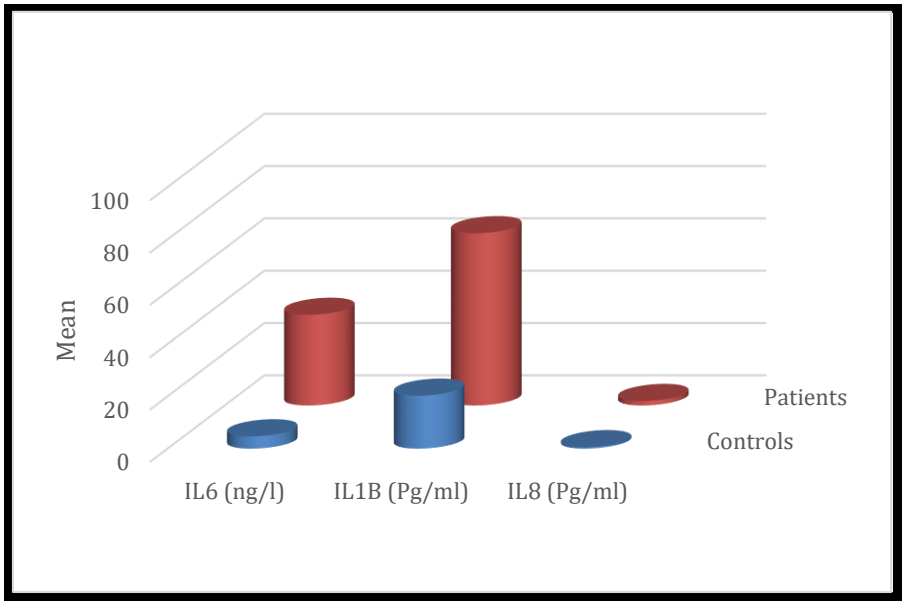

Figure 3: Interleukins' levels in severe COVID-19 patients and control group

3.3 Inflammatory biomarkers (CRP، D-dimer and ferritin)

Inflammatory biomarkers (CRP, ferritin, and D-dimer) were estimated in the current investigation. The number of individuals tested for each biomarker was 50/89 severe coronavirus disease-2019 patients and 40 control persons. The mean of serum ferritin level in severe patients was 342.6 (ng/ml), while in the control group, it was 108.3 (ng/ml). The mean CRP of COVID-19 patients was 41.6 (ng/ml)., while the mild and healthy control group was 5.0 (ng/ml). D-dimer levels also show higher records in severe patients (mean = 1131.0) (ng/ml) than in the control group (mean = 210) (ng/ml). Highly significant differences between patients and the control group were reported ($P<0.01$) for the three tested inflammatory parameters (Figure 4).

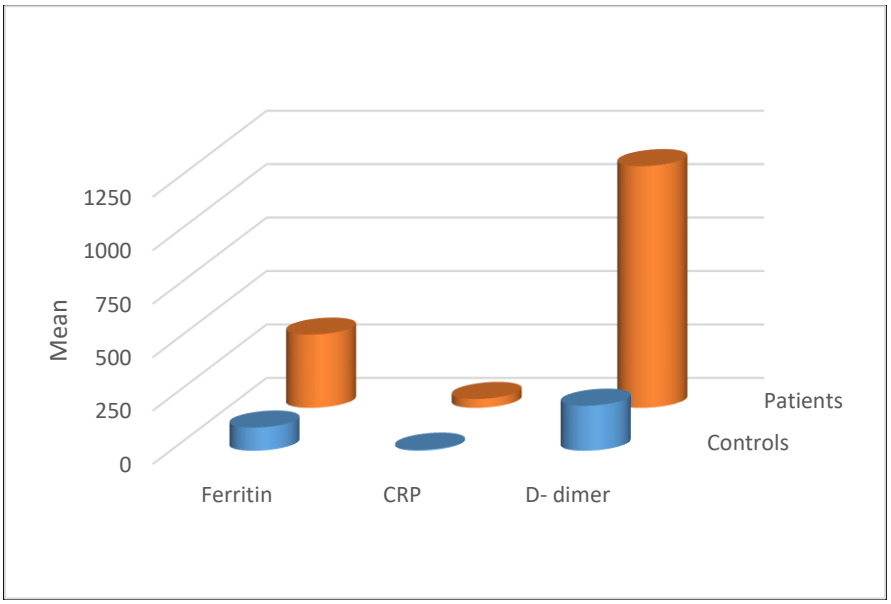

Figure 4: Inflammatory biomarkers distributions in COVID-19 patients and control groups

3.4 The correlation between log10 viral load and cytokine storm parameters

Pearson correlation (r) was calculated to reveal the relation between the log10 viral load of the virus and pro-inflammatory cytokines levels in severe COVID-19 patients. A positive correlation was revealed between the SARS-CoV-2 log10 viral load (copies/ml) and each of the Il-6, Il-8, and IL-1β levels. Differences were highly significant ($p<0.001$) between the log10 viral load and each pro-inflammatory cytokine included in the current study, according to Pearson correlation (r) (Table 4). Pearson correlation was also determined between log10 viral load presented as copies/ml and some inflammatory biomarkers that were estimated in the serum of severe cases and controls. The parameters include D-dimer, CRP, and ferritin. The findings showed a positive relationship between the viral load and serum ferritin levels, as well as a favorable relationship between the viral load and CRP. In contrast, D-dimer showed a negative correlation with log10 viral load. All the correlations are non-significant ($p < 0.001$). In addition, IL-6 and IL-1β demonstrated a strong positive association. IL-6 and the chemokine IL-8 presented a positive correlation (Table 4).

Table 4: Correlation study among log 10 SARS-CoV-2 viral load, pro-infammatroy cytokines and inflammatory biomarkers in severe COVID-19 patients

Pearson Correlation		Viral Load (Copies /ml)	IL-6 (ng/ml)	IL-8 (Pg/ml)
IL-6 (ng/ml)	r	0.903		
	P – Value	0.00		
	Significance	HS		
IL-8 (Pg/ml)	r	0.531	0.510	
	P – Value	0.00	0.00	
	Significance	HS	HS	
IL-1β (Pg/ml)	r	0.674	0.733	0.413
	P – Value	0.00	0.00	0.003
	Sign.	HS	HS	NS
CRP (ng/ml)	r	0.019		
	P – Value	0.862		
	Significance	NS		
D-Dimer (ng/ml)	r	-.040		
	P – Value	.782		
	Significance	NS		
Ferritin (ng/ml)	r	0.977		
	P – Value	0.593		
	Significance	NS		

***(ng/ml)=** nanogram per millilitre, **NS**= Non-significant, **HS**= highly significant, (Pg/ml)= Picograms/millilitre.

3. Discussion

The meshing between viral load (the number of particles of SARS-CoV-2 found in an affected person) and cytokines storm is not yet fully understood. The present study estimated the viral load from nasopharyngeal swabs of hospitalized severe cases of the disease. The relationship between all pro-inflammatory cytokines and viral load studied was positive. The result revealed that viral load is positively associated with cytokine storm.

In the current study, the log10 viral load of SARS-CoV-2 was estimated in severe cases of the disease. The specimens were nasopharyngeal swabs. Results reveal a range from one thousand (10^3) to one million (10^6) copies/mL. During the peak of infection, a study confirmed that an infected person can hold from 1 billion (10^9) to 100 billion (10^{11}) virions. The results of qPCR range from one thousand (1,000) to one hundred thousand (100,000) viral copies per millilitre to progress to a viable infection of coronavirus disease in 2019 [18]. Dealing with age and sex, the results of log10 viral load for SARS-CoV-2 revealed no differences between males and females and demonstrated non-significant differences among all age groups. A study dealing with SARS-CoV-2 found that the viral load in females is higher compared to males [19]. However, the age groups statistically show no significant differences. This result is compatible with our findings. Viral load quantification is an important step in providing physicians with information on treatment responses and informing infection control strategies and policies for diseases having epidemiological implications. Regarding the measurement of SARS-CoV-2, many challenges should be taken into consideration, including specimen collection mistakes, inadequate reference materials, unknown virus variants, and states *in vivo* that can affect the quantification [20].

Cytokine storm have been widely gained interest by researchers and the media. It means an uncontrolled generalized inflammatory response and huge activation of the immune system [21, 22]. Interleukins stimulate epithelial cells, initiate the acute phase of signalling, direct the migration of cellular immunity to the precise location of the infection, and regulate the creation of several other cytokines [23]. Among these cytokines, IL-6 merits a longer explanation because of its part in the storm of cytokines caused by the coronavirus. In the present investigation, the IL-6 mean records of hospitalized cases were eightfold higher than in normal healthy individuals. It essentially contributes to acute inflammation due to its

impact on mediating acute phase immunity [23]. It has implications for the pathology and physiology of the cytokine storm because of its pleiotropic features. A number of investigations have shown that coronavirus disease patients have higher blood products of IL-6 and that the degree of the illness is directly correlated with these levels [24-26]. For this reason, elevated blood IL-6 records have been proposed as a marker of illness severity [27]. In fact, suppression of the IL-6 transcription factor and, therefore, decreased generation of the virus were linked to lower mortality in animal experiments related to SARS-CoV infection [28].

The present study demonstrated that chemokines (IL-8 or CXCL8) increased in hospitalized cases of COVID-19 patients. This result is compatible with an article suggesting that IL-8 is a potential prognostic marker for ARDS [29]. Dealing with ARDS, the patients showed that the bronchoalveolar lavage fluid and plasma both had increased levels of IL-8. [30].
Also, one of the essential features of cytokine storm and severe COVID-19 cases is the massive production of IL-1β (inflammatory cytokine). Our study suggested that IL-1β was produced in critical cases of COVID-19 three times more than in normal control individuals. This result is compatible with a study that proposed that the pro-inflammatory cytokines IL-1β and IL-6 increased during severe COVID-19 illness and suggested that these cytokines be considered as indicators of illness progression and cytokine storm risk [31]. In 2020, Liao M. *et al.* suggested that critical COVID-19 illness had massively higher amounts of inflammatory cytokines than mild cases of the disease, especially IL-8, IL-6, and IL-1β [32].
An inflammatory response that progressed during COVID-19 not only included the elevation of cytokines but was also synchronized with increasing the production of many other biomarkers. Some of these biomarkers include CRP, D-dimer, and ferritin levels in the blood. Present research also determined the records of these markers in the blood. The amounts of CRP, D-dimer, and ferritin were estimated in patients and healthy control groups. The results revealed highly significant differences between the patients and controls for each CRP, D-dimer, and ferritin. The present findings agree with another study conducted in Iraq as well. They found that the products of IL-6, ferritin, and CRP could indicate risky complications of COVID-19 [33]. Therefore, the majority of COVID-19 hospitalized patients had raised levels of pro-inflammatory cytokines (IL-6 and IL-1β) and the chemokine (IL-8), and elevated inflammatory biomarkers (CRP, D-dimer, and ferritin) [34]. The increased production of these parameters could progress to cytokine storm and increased severity.

This complicated relationship could be explained by angII-related signaling (ACE2). ACE2 is predominant in lung epithelia and small intestinal enterocytes. It is considered the receptor that lets SARS-CoV-2 enter the lung [35]. Through its glycoprotein spike, the virus binds to ACE2. Once attached, the spike protein is cleaved by a co-receptor called transmembrane protease, serine 2 (TMPRSS2), or a related protease enzyme of the host, causing membrane fusion and endocytosis [36]. Following entry into the host cell, the virus multiplies immediately, which can be linked to pyroptosis, cell death, and the release of chemokines and proinflammatory cytokines [37]. The virus spreads from cell to cell and generates high viral particles by taking advantage of the elevation of ACE2 in the respiratory environment (such as the initial phases of an innate immune response) [38]. Conversely, the virus downregulates ACE 2 in the latter stages of replication [39,40]. Actually, ACE2 suppresses the synthesis of angiotensin II (AngII) and acts as a negative regulator of the renin-angiotensin system. [41]. So, when ACE2 is downregulated, Ang II will be activated, and when Ang II is activated, the signaling molecule NF-κB is activated as well, which increases the synthesis of pro-inflammatory cytokines, chemokines, and inflammatory molecules secreted during the infection and progresses to a cytokine storm [42]. One of these

pro-inflammatory cytokines that are elevated during coronavirus disease 2019 is IL-6 [43]. Ferritin and CRP levels in the blood are elevated, and this is mostly due to IL-6. Hepatocytes' receptor for IL-6 binds to it and activates intracellular signaling pathways, which induce the expression of the CRP gene [44]. In addition, IL-6 inhibits the action of TNF-α (tumor necrosis factor-alpha). This inhibition induces ferritin protein production. IL-6 can induce ferritin production, affecting iron homeostasis during inflammation [45] (Figure 5). The limitation of the present study was defined by the low number of severe COVID-19 cases in the hospital since the pandemic was over and the severe cases were lessened.

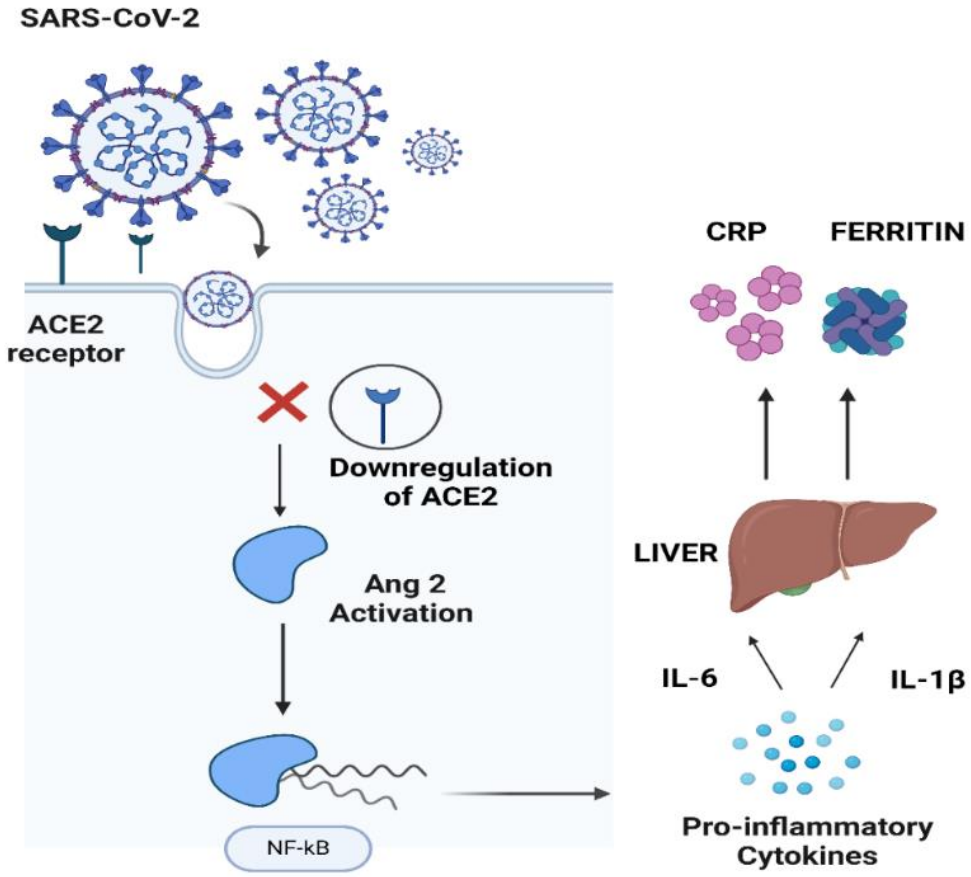

Figure 5: SARS-CoV-2 increased viral load and the generation of cytokines that promote inflammation. The elevated viral load of the virus leads to downregulation of the ACE2 receptor, which in turn results in increased synthesis of angiotensin II (AngII) and hyperactivation of NF-kB. The activation of NF-kB results in enormous production of pro-inflammatory cytokines in severe COVID-19 infection. IL-6 and IL-1β trigger, directly and indirectly the hepatocytes to produce CRP and ferrtin. All the presented pro-inflammatory cytokines and biomarkers prominent in a cytokine storm. This figure was created by www. Biorender.com

4. Conclusion

The present study demonstrates the impact of the SARS-CoV-2 viral load on cytokine storms in severe cases of coronavirus disease in 2019. Increased levels of pro-inflammatory cytokines and some inflammatory biomarkers are indicative of the cytokine storm that are tested in severe COVID-19 patients. The findings showed a substantial correlation between all examined pro-inflammatory cytokines and chemokines (IL-6, IL-1β, and IL-8) and the

log10 viral load of SARS-CoV-2. In addition, inflammatory biomarkers (CRP, ferritin) levels in the blood were determined and positively correlated with the viral load. In contrast, D-dimer showed a negative correlation with the viral load. All the pro-inflammatory cytokines and chemokines and all the studied inflammatory biomarkers are significantly expressed in severe cases of COVID-19. Further studies need to be conducted in the field of viral load and its impact on the severity of coronavirus disease-2019.

Conflict of interest: None.

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