Impact of SARS-CoV-2 on Hepcidin and others Inflammatory Markers in Baghdad

Luma A. Yasir[1], Aseel M. Hameed[2], Zaizafoon Nabeel Nasif[3]; Najlaa Qassim Muftin[4]
[1]College of medicine. Mustansiriyah University/Iraq
[2]National center of hematology, Mustansiriyah University/Iraq
[3, 4]College of science, Mustansiriyah University/Iraq
Email: luma.amer77@gmail.com

Abstract

Coronavirus disease 2019 (COVID-19) is a systemic inflammatory disorder affects both hematopoietic system and haemostasis process. The better understanding the natural history of COVID-19 would open the fields of treatment and prevention of this disease. COVID-19 patients suffer a mild to severe failing in the respiratory system after short period upon infection. Our study is focused on the early prediction of inflammatory status and respiratory impairment through the usage of inflammatory markers in COVID-19 patients. The current clinical study has included 60 COVID-19 patients and the results were controlled with 30 healthy people. The parameters of this case-control study was included white blood cells (WBCs), lymphocytes percentage (LYM %), D-dimer, interleukine-6 (IL-6), ferritin, hepcidin, vitamin D3 (Vit D), prothrombin time, and INR. The study was also included the IgG and IgM antibodies detection of COVID-19, and the other variables were statistically compared according to the quartiles of these antibodies, which enables a better elucidation for each parameter from onset of the disease up to the severe conditions, then down to the recovery. The predominant decrease was observed significantly in Vit D and non-significantly in LYM%. Areas under (AUC) the receiver operating characteristic (ROC) curve of WBCs, D-dimer, IL-6, Ferritin, PT, INR, Hepcidin, Vit D, LYM% and PTT had been analyzed. ROC analysis identified that Hepcidin and D-dimer has the excellent sensitivity and specificity (100%) with AUC value =1; cut off values were (72.28 and 53.37, respectively). Results appeared that there was a significant variation in the levels of D-dimer, IL-6, Hepcidin in four divided groups depend on IgM classification, on other direction, IgM shows a significant variation between four groups depend on IgG classification with non-significant changes in the other parameters. This study also confirmed that IgG titers in severe COVID-19 patients were significantly higher than those in non-severe patient's post-symptom onset and showed that Q2 group according to IgG was the worst case among other groups depend on hematological and immunological tests, whereas the Q4 group was the worst according to IgM. Pearson correlation coefficient (r) revealed the following results: there was a significant negative correlation between LYM% and WBC (r=-0.413), Hepcidin and VitD (r=-0.387), IL-6 and LYM% (r=-0.419), PT and LYM% (r=-0.465), INR and LYM% (r=-0.458), as well as, a significant positive correlation between IL-6 and INR (r=0.433), PT and INR (r=0.957), PT and PTT (r=0.623), Hepcidin and D-dimer (r=0.537), IL-6 and WBC (r=0.579), ferritin and VitD (r= 0.370). In conclusion, COVID-19 is accompanied with inflammatory increasing from the moment of onset to its severity influences, yet the inflammatory status remains elevated a while post-recovery and does not decrease in fast rhythm.

Keywords: SARS-CoV-2; COVID-19; Coronavirus; Lymphocytes; Coagulation; WBCs; D-dimer; IL-6; Ferritin; PT; INR; Hepcidin; Vit D; LYM%; PTT.

1. Introduction

Coronavirus disease 2019 (COVID-19) is a morbidly infection of the SARS-Cov-2 virus which may lead to a serious dysfunction in many organs, and can drive death in critical cases [1]. From its beginning in Dec 2019 in Wuhan city, the pandemic has been spread widely in very rapid manner [2].

The SARS-CoV-2 is a member of the Coronaviridae family, it is a novel positive single-stranded RNA virus with non-segmented genome and has protein envelop. The identification of this virus came after an earlier diagnostic of two former members in the family, SARS-CoV and MERS-CoV. It has a diameter range of 65-125 nm and possess a crown-like spike proteins on the exterior surface [3]. Four main proteins are detected in the structure of SARS-CoV-2; the membrane (M) protein, the spike (S) glycoprotein (which facilitates the binding of enveloped virus to the host cells through attraction with angiotensin-converting enzyme 2 that expressed predominantly in the cells of the lower respiratory tract), the nucleocapsid (N) protein and the small envelope (E) glycoprotein, in addition to a number of accessory proteins [4]. Severe COVID-19 is characterized by an acute activation of the innate immune system which accompanied with a prothrombotic state [5]. The scientific community has dedicated the efforts to reveal the mysteries of COVID-19 pandemic as its demoralizing effects are grown rapidly. The efforts are divided onto two teams, the first is searching for optimal therapeutic approach to reduce and eliminate this pandemic and the second team are searching for disease pathophysiology and predicts the best biomarkers regarding disease progression and risks in patients [6-8]. This could improve management's targets and overcome the insufficiency of material and medical resources that has been specifically clear during this global pandemic.

Hepcidin (Hep) is the protein which encoded using the gene of HAMP in humans. It is the key regulator of the entry of iron (Fe) in blood of mammals [9]. For the first time, it was discovered serum and urine of human in 2000 [10]. It was discovered in samples of human blood ultra-filtrate and urine as a small bactericidal peptide (defensing, and cathelicidin) which called LEAP–1 peptide (liver-expressed antimicrobial peptides) [11].

The recently discovered Hep biomarker regulates the

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absorption of Fe through inhibition of Fe transport from gut and trapping Fe in cells. These two pathways are significant in maintain Fe homeostasis in intra- and extracellular compartments. Hep is also expressed abnormally during inflammatory episodes that caused mainly by infection [12].

Ferritin (FER) is another biomarker of Fe storage, it has a key role in mediating immune dysregulation, particularly in very high levels of FER, through immediate immunosuppressive and pro-inflammatory impacts, participating to the cytokine storm syndrome (CSS) [13]. The lethal role of COVID-19 has been documented to be associated with the CSS, that way the researches have established that the severity of COVID-19 is dependent on the degree of the CSS [14].

It has been documented that the FER is a sensitive biomarker in the predication of severity and mortality upon the risk's assessment of COVID-19 [15]{Lin, 2020 #15}. Upon the infectious with SARS-Cov-2, the CSS is associated with hyperferritinemia [13].

FER is a protein found mainly in cytosol of most tissues, with small amount in the mitochondria (nuclear FER has been proposed) [16]. Although it is broadly distinguished as an agent of iron storage in the body, its diagnostic signify is linked with acute- and chronic- inflammatory events and is raised generally in a assortment of inflammatory disorders, e.g., rheumatoid arthritis, chronic kidney disease, and autoimmune disorders [17].

Cytokine release syndrome (CRS) is a term used to describe the response to a systemic inflammation that can be activated via diversity of factors including toxin, infection or special response to drugs, and is characterized by overexpression of pro-inflammatory cytokines such as interleukins 6 and 1β (IL-6 and IL-1β), and tumor necrosis factor (TNF). IL-6 participates in the defense system via modulation of acute phase responses, immune reactions and haematopoiesis. IL-6 is expressed hastily and transiently in response to tissue injuries and infections. The regulation of IL-6 genome occurs at transcriptional and posttranscriptional stages, yet it has a morbidly effect on autoimmunity and systemic inflammation. IL-6 is the key cytokine whose expression has been associated with numerous inflammatory disorders. Hence, elevated levels of IL-6 has been reported in COVID-19 patients which has attributed to pulmonary inflammation and severe lung damage [18, 19].

The nuclear 1,25(OH)2D receptors (VDR) are located in many immune cells such as dendritic, T and B cells, and macrophages [20]. Vitamin D (Vit D) is a key modulator of defending via phagocytosis against bacteria, including Mycobacterium tuberculosis[21] [22] and Escherichia coli [23], by macrophages. A significant portion of this antibacterial impact is related to the VDR-mediated induction via 1,25(OH)2D of cathelicidin (LL-37), which is an antibacterial peptide with positive net charge [24]. LL-37 is expressed predominantly in macrophages and to a lesser extent in the epithelium, and is also has an antiviral properties, especially against those with envelop structure such as SARS-CoV-2 [25]. Vit D has shown to triggers the antiviral activity against rhinovirus in cultured respiratory epithelial cells [26], a result that can also be confirmed by the introducing of LL-37 exogenously [27]. The antiviral activity of LL-37 against influenza virus has been documented [28].

One of the fibrin degradation products is the D-dimer, which is used widely as a marker for thrombotic diseases. The cut-off normal value of D-dimer was assigned as 0.5 μg/mL, and this value is elevated with age and also in pregnancy. It has been established that D-dimer level is elevated upon the severity of community-acquired pneumonia. During the global pandemic of COVID-19, the D-dimer has been used as potential marker for indicating the severity of COVID-19 in patients. Nemours studies have indicated the usefulness of D-dimer in the prediction of COVID-19 severity [29-32]

The immunity system of humans is stimulated in the presence of SARS-CoV-2 antigens, which ultimately leads to the generation of IgM and IgG antibodies for this virus. In general, the IgM antibodies are generated during the first term of viral infection (during the acute phase of the disease), and followed by the production of the IgG antibodies during the convalescence phase [33].

Conversely, the responses of antibodies against the other human corona-viruses were documented to fade over time [34]. According to the findings of seroprevalence studies on SARS-CoV indicated that the activity of its specific IgG is positive for finite time, as maximum as two years. After that, the incidence of a second infection is very conceivable due to the negativization of IgG antibodies [35, 36].

The blood picture analysis is a common procedure falls under the routine test's checkup of human health. The count of blood cells could be used in the detection of numerous diseases such as anemia, infections, leukemia, and immunity disorders [37].

Our work is designed to study the inflammatory status of COVID-19 patients in order to incursion in a better understanding of this virus influences. A number of inflammatory biomarkers are selected to be evaluated in the serum of COVID-19 patients including; Hep, FER, D-dimer, and IL-6. Vit D was also selected to investigate its role in the inflammatory status of COVID-19 patients. Furthermore, the relationship of these parameters with each other and with COVID-19 antibodies (IgG and IgM) are subjects to study in this work, to predict their usefulness in detecting the severity of the disease.

2. Methods

Patients

The study was included 30 patients with confirmed SARS-CoV-2 infection who were resident at Al-Yarmook Teaching Hospital (Baghdad, Iraq) from Jan 28th to the 05th of Mar, 2020. The cases were confirmed according to a positive real-time reverse transcription polymerase chain reaction (RT-PCR) assay for either respiratory or blood samples, or a positive virus gene sequencing as clinical diagnostic criteria based on the Novel Coronavirus Pneumonia Diagnosis and Treatment Guideline (6th ed.) [38]. The onset of symptoms is specified by the initial clinical aspects harmonious with COVID-19, including dyspnea, cough, fever, muscle pain, diarrhea and tiredness, registered in the anamnesis on admission. Patients with tumors, pregnancy, hematological disorders, liver dysfunction, heart diseases, trauma or surgery within a month, as well as those without D-dimer test result in the records. We have collected and noted patient's characteristics, clinical outcomes, laboratory tests, images of chest computerized tomography (CT) and prognosis out of the patient's medical records by using a standard data form. The laboratory tests were included; WBC, LYM%, coagulation profile, D-dimer, IL-6, Ferritin, Hepcidin, and Vit D. The level of D-dimer was determined by using a Sysmex, CS5100 immunoturbidimetric assay with 0.0-0.5 mg/L as reference range. Doppler ultrasound and computed tomography of the lungs angiography were performed for any patient clinically suspected of a high degree of pulmonary embolism/deep vein thrombosis (PE/DVT). A chest CT scan was performed for all patients. We controlled the study with 30 healthy people were volunteered from Mustansiriyah University (Baghdad, Iraq).

3. Statistical Analysis

The results are placed in form of mean±standard deviation (SD) and CV%. The differences between COVID-19 patients and control subjects were analyzed by using independent sample t-test. The results of IgM and IgG antibodies were divided onto four groups with Q1 being the group of patients with lower values and Q4 contains the patients with higher values, the other variables were compared between these quartiles of antibodies by using analysis of variance (ANOVA) and the post-Hoc LSD test. Pearson's correlation coefficient (r) was determined between each two variables in COVID-19 patients to investigate their relationships. Parameters were analyzed by receiver operating characteristics (ROC) test to determine the sensitivity of the parameters as diagnostic markers for COVID-19, the area under the curve (AUC), and cut-off values with sensitivity% and specificity% were calculated.

4. Results

The prognosis of patients was confirmed by a positive RT-PCR tests performed by Al-Yarmook Teaching Hospital. The age of selected subjects (patients and control, N=60) were ranged from 22 to 87 years with mean value equal to 48.7±16.43 years. The comparison of demographic and clinical characteristics between the normal group and COVID patients group are shown in Table 1.

Table 1. Clinical laboratory data of participants.

Parameters		Patients N=30	Control N=30	p-value
Age (year)	Mean±SD	48.7±16.43	47.33±14.20	0.732
	SE	2.999	2.593	
	Range	22-87	22-71	
	CV%	33.74	30	
W.B.C	Mean±SD	6.52±2.50	5.34±0.72	0.016
	SE	0.457	0.131	
	Range	2.50-12.30	4.20-7.10	
	CV%	38.34	13.48	
LYM%	Mean±SD	20.95±9.03	23.59±3.78	0.145
	SE	1.65	0.69	
	Range	4.0-41.20	16.35-32.50	
	CV%	43.10	16.02	
Vit D3 (ng/mL)	Mean±SD	19.57±12.31	27.90±6.57	0.002
	SE	2.25	1.20	
	Range	5.20-70.0	16.84-41.80	
	CV%	62.90	23.55	
D. Dimer (ng/mL)	Mean±SD	440.15±247.06	0.40±0.082	0.0001
	SE	45.11	0.015	
	Range	106.23-970	0.18-0.52	
	CV%	56.13	20.5	
IL-6 (pg/mL)	Mean±SD	3.55±2.88	0.74±0.21	0.0001
	SE	0.522	0.039	
	Range	0.30-9.24	0.44-1.15	
	CV%	81.13	28.38	
Ferritin (ng/mL)	Mean±SD	358.78±299.65	89.71±32.59	0.0001
	SE	54.71	5.95	
	Range	16.55-1000	23.70-164.90	
	CV%	83.52	36.33	
PT	Mean±SD	17.36±8.11	12.39±0.98	0.002
	SE	1.48	0.178	
	Range	11-44.60	10.60-13.80	
INR	Mean±SD	1.77±1.43	0.99±0.07	0.004
	SE	0.26	0.012	
	Range	1-7	0.8-1.20	
	CV%	80.79	7.071	
PTT	Mean±SD	31.41±6.64	29.83±4.01	0.270
	SE	1.21	0.73	
	Range	20-46	24-38	
	CV%	21.14	13.44	
Hepcidin	Mean±SD	291.49±138.87	24.43±10.29	0.0001
	SE	25.35	1.88	
	Range	92.33-560.90	13.50-52.24	
	CV%	47.64	42.12	

W.B.C × 1000

Major laboratory markers shows that there was a significant increase in the level of WBCs , D-dimer ,IL-6 ,Ferritin ,PT, INR ,Hepcidin and non-significant increase in Age and PTT .The predominant decrease were seen significantly in Vit D and non-significantly in LYM% .The coefficient of variation percent shows that the control group was more accuracy than patient group in all studied parameters . ROC curves were used in the analysis of prediction efficacy and the optimal prediction threshold for worsening of condition in COVID-19 patients. ROC analysis identified that Hepcidin and D-dimer has the excellent sensitivity and specificity (100%) in AUC value =1 ; cut off values were (72.28 ,53.37) , indicating that it better predicts the development of severe pneumonia in COVID-19 , good sensitivity was noticed in ,IL-6, Ferittin, Vit D ,INR with AUC value (0.895 , 0.842 , 0.809 , 0.807) ; cut off value (0.965 120.915 , 21.235 1.05,) respectively upon admission as the optimal cutoff level to discriminate covid pateints from non-covid persons (control) . The non-significant poor sensitivity result was noticed only in WBCs (area under the ROC 0.612, standard error 0.078; cut off =5.25.

Table 2. ROC curve analysis of clinical biomarkers.

Area Under the Curve				
Test Result Variable(s)	Area	SE	Sensitivity	p-value
WBC	0.612	0.078	Poor	0.137
Ddimer	1.0	0.027	Excellent	0.000
IL6	0.895	0.044	Good	0.000
Ferritin	0.842	0.061	Good	0.000
PT	0.789	0.060	Fair	0.000
INR	0.807	0.056	Good	0.000
Hepcidin	1.0	0.038	Excellent	0.000
Vitamin D3	0.809	0.060	Good	0.000

Table 3. ROC curve analysis of clinical biomarkers.

Cut-off Values			
Test Result Variable(s)	Cut-off value	Sensitivity%	Specificity%
WBC	5.25	60%	56.7%
Ddimer	53.375	100%	100%
IL6	0.965	80%	86.7%
Ferritin	120.915	80%	83.3%
PT	13.35	70%	80%
INR	1.05	63.3%	90%
Hepcidin	72.284	100%	100%
Vitamin D3	21.235	90%	70%

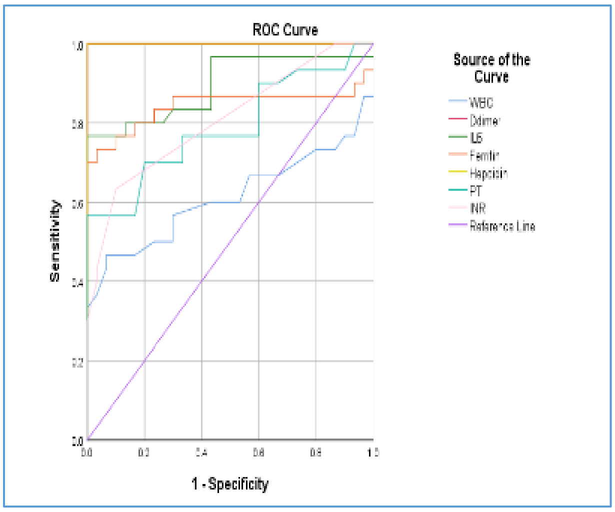

Figure 1: ROC curves comparing the prediction of severe COVID-19 variables for hepcidin combined with serum ferritin.

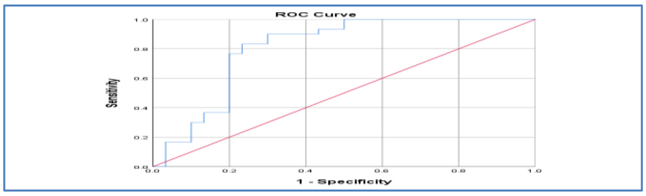

Figure 2: ROC curves comparing the prediction of severe COVID-19 variables for hepcidin combined with serum ferritin.

For predicting immunoglobulin role in COVID-19 patients , present work performed an analysis of demographic, clinical and laboratory parameters of COVID-19 hospitalized patients according to quartiles of serum IgG and IgM levels:[mild Q1(≤0.85) ,n=7, Q2 moderate (0.86-1.85),n=9 , sever Q3(1.86-2.68), n=7 and very critical infection Q4(>2.68), n=7 according to IgM] and [mild Q1(≤0.9) ,n=8, moderate Q2(0.91-3.9),n=7 , sever Q3(3.91-7.425),n=8 and very critical infection Q4(>7.425),n=7 according to IgG] (Table 4).

Table 4. Differences across groups dividing patients according to IgM levels.

Parameters	Q1 (≤0.85)	Q2 (0.86-1.85)	Q3 (1.86-2.68)	Q4 (>2.68)	p-value
N	7	9	7	7	-
Age	46.0±17.07	51.56±16.0	43.71±14.01	52.71±20.16	0.698
WBC	7.79±2.19	6.52±2.60	5.93±2.41	5.83±2.81	0.457
LYM	21.70±6.30	20.94±7.30	19.60±9.04	21.54±14.10	0.975
Vit. D3	21.70±6.30	20.94±7.30	19.60±9.04	21.54±14.10	0.777
D. dimer	294.72±164.66	351.56±122.86	509.09±276.08	630.56±295.43	0.031
IL-6	3.03±2.19	2.32±1.81	3.22±2.70	6.01±3.64	0.05
Ferritin	187.06±293.73	306.34±301.13	529.33±366.52	320.72±314.77	0.411
PT	15.93±8.96	18.41±7.55	17.23±3.62	17.56±12.01	0.951
INR	1.69±1.59	1.71±0.99	1.86±0.95	1.86±2.27	0.848
PTT	29.57±7.79	31.29±5.12	32.60±7.86	32.21±7.01	0.993
Hepcidin	158.34±82.95	280.59±81.55	388.79±148.08	341.35±143.99	0.006
IgG	7.03±3.94	5.73±5.73	4.36±4.60	2.73±3.82	0.366
IgM, Total samples =30 (results expressed as mean±SD)					

Table 5. Differences across groups dividing patients according to IgG levels.

Parameters	Q1 (≤0.9)	Q2 (0.91-3.9)	Q3 (3.91-7.425)	Q4 (>7.425)	p-value
N	8	7	8	7	-
Age	39.63±13.39	52.14±19.34	48.88±14.44	55.43±17.35	0.278
WBC	6.41±3.56	6.33±1.83	6.95±2.90	6.33±1.42	0.959
LYM	24.61±10.99	16.64±8.91	19.44±5.62	22.79±9.53	0.344
Vit. D3	18.43±8.17	16.35±8.70	25.98±19.35	17.14±8.18	0.411
D. dimer	491.34±286.13	466.28±268.90	416.06±263.07	383.06±193.92	0.847
IL-6	2.66±2.46	4.53±3.75	3.33±2.25	3.86±3.17	0.655
Ferritin	313.04±315.42	507.32±379.48	375.43±287.96	243.51±181.05	0.414
PT	14.59±2.52	19.50±11.41	18.45±8.78	17.13±8.53	0.687

INR	1.20±0.34	2.33±2.19	1.93±1.64	1.69±1.01	0.871
PTT	29.81±3.62	31.31±8.86	32.58±7.86	32.0±6.43	0.507
Hepcidin	289.29±151.16	355.20±107.51	247.47±158.93	280.588±134.39	0.530
IgM	2.19±1.09	3.21±1.82	1.19±0.59	1.53±1.10	0.018
IgG, Total samples =30 (results expressed as mean±SD)					

Results appeared that there was a significant variation in the levels of D-dimer, IL-6, Hepcidin in four divided groups depend on IgM classification, on other direction, IgM show a significant variation between four groups depend on IgG classification with non-significant changes in other parameters. This study also confirmed that IgG titers in severe COVID-19 patients were significantly higher than those in non-severe patients post-symptom onset, and showed that Q2 group according to IgG was the worst case among other groups depend on hematological and immunological tests whereas Q4 group was the worst according to IgM. Pearson correlation coefficient appeared the following results : there was a significant negative correlation between LYM&WBC (r=-0.413) , Hepcidin & VitD (r=-0.387),IL-6 & LYM (r=-0.419) ,PT & LYM (r=-0.465), INR & LYM (r=-0.458) and is a significant positive correlation IL-6 & INR (r=0.433), PT & INR (r=0.957), PT & PTT (r=0.623), Hepcidin & D-dimer (r=0.537), IL-6 & WBC (r=0.579), Firritin & VitD (r= 0.370).

Table 6. Correlation coefficient between all studied parameters.													
Age	**r**	**-0.114**	**-0.178**	**-0.005**	**0.232**	**-0.075**	**-0.323**	**0.031**	**-0.091**	**-0.151**	**-0.219**	**-0.036**	**0.312**
	p	**0.547**	**0.346**	**0.980**	**0.218**	**0.692**	**0.081**	**0.870**	**0.633**	**0.425**	**0.245**	**0.850**	**0.093**
WBC	r	-0.161	-	-0.413*	-0.063	0.117	0.579**	-0.039	-0.031	0.044	-0.010	-0.216	-0.048
	p	0.396	-	0.023	0.714	0.539	0.001	0.838	0.871	0.818	0.958	0.253	0.802
LYM	r	-0.340	-0.413*	-	0.025	-0.210	-0.419*	-0.349	-0.465**	-0.458*	-0.253	-0.212	-0.010
	p	0.066	0.023	-	0.894	0.265	0.021	0.058	0.010	0.011	0.178	0.261	0.957
Vit. D_3	r	-0.387*	-0.063	0.025	-	-0.22	-0.060	0.370*	-0.172	-0.162	0.085	0.038	-0.069
	p	0.035	0.714	0.894	-	0.082	0.755	0.044	0.363	0.394	0.654	0.843	0.716
D. dimer	r	0.537**	0.117	-0.210	-0.22	-	-0.093	-0.184	0.039	0.061	0.044	0.000	0.097
	p	0.002	0.539	0.265	0.082	-	0.624	0.330	0.837	0.750	0.819	0.999	0.608
IL-6	r	0.129	0.579**	-0.419*	-0.060	-0.093	-	0.151	0.355	0.433*	0.350	0.283	-0.112
	p	0.496	0.001	0.021	0.755	0.624	-	0.427	0.055	0.017	0.058	0.129	0.556
Ferritin	r	-0.143	-0.039	-0.349	0.370*	-0.184	0.151	-	0.284	0.361	0.129	0.114	-0.196
	p	0.450	0.838	0.058	0.044	0.330	0.427	-	0.128	0.050	0.495	0.548	0.299
PT	r	0.285	-0.031	-0.465**	-0.172	0.039	0.355	0.284	-	0.957**	0.623**	0.124	0.137
	p	0.126	0.871	0.010	0.363	0.837	0.055	0.128	-	0.000	0.000	0.515	0.470
INR	r	0.297	0.044	-0.458*	-0.162	0.061	0.433*	0.361	0.957**	-	0.681**	0.143	0.032
	p	0.111	0.818	0.011	0.394	0.750	0.017	0.050	0.000	-	0.000	0.450	0.866
PTT	r	0.312	-0.010	-0.253	0.085	0.044	0.350	0.129	0.623**	0.681**	-	0.182	0.015
	p	0.093	0.958	0.178	0.654	0.819	0.058	0.495	0.000	0.000	-	0.334	0.938
Hepcidin	r	-	-0.161	-0.340	-0.387*	0.537**	0.129	-0.143	0.285	0.297	0.312	0.271	0.093
	p	-	0.396	0.066	0.035	0.002	0.496	0.450	0.126	0.111	0.093	0.148	0.626
IgM	r	0.271	-0.216	-0.212	0.038	0.000	0.283	0.114	0.124	0.143	0.182	-	-0.340
	p	0.148	0.253	0.261	0.843	0.999	0.129	0.548	0.515	0.450	0.334	-	0.066
IgG	r	0.093	-0.048	-0.010	-0.069	0.097	-0.112	-0.196	0.137	0.032	0.015	-0.340	-
	p	0.626	0.802	0.957	0.716	0.608	0.556	0.299	0.470	0.866	0.938	0.066	-

5. Discussion

One of the most common symptoms during the clinical course of COVID-19 is the respiratory deterioration, which occurs within short time. Therefore, it is necessary to predict an initial step in the identification whether the patients would suffer a severe or mild infection. Regarding this topic, a number of studies have indicated the possibility of predicting respiratory failure by using some blood markers in COVID-19 patients [39, 40]. furthermore, the usefulness of several pro-inflammatory cytokines in the characterization of severity in COVID-19 has been documented [41]. Nevertheless, in some cases, the overall status rapidly deteriorates over the course of a few days, resulting in severe respiratory failure. It's still unclear how to forecast fast respiratory collapse. As a result, identifying accurate blood indicators that can predict respiratory problems in the close future in medical settings is a top focus. The focus of this research was to see if laboratory indicators might indicate respiratory failure in COVID-19 subjects in the short term.

In our research, laboratory examination of leukocytes, lymphocytes, D-dimer, IL-6, FER, Hep, PT, PTT, INR, and COVID-19 antibodies (IgG and IgM) was shown to be an important tool in the diagnosis, monitoring, and identification of extreme symptoms of hematological, immunological consequences [42]. Since the initial articles, quantitative hematological anomalies have been described; all blood cells, primarily leukocyte and platelet cells, can be impacted by COVID-19 [43].

COVID-19 individuals had non-significant lymphocytopenia, according to our findings. While additional investigation into the underlying etiology is needed, various variables may play a role in COVID-19-related lymphocytopenia. In this context, lymphocytes have been demonstrated to have the ACE2 receptor on their surface [44]; As a result, SARS-CoV-2 might infect those cells directly, resulting in their destruction. Additionally, the CSS is characterised by significantly elevated levels of interleukins, particularly IL-6, that could induce lymphocyte apoptosis [45-47]. Moreover, lymphocytopenia was seen in nearly 40 percent of total of the very first 18 COVID-19-infected hospitalised in Singapore [48]. The percentage of individuals with lymphocytopenia was verified in a recent study of 69 cases, while 20% had moderate thrombocytopenia. Apparently, 69 percent of cases with a low lymphocyte count had a responsive lymphocyte population, which included a lymphoplasmacytoid subset, which was not seen in the peripheral circulation of SARS cases in 2003 [43, 49, 50]. The CD4+/CD8+ lymphocytes ratio did not invert according to flow cytometry [43]. Nevertheless, functional investigations reveal that SARS-CoV-2 may affect the function of CD4+ helper and regulatory T-cells, as well as enhance the rapid hyperactivation of cytotoxic CD8+ T-cells, which is followed by fast exhaustion [51, 52]. We studied the blood regular indicators from COVID-19 patients in this study, and found that WBC was considerably greater in COVID-19 patients than in healthy controls, and that LYM% was comparatively lower in COVID-19 patients than in healthy controls. WBC levels rise in response to acute inflammatory response, which might indicate pulmonary and extra - pulmonary injuries, such as respiratory failure, acute ischemic damage, and acute renal damage. High IL-6 levels indicated the requirement for mechanical ventilation, according to Herold et al. [53]. Our findings imply that IL-6, a crucial cytokine upfront of the pro-inflammatory cytokine pathway, rises in severe COVID-19 patients before to acute respiratory distress syndrome (ARDS), accompanied by an increase in acute-phase protein levels, such as CRP [54, 55]. SARS-CoV-2 has the ability to directly stimulate pathogenic Th1 cells, causing them to release inflammatory cytokines including IL-6 [56]. Because the shift from viral pneumonia to ARDS is so sudden, identifying possibly deteriorating patients between many stable patinets must be a medical priority. In pandemics with limited medical supplies, screening IL-6 as an initial inflammatory biomarker for ARDS can become a helpful method for recognizing collapsing patients and preparing suitable interventions. In COVID-19, a cytokine storm producing severe respiratory distress is an immunological illness associated with high immune cell activation and enormous synthesis of pro-inflammatory cytokines and biochemical mediators [57, 58].

Patients with COVID-19, particularly those with severe illness, are more likely to have coagulation problems [29, 59]. Above results noticed a raised in D-dimer, PT, PTT, INR level in patient group compare with control and become worse in Q4 group compare with the rest group according to both classification IgG and IgM. D-dimer, FDP fibrin breakdown products, and fibrinogen concentrations were significantly higher in patients with COVID-19 compared to healthy controls (p0.001 for all three comparisons) in prospective research assessing the coagulation profile of COVID-19 patients. D-dimer and FDP levels were greater in patients with severe illness than in those with milder symptoms (p0.05 for both comparisons) [60].

Some researchers believe that the significantly elevated D-dimer concentrations and high rates of micro- and macrovascular thrombosis, in contradiction of the modestly elevated median IL-6 concentrations, indicate that COVID-19 pathophysiological sequelae are because of a reduction of vasculopathy inflammatory is more willingly than a CSS [61]. The ACE2 receptor plays a critical role in viral cell entry, and the complex vascular alterations reported in autopsy investigations, including as intussusceptive angiogenesis and extensive endothelial membrane rupture, which imply a key role for vasculopathy in COVID-19-related morbidity [62].

The CRS is a term used to describe the response to a systemic inflammation that can be activated via diversity of factors including toxin, infection or special response to drugs, and is characterized by overexpression of pro-inflammatory cytokines. IL-6 participates in the defense system via modulation of acute phase responses, immune reactions and haematopoiesis. IL-6 is expressed hastily and transiently in response to tissue injuries and infections. The regulation of IL-6 genome occurs at transcriptional and posttranscriptional stages, yet it has a morbidly effect on autoimmunity and systemic inflammation. IL-6 is the key cytokine whose expression has been associated with numerous inflammatory disorders. Hence, elevated levels of IL-6 has been reported in COVID-19 patients which has attributed to pulmonary inflammation and severe lung damage [18, 19].

A major indicator of inflammation is IL-6, a chemokine secreted by macrophages and T-cells to stimulate an immunological response. It also consists from a variety of cell types that adapt to a variety of pathological situations, including inflammatory processes, infections, and malignancies [63, 64]. By promoting cytolytic dysfunction, IL-6 may trigger CRS. In intense COVID-19, high concentrations of IL-6 administration have been found to decrease the cytotoxicity of natural killer (NK) cells whereas, it downregulates the production of granzyme B and perforin. The failure of cytotoxic NK cells or T lymphocytes to destroy target cells by perforin or granulase-induced apoptosis, resulting in increased target cell survival and improved antigen stimulation. Overall, it leads to an overproduction of pro-inflammatory cytokines, which finally leads to ARDS and MODS [65-67]. Fujino et al. [57], observed that the concentrations of IL-6, CRP, LDH, WBC, and D-dimer were linked to the intensity of COVID-19 infectious diseases over 3 days, and that they were much higher in HFG than in LFG. Multiple organ illness produced through the cytokines responding in the context of serious COVID-19 infection. Elevated D-dimer concentrations have been linked to deadly DIC-related consequences other than ARDS, according to Querol et al. [68]. According to Poudel et al., the D-dimer value upon hospital entrance is an efficient predictor for identifying morbidity in COVID-19 subjects. The optimum D-dimer cut-off value for prediction morbidity in COVID-19 subjects is 1.5 g/ml. On admissions, the AUC of the ROC curve for D-dimer was 0.807 [69]. Viremia and the CSS, whereby the increase in pro-inflammatory cytokines is insufficiently regulated by anti-inflammatory agents, overwhelming the coagulation cascade, are the most prevalent reasons reported in the literatures for the increasing of D-dimer levels [70]. Hypoxia activates the hypoxia-inducible transcriptional factor-dependent signaling cascade, which increases the risk of thrombosis. Elderly and comorbid individuals are the most typically affected. Patients might be predisposed to thrombosis as they get older and have common complications including hypertension, metabolic disorders, and cardiovascular problems [69].

Hepcidin's primary role is to protect the cells from bacteremia by lowering the availability of free iron around the cells, limiting the bacteria's capacity to replicate. Macrophages that take in iron and release it as FER have the same effect [71]. In addition, direct generation of IL-6 and Hep reduces intralveolar iron in pneumocytes; in particular, ferroportin is produced on the alveolar surface [72, 73] In Hirano's proposed commentary [74], The SARS-CoV-2 virus activates the NF-B factor, which connects the ACE2 receptor of the alveolar cells and allows it to replicate. For the complex IKK - Iβ - NF-β to activate the NF-β, it needs iron [75]. The result of NF-kβ triggering induces Hep and IL-6 expression [76]. Hep and transferrin receptor 1 are likewise stimulated by IL-6, resulting in transferrin incorporation [77] and increase the levels of iron in the alveolar cells. The NF-β activation is maintained by intracellular iron [75]. Whenever this process is amplified very quickly, cellular iron gets overcharged, resulting in ferroptosis [78]. Hep production in macrophage is maintained through ferroptosis and IL-6 expression, with the purpose of iron trapping and conversion to FER [71]. When the alveolar tissue is disrupted by ferroptosis, many inflammatory mediators, pro-coagulant compounds, and free iron ions are released into the bloodstream [79]. The current definition of Hep as a virus helper in replicating and a defensive immunity enhancer is the result of all of these events, resulting in a "Hep paradox and an iron storm." Identifying if the cytokine storm is limited to an iron storm might be a focus for improving our therapies. Some researchers [80, 81] have already conclude, confirming the NF-B pathway's dual role in viral replication and immune response. Comprehending the two unique stages of COVID infection might be beneficial in comprehending COVID infection.

Vit D's effects on macrophage defence versus viral infections have been found to have a greater influence on cytokines response than on viral elimination [82]. Some of the studies have been focused on dengue infection, a virus disease that leads to massive of cytokines release [83, 84]. Vit D deficiency is associated with increased cytokine production by the dengue virus in vitro, despite one research finding a lower risk of septic shock in vit D dengue disease patients [85]. Vit D has been found to reduce the proinflammatory responses to infections in macrophage, T - lymphocytes, and diverse animal species of pneumonia and pneumonitis [86-88]. In response to an intratracheal administration of bacterial lipopolysaccharide, vitamin D-deficient animals experience more severe lung damage. Intraperitoneal injections of cholecalciferol alleviated these pathoeffects [89]. The elimination of vitamin D's regulatory action causes a several-fold elevation in the proinflammatory interleukin (IL-6) response to intra-peritoneal lipopolysaccharide, according to the work of Kong et al. [90]. L1-10, an angiotensin-2 inhibitor, blocks this effect. The hyper-inflammatory lung injury that defines severe COVID-19 is hypothesized to be caused by increased angiotensin-2 activity as a result of the interaction between SARS-CoV-2 and its receptor, ACE2 [91].

FER is a biomarker of iron storage, it has a key role in mediating immune dysregulation, particularly in very high levels of FER, through immediate immunosuppressive and pro-inflammatory impacts, participating to the CSS [13]. The lethal role of COVID-19 has been documented to be associated with the CSS, that way the researches have established that the severity of COVID-19 is dependent on the degree of the CSS [14]. Several people with diabetes have high levels of FER in their blood [92-94], and it is well recognized that they are more likely to have significant COVID-19 problems [95]. On this premise, we evaluate the evidence for the idea that FER levels may be a critical determinant affecting COVID-19 severity. When FER levels were measured in the peripheral circulation of 69 individuals with severe COVID-19, they were found to be higher than in patients with mild symptoms. As a result, circulating FER concentrations were shown to be closely linked to the intensity of COVID-19 [96]. Furthermore, laboratory results in COVID-19 patients indicated evidence consistent with a CSS with elevated inflammatory mediators, such as FER, which was linked to serious and life-threatening disease [97].

What does this have to do with increasing the biology of Hep? To begin with, one of the first discoveries of Hep was that it is influenced by anemia, ischemia, and infection [98]. Inflammation caused by infections boosts Hep synthesis, which can contribute to inflammation-related anemia, according to a broad knowledge of this regulatory network [99, 100]. Hep fabrication in the liver is stimulated by IL-6 [101, 102], and it has been reported that hepatic heparan sulfate affects and regulates IL-6-stimulated Hep expression [103]. Heparin, a glycosaminoglycan anticoagulant that is an intensely sulfated version of heparan sulfate, has also been found to be a powerful suppressor of Hep production [104]. Anti-coagulant therapy has been shown to be helpful in a subgroup of COVID-19 cases, and studies have discovered that the pro-coagulant transferrin is involved [105-107], in which is upregulated in COVID-19 [108]. There have lately been recommendations based on ACE2 and the vasopressor system protein bradykinin in relation to such problems with inflammation and coagulation in COVID-19 [109, 110].

It's also worth noting a number of circumstantial but potentially significant results in the literature about lung disease. These include (i) a relationship involving SARS and impaired liver function [111], (ii) the correlation between pulmonary iron excess and chronic obstructive pulmonary disease [112-114], (iii) iron's function in pulmonary fibrosis (iv) modulation of pulmonary artery smooth muscle cell proliferation by Hep [115], and (v) Hep has a critical role in alveolar macrophage activity [116]. Moreover, (vi) In influenza infections, hep overexpression has been linked to a drop in blood iron levels [117, 118]. Iron dysregulation alterations, on the other hand, may only occur at a specific cellular/tissue level and not result in a systemic reaction [119]. During diverse viral infections, it may show specific tissue tropisms [120].

In COVID-19, the pro-inflammatory response is important in the transition from moderate to severe symptoms [121]. Iron homeostasis is regulated by inflammation. Hep biomarker regulates the absorption of Fe through inhibition of Fe transport from gut and trapping Fe in cells. These two pathways are significant in maintain Fe homeostasis in intra- and extracellular compartments. Hep is also expressed abnormally during inflammatory episodes that caused mainly by infection [12]. Patients in the severe group had considerably greater levels of Hep and serum FER than those in the mild subgroup. The ROC curve integration of severe COVID-19 in combined prognosis using both Hep and serum FER was excellent (AUC=1.0, p0.01), showing that they are potential indicators in the prognosis of severe COVID-19 and that combined detection yields an excellent performance.

In this work, the date of the beginning of COVID-19 signs was used as a point of reference for evaluating immunological profiling. The duration between the beginning of symptoms and the immunological examination in a specific subject ranged from 12 to 86 days, and the immunological tests (IgG and/or IgM) were positive in all cases [122].

Many research have looked at the issue of IgM and IgG seroconversion in COVID-19 patients. IgM seroconversion could be identified as soon as 5 days after the beginning of symptoms, but IgG seroconversion takes 14 days [123]. Nevertheless, a comprehensive study of the initial antibody response to SARS-CoV-2 in 285 COVID-19 patients found that seroconversion of IgM and IgG might happen concurrently or sequentially in COVID-19 patients, with IgM preceding or even succeeding IgG. This study also found that IgG rates in severe COVID-19 cases were considerably greater than those in non-severe COVID-19 cases post-symptom start, and that the Q2 group was the worst case among the other groups based on IgG, while the Q4 group was the worst based on IgM [124]. The level and longevity of humoral immunity in COVID-19 individuals is, certainly, a prominent focus of interest. Within 2 to 3 months after infection, IgG and neutralize antibody levels begin to fall considerably, according to Long et al. [125]. Seow et al. corroborate these findings [126], demonstrating the temporary nature of the SARS-CoV-2 antibody response. This trait more closely resembles the immune reaction to endemic annual viral pathogens (i.e., those that cause the common cold), which have also been shown to be temporary. Another study found that roughly 6–7 weeks following ailment start, neutralize antibody titers considerably decreased in convalescent people (4 out of 8 tested subjects) [127].

6. Conclusions

The high levels of WBCs, D-dimer, IL-6, FER, PT, INR, Hep, Vit D, LYM %, and PTT were assessed in this research that concentrating on initial hyper-inflammation in COVID19 patients. Thus, our data imply that Hep and serum FER levels are suitable being used in the prognosis of COVID-19 severity as a marker for the cytokine storm syndrome. In individuals with severe COVID-19, iron management tends to have diagnostic value. In individuals with COVID-19, the concentrations of Hep and serum FER can be evaluated and utilized as clinical indicators to determine disease severity when appropriate. This emphasizes the necessity of future research focused on the balance of pro- and anti-inflammatory agents, as well as how this may affect disease development. The levels of IgM and IgG antibodies in a population of 30 COVID-19 patients were examined in this investigation. Ultimately, this study found that IgG titers in severe COVID-19 patients were considerably greater than those in non-severe COVID-19 patients post-symptom start, and that the Q2 group was the worst among the other groups based on IgG, whereas the Q4 group was the worst based on IgM. Given the limited number of participants, numerous unusual features of the humoral response in COVID-19 patients appeared, such as substantial inter-individual variability, short IgG half-lives in some people, and long-lasting IgM titers in others.

Finally, the goal of this investigation was to see if routine supplementary tests might be used to discover any meaningful role for prognostic risk. The severity of inflammation in the days following hospitalization may aid in identifying individuals at higher risk and making early therapeutic choices about preventative interventions for these patients.

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