Original Research Article

Title: INTERLEUKIN-6 and FERRITIN as prognosticators in patients with COVID -19 in Kashmir

Abstract

Background:

Many laboratory findings or Pro- and inflammatory markers can be used ~~to better~~to better understand the causes of poor disease outcomes and in the management of COVID-19 disease.

Objectives:

1. To estimate ~~ferritin and~~ferritin and IL6 ~~levels~~6 ~~for~~levels for predicting inflammatory ~~response and~~response and pro-inflammatory immune response in RT- PCR confirmed SARS-CoV-2 infected patients for deciphering their role in disease pathology.

2. Correlate the above ~~pro~~ pro-inflammatory cytokines, and inflammatory ~~markers with~~disease severity and outcome in SARS-CoV-2 infected patients

Methods:

A total of 100 SARS-CoV-2 infected (RT-PCR confirmed) patients from Kashmir that ~~were followed~~were followed for a period of I ~~month (~~month (14thand 28th~~th~~ day) were included in this ~~cohort study~~study cohort study. The sociodemographic ~~features and~~and features and co-morbiditiesof the infected patients were ~~recorded .~~recorded. Clinically patients were of 3 ~~stages .~~stages.

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Results:

One of the hallmark features of COVID-19 disease is an association of viral load and disease severity with an inflammatory cytokine response which explains many underlying SARS-CoV-2 ~~pathophysiology~~pathophysiologies.Here, we estimated ferritin and IL6 levels by ELISA and fully automated ~~immunoanalyzer~~ immune analyzer in a cohort of 100 RT-PCR confirmed SARS-CoV-2 infected patients, followed for a period of I month (14th and 28 th day), from Kashmir, North India. ~~On the basis of~~Based on estimated ferritin levels, the cohort was categorized ~~into Mild~~intoMild= <500 ng/ml, Moderate=≥500-<1500, and High = ≥1500ng/ml.Also patients were grouped as Mild= 0-<10pg/ml, Moderate=≥10-<80pg/ml and High=≥80pg/ml based on Interleukin IL-6 levels. Correlation analysis of SARS-CoV-2 infected patients of varying ferritin levels with disease severity revealeda percent increase in the number of patients of stage 3 severity as ferritin levels increased from mild, to moderate to high levels. Similarly,a percent increase in the number of SARS-CoV-2 infected patients of increased severity was found as IL6 levels increased from mild to moderate and high levels. Further, the ROC analysis of ferritin and IL-6 levels with disease outcome suggested both ferritin and IL6 as early predictive markers of poor disease outcome. However, IL-6, with AUC =0.70 and sensitivity of 70% ~~&~~andspecificity of 62~~%, is~~%, is a better early predictive marker of poor disease outcome than ferritin with AUC=0.66 ~~at sensitivity~~at sensitivity of 60% and specificity of 64% in SARS -CoV-2 ~~infected patients~~patients infected patients from Kashmir. Further ROC analysis of patients with very high ferritin levels (>1500ng/ml) alone suggests it as an early marker of patients with hyperinflammatory phenotype.

Conclusion:

~~Study~~ The study concluded that the estimation of ferritin and IL6 levels ~~as~~ is a simple complementary early prognostic tool that can help in clinical ~~decision~~ decision-making and selecting appropriate treatment options in SARS-CoV-2 infected patients from our place, Kashmir, North India.

KEY WORDS: Ferritin,IL6,Cytokine storm

1. INTRODUCTION

In December 2019, Wuhan, China, reported an outbreak of a distinct coronavirus-based sickness. The ~~WHO named~~WHO named this coronavirus "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) and the condition it caused "coronavirus disease 2019 (COVID-19~~).Globally~~). Globally, as on 27 May 2022, there have been 525,467,084 confirmed cases of COVID-19, including 6,285,171 deaths, reported to ~~WHO.The~~WHO. The majority of COVID-19 patients either have no symptoms or have had mild to severe respiratory illnesses. However, multi-organ and systemic symptoms like sepsis, septic shock, and multiple organ dysfunction syndromes (MODS), which can be lethal, have also been documented [1,2].

The mechanism implicated in COVID-19 is the dysregulated host Immune Response (IR) reflected by the excessive innate and inadequate adaptive immune ~~response.In~~response. In patients with SARS-CoV-2 infection, a rapid course of acute lung injury (ALI) and ARDS has been ~~hypothesised~~ hypothesized to be caused by an excessively proinflammatory host response.There ~~has~~ have been overwhelming evidence~~s~~ supporting the theory that not ~~the~~ SARS-COV-2 itself but the widespread inflammatory response that it triggers is reflected by massive cytokine or chemokine release that causes the organ damage. The ~~so~~ so-called “cytokine storm” is the leading factor~~s~~ that trigger the pathological processes leading to plasma leakage, vascular permeability, and disseminated vascular coagulation observed in COVID-19 patients and accounting for life-threatening respiratory symptoms.IL-6 is one of the important pro-inflammatory cytokines., and ferritin is an important inflammatory marker in Covid-19. Meta-~~analysis of~~analysis of various studies conducted worldwide have recommended the estimation of these immune markers during hospitalization to recognize high-risk individuals that can develop ARDS and are thus ~~are~~clinical predictors of severe and fatal COVID-19

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[2].Despite the available information about predictive/prognostic markers in COVID-19, it has been observed people belonging to geographically diverse regions and of different ethnicity and ~~geneticity~~genericity behaves differently against the SARS-CoV-2 virus. While some ~~are able to~~can resolve it quickly others succumb to the disease.Furthermore, there is a possibility that SARS-CoV-2 could modify host innate immune responses ~~in order~~ to avoid immune identification and weaken human ~~defences~~defines.To find these differences, which may be unique to our population, it is essential to assess the immune response in SARS-CoV-2 infected patients from our ~~own~~ Kashmiri population.As a part of this initiative herein we unraveled the prognostic value of ferritin and IL-6 asmarkers of inflammatory and pro-inflammatory immune response in SARS-CoV-2 infected patients from Kashmir.

2. Materials and methods

The present study was initiated after the approval obtained from the~~Institutional~~institutional Ethical ~~Committee~~committee, ~~Goverment~~Government Medical College, Srinagar (IEC-GMC-Sgr/27,19thDec,2020)and included patients with COVID-19hospitalized at SHMS Hospital between Oct. 2020 and Oct. 2021. In total 100 SARS-COV-2 infected (RT-PCR confirmed) patients from Kashmir followed for a period of I month were included in this cohort study. The median age of the cohort was 63.41+13.85yrs with a ~~female:~~female: male ratio of 1.85. The socio-demographic features viz age, gender, dwelling, and clinical features like symptoms (fever, cough, myalgia, pneumonia, ~~diarrhea,hypoxia~~diarrhea, hypoxia) and co-morbidities (diabetes, hypertension,COPD,CKD,hypothyroidism) of the infected patients were recorded.Clinically patients were of 3 stagesviz Stage 1 patients with symptoms like myalgia, dry cough, headache;Stage2 (IIa+IIb) patientswith symptoms like high fever, cough, ~~lymphopenia,~~

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raisedlymphopenia, raisedCRP levels, with hypoxia in the subgroup (IIb) and withoutand withouthypoxia inhypoxia inthe subgroup (IIa); Stage3 patients include those with the severe systemic inflammatory syndrome, culminating into severe respiratory failure. The patients after following for a period of 28 days (14th day and 28th day) were either discharged or dead. The levels of ferritin and IL6 were estimated by an IL6 Elisa kit (Diaclone) and a fully automated analyzer (Siemens).

3. Results

3.1General The general characteristic feature of SARS-CoV-2 infected patients (n=100) and their correlation to clinical staging

A total of 100 SARS-COV-2 infected (RT-PCR confirmed) patients from Kashmir followed for a period of I month were included in this cohort study. The median age of the cohort was 63.41±13.85yrs with a female:male ratio of 1.85. The socio-demographic features viz age, gender, dwelling. and clinical features like symptoms (fever, cough, myalgia, pneumonia, diarrhea,hypoxiadiarrhea, hypoxia) and co-morbidities (diabetes, hypertension,COPD,CKD,hypothyroidism) ofhypothyroidism) of the infected patients were recorded and is represented by (table1). Most of the SARS-CoV-2 patients in this cohort were >55 years of age, mostly females,of of females, of urban origin, and without comrbiditiescomorbidities. Among comorbidities, hypertension was the most common finding in this cohort. Clinically patients were of 3 stagesviz Stage 1 patientswith symptoms like myalgia, dry cough, headache;Stage2 (IIa+IIb) patientswith symptoms like high fever, cough,

lymphopenia, raised CRP levels, with hypoxia in the subgroup (IIb) andwithout hypoxiain the subgroup (IIa); Stage3 patients include those with the severe systemic inflammatory syndrome, culminating into severe respiratory failure. The patients after following for ~~a period of~~ 28 days (14th day and 28th day) were either discharged or dead. Correlation analysis of SARS-CoV-2 infected patients of ~~varying severity~~varying severity/stages with sociodemographic and clinical characteristics ~~revealed significant~~revealed significant no. of SARS-CoV-2 patients of stage 2 severity among females compared to males which were mostly of stage 3 severity. Most of the patients ~~of~~ withstage3 severity were of urban origin and no link between the presence of comorbidities and stage/ disease severity was observed (table 2).

3.2 Correlation of estimated ferritin and IL6 levels in SARS-CoV-2 infected ~~patients with~~patients with sociodemographic and clinical features

The inflammatory marker ferritin levels in each SARS-CoV-2 infected patient ~~was~~ wereestimated and ~~on the basis of~~based on estimated ferritin levels the cohort was categorized into three groups those with Mild =<500 ng/ml, Moderate=≥500-<1500 and High levels= ≥1500ng/ml of ferritin as represented by Box and whisker plot (fiq1).Correlation analysis of patients with varying ferritin levels with socio-demographic and clinical features of SARS-CoV-2 infected patients revealedasignificant number of patients with very high ferritin levels (>1500ng/ml) were usually from rural areas, whereas patients from urban areas had mild ferritin levels(<500ng/ml) (p=0.00). In addition, although not ~~significant,in~~significant ~~patients~~in patients with ~~mild and~~mild and moderate levels of ferritin cough ~~was~~ wereone of the ~~symptom~~symptoms whereas ~~puenmonia~~pneumonia was usually found in patients

withextremelywith extremely high levels of ferritin. Further, noFurther, no association was found between the varying levels of ferritin andferritin andthe presence or absence of fever or comorbidities (table3).

Interleukin, IL-6 level in each patient was estimated and on the basis ofbased on their levels patientspatients were categorized into three groups viz:mild=0-<10pg/ml,moderate=≥10-<80pg/mland high=≥80pg/ml, the estimated values in eachis represented by box and whisker plots (fig 2). Correlationanalysis of SARS-CoV-2 infected patients with varying IL6 levels with socio-demographic and clinical featuresrevealed no significant association between very high ILhigh IL6 levels age, gender, ororiginor origin however moderate levels of IL6 were typically found in patients >55 years of age. Except for Cough that whichwas usually found in patients withpatients with very high IL6 levels no significant link waswerefound betweenfound between IL-6 levels and the presence of symptomsof symptoms (fever,hypoxia,puenmoniapneumonia) and/ comorbidities (HTN,COPD,Diabetes,Chest disease). Table 4.

3.3Correlation analysis of SARS-CoV-2 infected patients with varying ferritin and IL6 levels and disease severity /staging

Although not statistically significant, correlation analysis of SARS-CoV-2 infected patients of varying ferritin levels with disease severity revealed percentrevealed percent increase in number of patientsof patients of stage 3 severity as ferritin levels increased from mild,moderatemild, moderateto high levels (table 5). Similarly percentSimilarly percent increase in the number of SARS-CoV-2 infected patients of higher stage or increased

~~severity~~increased severity was found as IL6 levelsincreased from mild to moderate and high levels(table 6).

3.4 ROC analysis of ferritin and IL-6 with disease outcome in SARS-CoV-2

For determining the predictive or prognostic value of the inflammatory marker, ferritin, and ~~pro-~~pro-inflammatory marker, IL6, the ROC analysis of ferritin and IL-6 levels of ~~SARS~~ SARS-infected patients(n=100) (followed for ~~a period of~~ 1 month) with disease outcome (death~~-~~/discharge) w~~as~~ ere done (fig 3).The cutoff value for IL6 was 23.5with the sensitivity of 70% and specificity of 62%. The AUC of IL-6 was ~~0.70 .~~0.70. The cut off value ~~of ferritin~~of ferritin was 26.5with the sensitivity of 60% and ~~specificityof~~specificity of 64%.~~The AUC~~The AUC of ferritin was equivalent to ~~0.66 .~~0.66. The AUC analysis suggested both ferritin and IL-6 as early predictive markers of poor disease outcomes. ~~However~~However, among the two markers based on ~~AUC, IL~~AUC, IL-6 (0.70) is a better early predictive marker of poor disease outcome than ferritin (0.66) in SARS-CoV-2 ~~infected patients~~infected patients from ~~kashmir~~Kashmir. Further ROC analysis of patients with very high ferritin levels (>1500ng/ml) suggests it ~~as~~ is early marker of patients with the hyperinflammatory phenotype (fig 4).

4. DISCUSSION

SARS-COV-2, also known as COVID-19, is a novel form of coronavirus that is responsible for the world's most recent pandemic [3, 4].The practical management of these individuals requires the identification of risk factors for early progression to severe disease and/or poor disease ~~outcome.In~~outcome. In COVID-19, ~~a number of~~several clinical features and laboratory data have been linked to illness severity, hospital stay, and mortality in various

populations[5].According to numerous ~~studiespro~~studies-inflammatory cytokines are also implicated in the pathogenesis of lung injury in SARS-CoV-2 infected individuals. However, it remained to be determined whether clinical features, laboratory findings, or levels of inflammatory markers are the better ~~prognosticaters~~prognosticators in SARS-CoV-2 infected patients from Kashmir, North India.The present study for the first time determined IL6 and ferritin as an early predictive inflammatory marker~~s~~ of disease severity and negative disease outcome in SARS-CoV-2 patients from our region with IL-6 being a better prognostic marker of negative disease outcome in comparison to ferritin and high ferritin (>1500ng/ml) as an early indicator of hyper-inflammatory phenotype in SARS-CoV-2 infected patients.

Among the clinical ~~characteristicscough~~characteristics cough (57%) and ~~puenmonia (~~pneumonia (55%) were dominant symptoms observed in SARS-CoV-2 infected patients of our population. In this study high levels of ferritin [≥1500 ng/mlie > 3fold] were foundin patients with ~~puenmonia~~pneumonia while as cough was more common in patients with mild and moderate levels of ~~ferritin ,~~ferritin.Further patients with h~~igh~~high levels of ferritin usually had severedisease (table 5) and negative ~~disease outcome~~disease outcome (fig 4). This finding is consistent with worldwide studies that report an increase in ferritin levels in SARS-CoV-2 infected patients which then began to decrease as patients began to recover [6,7].ThusFerritin proved to be a prognosticator of disease severity and very high ferritin was an indicator of hyperinflammatory phenotype.Hyperferritinemia causes inflammatory states in SARS-CoV-2 infection, particularly in the lungs, as demonstrated by the presence of a high number of macrophages in the lung parenchyma of SARS-CoV-2 patients, and high ferritin also potentiates the production of IL6[8]. Further, the finding from this study ~~i.e~~i.e. percent increase in the number ~~of SARS~~of SARS-CoV-2 patients ~~of stage~~of stage 3 ~~disease severity~~disease

~~as~~severity as ferritin levels increased from mild, ~~moderate to~~moderate to high level suggest the relationship between disease severity and ferritin ~~levels.Ferritin~~levels. Ferritin and cytokines have been discovered to have feedback mechanisms in the control of pro-inflammatory and anti-inflammatory responses, with cytokines triggering ferritin expression but ferritin promoting both pro-inflammatory and anti-inflammatory cytokine expression[8].Iron overload, in addition to ferritin, is a factor to consider in viral infections. Viral replication, mitochondrial activity, ATP ~~production, synthesis~~production, synthesis and repair of DNA and RNA, and cell survival/ferroptosis are all processes that require iron[9].Iron overload has been associated ~~to~~ with a worse prognosis in HBV and HCV infections, whereas iron supplementation has been linked to an increase in HIV patient mortality[10-13].Iron is necessary for the replication of virus and function in SARS-CoV-2 infections, hence iron chelation therapy is indicated to prevent infection in these patients [14].

In SARS-CoV-2-infected patients, cytokine storm is an interesting feature. The immune response is triggered by cytokines, which result~~s~~ in macrophage and monocyte invasion of the alveoli, causing lung inflammation, lung damage, and ultimately multiorgan failure [15-16].Inconsistent to the present study Patients infected with SARS-CoV-2 have been reported to have cytokines, particularly those with severe disease requiring mechanical ventilation or intubation. According to prior studies, binding the SARS-CoV spike (S) protein to angiotensin-converting enzyme (ACE) 2 causes the production ~~of inflammatory~~of inflammatorycytokines [17-18].As a result of the hyperinflammatory response, the endothelium barrier is ~~disrupted~~disrupted, leading to hypercoagulability. Pulmonary hypertension~~,~~ increased dead space ventilation, and ultimately right heart failure can be caused by diffuse constriction of the pulmonary vascular bed's microcirculation. Acute respiratory distress syndrome develops as a

result of substances in the blood called von Willebrand factor (vWF), soluble thrombomodulin, and soluble P-selectin (sP-sel) (ARDS) [19]. Taken together, our data point toCOVID-19 causes inflammatory ~~endothelialitis~~endotheliosis, which is ~~characterised~~characterized by a direct viral infection of pneumocytes, the production of inflammatory cytokines by epithelial cells, and immune-mediated damage to the surrounding tissue [19].

IL6 is a key mediator of the inflammatory and immunological responses triggered by infection or damage, and it is reported to be elevated in more than half of COVID-19 patients.~~Interestingly,in~~Interestingly, inconsistent~~to~~ withvarious ~~others~~otherstudies, high IL-6 levels (=>80mpg/ml) in our patients was associated ~~to~~ withsevere disease and negative disease outcome [7].Further study~~another~~anotherpro-inflammatory cytokines such as IL1α, IL8, IL10, VEGF, and TNFα is underway in SARS-CoV-2 infected patients from our populationthe results of which will publish~~ed~~ in our future papers [20].

Further,the ROC a~~nalysis~~analysis revealed IL6as a predictor oftransition from mild to severe infection and a prognostic marker ~~of negative~~of negative disease ~~outcome which~~outcome which is consistent ~~to the~~to the meta-analysis (9 studies) reporting > 3fold increase (≥80pg/ml)in IL6 levels as a mortality risk factorin SARS-CoV-~~2 infected~~2 infectedpatients[21].

Inconsistent to our study a study reported that a cut-off value of ferritin level ≥272.5 ng/mL predicted disease severity on admission with a sensitivity of 96%~~,~~ and a specificity of 70% (AUC=0.873) [22-26].In our study, the optimal threshold at 23.5,26.5 for IL6 and ferritin respectively showed a superior prognostic possibility for IL6 over ferritin for patients to change from mild to severe, with an AUC curve of 0.70 at a sensitivity of 60% and specificity

of 64% for IL-6 and AUC for ferritin= 0.66 at sensitivity(70%) and specificity(62%). Further ROC analysis of patients with very high ferritin levels (>1500ng/ml) alone suggests it as an early marker of patients with hyperinflammatory phenotype. So, these patients must be closely monitored by the clinician. Therefore, our results proved IL6 and ferritin are independent early prognostic biomarkers of disease severity and poor disease outcome in SARS-CoV-2 infected patients from our population.

5. Conclusion

We, therefore, conclude~~estimation~~estimation of ferritin and IL6 ~~levels as~~as levels as anearly prognostic tool that can help in clinical ~~decision~~decision-making and choosing treatment options.Tocilizumab, an anti-IL6 drug, and iron chelation therapy, which target the cytokine storm caused by the SARS-CoV-2, are further supported by the study as viable therapeutic options for treating ~~hyperferritinemia~~ ferritinemia hyperserotonemia and targeting the SARS-CoV-2 cytokine storm, respectively, for COVID-19 patients' treatment outcomes.

Declarations

Informed Consent Statement: Written informed consent has been obtained from the patients to publish this paper

Ethic declaration:

This study was approved by the Institutional Ethics Committee (Ref No.IEC-GMC-Sgr/27).

References

1. Yang X,Yu Y , Xu J,Shu H,Xia J. Clinical course and outcomesof critically ill patientswithSARS-CoV-2pneumoniainWuhan,China:a single centered,retrospective,observational study. Lancet Respir Med.2020;8: 475-481.
2. Khan AU, Akter R, Khan FU, Khanom S, Das B, Khan AU and Afsana AS. 2022. Comorbidities, Infections, and Mortalities of COVID-19 in Bangladesh During May-June 2021. *International Journal of Infection*. 9(2): e118083. Doi: 10.5812/iji-118083.
3. HenryBM,OliveiraMD,BenoitS,PlebaniM,LippiG.Hematologic,biochemicalandimmune biomarkerabnormalitiesassociatedwithsevereillnessandmortalityincoronavirusdisease2019(COVID19):ametaanalysis, Clin.Chem.LabMed.2020;58: 1021-1028.
4. **Khan AU**, Proma AA, Akter M, Rahaman MM, and Das S. 2020. A Review on Coronavirus Disease (COVID-19) Epidemic Threat for Global Health in 2020. *American Journal of Microbiological Research*. 8(2): 57-62. Doi: 10.12691/ajmr-8-2-3.
5. RaoultD,ZumlaA,LocatelliF,IppolitoG.Coronavirusinfection:epidemiological,clinical and immunological featuresandhypotheses.cellstress .2020;4:66.
6. Cecconi M ,Piovani D ,Brunetta E , Aghemo A . Early predicators of clinical deteriotion in a cohort of 239 patients hospitalized for COVID-19 infection in lambordy Italy.JclinMed 2020;9:1548
7. Zhou F , Yu T, Du R , Fan G , Liu Y . Clinical course and risk factors for mortality of adult in patients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395: 1054–1062.

8. Liu T, Zhang J, Yang Y, Ma H .The potential role of IL-6 in monitoring severe case of coronavirus disease .EMBO Mol Med 2020;12: e12421.
9. RosarioC,ZandmanGoddardG,MeyronHoltzEG.TheHyperferritinemicSyndrome:macrophage activation syndrome, Still's disease, septic shock and catastrophicantiphospholipidsyndrome. BMCMed 2013;11:185.
10. Khodour Y , Kaguni LS , StibanJ.Iron-sulfur clusters in nucleic acid metabolism: varying roles of ancient cofactors,Enzymes.2019; 45:225–256
11. ThurszM.Iron, haemochromatosis and thalassaemia as risk factors for fibrosis in hepatitis C virus infection.Gut.2007; 56:613–614
12. Galli A, Svegliati-BaroniG , Ceni E , Milani S . Oxidative stress stimulates proliferation and invasiveness of hepatic stellate cells via a MMP2-mediated mechanism. Hepatology 2005;41:1074–1084.
13. KaufmannSH ,McMichaelAJ.Annulling a dangerous liaison: vaccination strategies against AIDS and tuberculosis. NatMed.2005;11:S33–S44.
14. Haider BA , Spiegelman D , Hertzmark E, Sando D . Anemia, iron deficiency, and Iron supplementation in relation to mortality among HIV-infected patients receiving highly active antiretroviral therapy in Tanzani. Am JTrop Med Hyg. 2019;100 : 1512–1520.
15. Liu W , ZhangS , Nekhai S , Liu S.Depriving Iron supply to the virus represents a promising adjuvant therapeutic against viral survival,CurrClin MicrobiolRep 2020;20 : 1–7.
16. ChannappanavarR,PerlmanS. Pathogenichumancoronavirus infections: causes and consequencesofcytokinestormandimmunopathology. SeminImmunopathol 2017;39:529 –539.

17. Lu R , Zhao X, Li J,Niu P.Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding.Lancet 2020;395:565–574.
18. Colling ME, Kanthi Y .COVID-19-associated coagulopathy: An exploration of mechanisms.Vasc Med 2020;25:471478.
19. He L,Ding Y,Zhang Q,Che X.Expression of elevated levels of pro-inflammatory cytokines in SARS-CoV-infected ACE2+ cells in SARS patients: relation to the acute lung injury and pathogenesis of SARS. JPatho.2006;210 :288–297
20. Goshua G,Pine AB,Meizlish ML,Chang CH.Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study, Lancet Haematol.2020; 7 : e575–e582.
21. WongCK,LamCWK,Wu AKL,Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome.Clin ExpImmunol 2004;136:95–103
22. Aziz M ,Fatima R,Assaly R .Elevated interleukin -6 and severe COVID 19:a meta-analysis.JMed Virol2020;92:2283-2285.
23. Cao P,Wu Y,Wu S.Elevatedserum ferritin level effectively discriminates severity illness and predicts prognosis of COVID-19 patients .Res Sq 2020
24. Khan AU, Akter R, Khan FU, Khanom S, Khan AU, Afsana AS. 2021. Second wave and pandemic situation of COVID-(2020-2021) in Bangladesh. *Qubahan Academic Journal*. 1(4): 25–31. https://doi.org/10.48161/qaj.v1n4a74.
25. Khan AU, Akter R, Khan FU, Khanom S, Das B, Khan AU and Afsana AS. 2022. Comorbidities, Infections, and Mortalities of COVID-19 in Bangladesh During May-June 2021. *International Journal of Infection*. 9(2): e118083. Doi: 10.5812/iji-118083.

26. Khan AU. 2022. Due to the COVID-19 Situation, the Death Rate in Bangladesh has Decreased from July-December 2021. *Clinical Medicine and Health Research Journal (CMHRJ)*. 4(2): 155-159. Doi: https://doi.org/10.18535/cmhrj.v2i4.67

Feature	Cases n=100 n=%
Age	
≤55	25
>55	75
Gender	
Male	35
Female	65
Residence	
Rural	42
Urban	58
Symptoms	
a) Cough	
Yes	57
No	43
b) Fever	
Yes	47
No	53
c) Myalgia	
Yes	10
No	90
d)Hypoxia	
Yes	33

No	67
e) Pneumonia	
Yes	55
No	45
f)Diarrhoea	
Yes	6
No	94
Comorbidities	
a) Hypertension	
Yes	52
NO	48
b)COPD	
Yes	8
No	92
c)CD	
Yes	2
No	98
d)CKD	
Yes	5
No	95
e) CLD	
Yes	1
No	99
f) Hypothyroidism	
Yes	11
No	89

Table1: ~~Sociodemogra~~Sociodemographic and clinical features of SARS-CoV-2 infected patients(N=100) admitted ~~in~~ to SMHS hospital.

		Stages		
Features	Cases	2	3	Fisher exact test
		79	21	
Age				
≤55	25 (25%)	22 (27.8%)	3 (14.3%)	0.2
>55	75 (75%)	57 (72.2%)	18 (85.7%)	
Sex				
Female	65 (65%)	56 (70.9%)	9 (42.9%)	0.02
Male	35 (35%)	23 (29.1%)	12 (57.1%)	
Residence				
Rural	42 (42.0%)	37 (46.8%)	5 (23.8%)	0.08
Urban	58% (58%)	42 (53.2%)	16 (76.2%)	
Symptoms				
Cough				
Yes	57 (57%)	48 (60.8%)	12 (57.1%)	0.2
No	43 (43%)	31 (39.2%)	9 (49.9%)	
Fever				
Yes	47 (49.4%)	40 (50.6%)	7 (33.3%)	0.21
No	53 (53%)	39 (49.4%)	14 (66.7%)	
Myalgia				
Yes	10 (10%)	7 (8.9%)	3 (14.3%)	0.43
No	90 (90%)	72 (91.1%)	18 (85.7%)	
Hypoxia				
Yes	33 (33%)	28 (35.4%)	5 (23.8%)	0.43
No	67 (67%)	51 (64.6%)	16 (76.2%)	
Pneumonia				
Yes	55	45	10	0.46

No	**45 (45%)**	**34 (43.0%)**	**11 (52.4%)**	
Diarrhoea				
Yes	**6 (6%)**	**5 (6.3%)**	**1 (4.8%)**	**1.00**
No	**94 (94%)**	**74 (93.7%)**	**20 (95.2%)**	
Diagnostic				
RT-PCR	**78 (78%)**	**65 (83.3%)**	**13 (16.7%)**	**0.07**
RAT	**22 (22%)**	**14 (63.4%)**	**8 (36.4%)**	
Comorbidity				
HTN				
Yes	**52 (52%)**	**46 (58.2%)**	**6 (28.6%)**	**0.02**
No	**48 (48%)**	**33 (41.8%)**	**15 (71.4%)**	
COPD				
YES	**8 (8%)**	**6 (7.6%)**	**2 (9.5%)**	
No	**92 (92%)**	**73 (92.4%)**	**19 (90.5%)**	**1.00**
Diabetes				
Yes	**38 (38%)**	**30 (38.0%)**	**8 (38.1%)**	**1.00**
No	**62 (62%)**	**49 (62.0%)**	**13 (61.9%)**	
Chest Disease				
YES	**2 (2%)**	**2 (2.5%)**	**0 (0.0%)**	**1.00**
NO	**98 (98%)**	**77 (97.5%)**	**21 (100%)**	
CKD				
Yes	**5 (5%)**	**3 (3.8%)**	**2 (9.5%)**	**0.28**

NO	95 (95%)	76 (96.2%)	19 (90.5%)	
Hypothyroidism				
Yes	11 (11%)	10 (12.7%)	1 (4.8%)	0.45
No	89 (89%)	69 (87.3%)	20 (95.2%)	

Table 2: Correlation analysis of the SARS-CoV-2 infected patients of varying disease severity with sociodemographic and clinical features.

		Ferritin				
Features	Cases	Mild <500 (ng/ml)	Moderate ≥500-<1500 (ng/ml)	High ≥1500 (ng/ml)	~~Chi~~ Chi-square	P-value
		55	29	16		
Age						
≤55	25 (25%)	14 (25.5%)	7 (24.1%)	4 (25%)	0.01	0.9
>55	75 (75%)	41 (74.5%)	22 (75.9%)	12 (75%)		
Sex						
Female	65 (65%)	41 (74.5%)	16 (55.2%)	8 (50.0%)	5.0	0.81
Male	35 (35%)	14 (25.5%)	13 (44.8%)	8 (50.0%)		
Residence						
Rural	42 (42%)	16 (29%)	15 (51.7%)	11 (68.8%)	9.51	0.00
Urban	58 (58%)	39 (70.9%)	14 (48.3%)	5 (31.3%)		
Symptoms						
Cough						
Yes	57 (57%)	33 (60.0%)	18 (62.11%)	6 (37.5%)	2.9	0.22
No	43 (43%)	22 (40.0%)	11 (37.9%)	10 (62.5%)		
Fever						
Yes	47 (47%)	29 (52.7%)	12 (41.4%)	6 (37.5%)	1.6	0.4
No	53 (53%)	26 (47.3%)	17 (58.6%)	10 (62.5%)		
Myalgia						

Yes	10 (10%)	7 (12.7%)	2 (6.9%)	1 (93.5%)	1.01	0.60
No	90 (90%)	48 (87.3%)	27 (93.1%)	15 (6.3%)		
Hypoxia						
Yes	33 (33%)	21 (38.2%)	9 (31.0%)	3 (18.8%)	2.18	0.33
No	67 (67%)	34 (61.8%)	20 (69.0%)	13 (81.3%)		
Pneumonia						
Yes	55 (55%)	28 (50.9%)	16 (55.2%)	11 (68.8%)	1.59	0.45
No	45 (45%)	27 (49.1%)	13 (44.8%)	5 (31.3%)		
Diarrhoea						
Yes	6 (6%)	4 (7.3%)	0 (0%)	2 (12.5%)	3.20	0.20
No	94 (94%)	51 (92.7%)	29 (100%)	14 (87.5%)		
Diagnostic						
RT-PCR	78 (78%)	46 (59.0%)	18 (23.1%)	14 (17.9)	6.1	0.04
RAT	22 (22%)	9 (40.9%)	11 (50.0%)	2 (9.1%)		
Comorbidity						
HTN						
Yes	52 (52%)	31 (56.4%)	17 (58.6%)	4 (25.0%)	5.60	0.06
No	48 (48%)	24 (43.6%)	12 (41.4%)	12 (75%)		
COPD						
YES	8 (8%)	4 (7.3%)	3 (10.3%)	1 (6.3%)	0.32	0.85
No	92 (92%)	51 (92.7%)	26 (89.7%)	15 (93.8%)		
Diabetes						
Yes	38	16	15	7		

	(38%)	(29.1%)	(51.7%)	(43.8%)	4.39	1.11
No	62 (62%)	39 (70.9%)	14 (48.3%)	9 (56.3%)		
Chest Disease						
YES	2 (2%)	1 (1.8%)	1 (3.4%)	1 (0%)	0.64	0.72
NO	98 (98%)	54 (98.2%)	28 (96.6%)	16 (100%)		
CKD						
Yes	5 (5%)	2 (3.6%)	1 (3.4%)	2 (12.5%)	2.25	0.32
NO	95 (95%)	53 (96.4%)	28 (96.6%)	14 (87.5%)		
Hypothyroidism						
Yes	11 (11%)	7 (12.7%)	3 (10.3%)	1 (6.3%)	0.54	0.76
No	87 (87%)	48 (87.5%)	26 (89.7%)	15 (93.8%)		

Table 3: Correlation analysis of ferritin levels (Mild <500 ng/ml, Moderate≥500-<1500, and High levels ≥1500ng/ml) with sociodemographic and clinical features of SARS-CoV-2 infected patients.

	IL-6 levels (pg/ml)					
Features	Cases	Mild 0-<10 (pg/ml)	Moderate ≥10-<80 (pg/ml)	High ≥80 (pg/ml)	~~Chi~~ Chi-square	P-value
		33	63	4		
Age						
≤55	25 (25%)	10 (30.3%)	13 (2.6%)	2 (50%)	2.4	0.2
>55	75 (75%)	23 (20.6%)	50 (79.4%)	2 (50%)		
Sex						
Female	65 (65%)	23 (69.7%)	40 (63.5%)	2 (50%)	0.77	0.67
Male	35 (35%)	10 (30%)	23 (36.5%)	2 (50%)		
Residence						

Rural	42 (42%)	16 (48.5%)	24 (38.1%)	2 (50%)	1.069	0.58
Urban	58 (58%)	17 (51.5%)	39 (61.9%)	2 (50%)		
Symptoms						
Cough						
Yes	57 (57%)	20 (60.6%)	34 (54%)	3 (75%)	0.94	0.62
No	43 (43%)	13 (39.4%)	29 (46.0%)	1 (25%)		
Fever						
Yes	47 (47%)	18 (54.5%)	27 (42.9%)	2 (50%)	1.2	0.54
No	53 (53%)	15 (45.5%)	36 (57.1%)	2 (50%)		
Myalgia						
Yes	10 (10%)	5 (15.2%)	5 (7.9%)	0 (0%)	1.71	0.42
No	90 (90%)	28 (84.8%)	58 (92.1%)	4 (100%)		
Hypoxia						
Yes	33 (33%)	12 (36.4%)	19 (30.2%)	2 (50%)	0.92	0.63
No	67 (67%)	21 (63.6%)	44 (69.8%)	2 (50%)		
Pneumonia						
Yes	55 (55%)	16 (48.8%)	38 (60.3%)	1 (25%)	2.74	0.25
No	45 (45%)	17 (51.5%)	25 (39.7%)	3 (75%)		
Diarrhoea						
Yes	6 (6%)	3 (9.1%)	1 (1.6%)	2 (50%)	16.4	0.00
No	94 (94%)	30 (90.9%)	62 (98.4%)	2 (50%)		
Diagnostic						
RT-PCR	78 (78%)	27 (34.6%)	47 (60.3%)	4 (5.1%)	1.8	0.4

RAT	22 (22%)	6 (27.3%)	16 (72.7%)	0 (0.0%)		
Comorbidity						
HTN						
Yes	52 (52%)	19 (57.6%)	32 (50.8%)	1 (25%)		
No	48 (48%)	14 (42.4%)	31 (49.2%)	3 (75%)	1.61	0.44
COPD						
YES	8 (8%)	3 (9.1%)	5 (7.9%)	0 (0%)		
No	92 (92%)	30 (90.9%)	58 (92.1%)	4 (100%)	0.4	0.8
Diabetes						
Yes	38 (38%)	13 (39.4%)	25 (39.7%)	0 (50%)	2.5	0.2
No	62 (62%)	20 (60.6%)	38 (60.3%)	4 (50%)		
Chest Disease						
YES	2 (2%)	0 (0%)	2 (3.2%)	0 (0%)	1.19	0.54
NO	98 (98%)	33 (33%)	61 (96.8%)	4 (100%)		
CKD						
Yes	5 (5%)	0 (0%)	3 (4.8%)	2 (50%)	18.7	0.00
NO	95 (95%)	33 (100%)	60 (95.2%)	2 (50%)		
Hypothyroidism						
Yes	10 (10%)	5 (15.4%)	6 (9.5%)	0 (0%)	1.2	0.5
No	90 (90%)	28 (84.8%)	57 (90.5%)	4 (100%)		

Table 4: Correlation analysis of IL6 levels (Mild 0-<10pg/ml,Moderate ≥10-<80pg/ml, and high levels≥80pg/ml) with the sociodemographic and clinical features of SARS-CoV-2 infected patients (N=100) admitted in SHMS hospital.

		Ferritin				Chi-square test	P value
		Mild	Moderate	High	Total		
Stage	2	47	22	10	79	4.1	0.1
		85.5%	75.9%	62.5%	79.0%		
	3	8	7	6	21		
		14.5%	24.1%	37.5%	21.0%		
Total		55	29	16	100		
		100.0%	100.0%	100.0%	100.0%		

Table5:Correlation analysis of SARS-CoV-2 infected patients with varying ferritin levels with the severity of disease

		IL6				Chi square test	P value
		Mild	Moderate	High	Total		
Stage	2	30	46	3	79	4.2	0.1
		90.9%	73.0%	75.0%	79.0%		
	3	3	17	1	21		
		9.1%	27.0%	25.0%	21.0%		
Total		33	63	4	100		
		100.0%	100.0%	100.0%	100.0%		

Table 6:Correlation analysis of SARS-CoV-2 infected patients of varying IL-6 levels with the disease severity.

Figure 1.

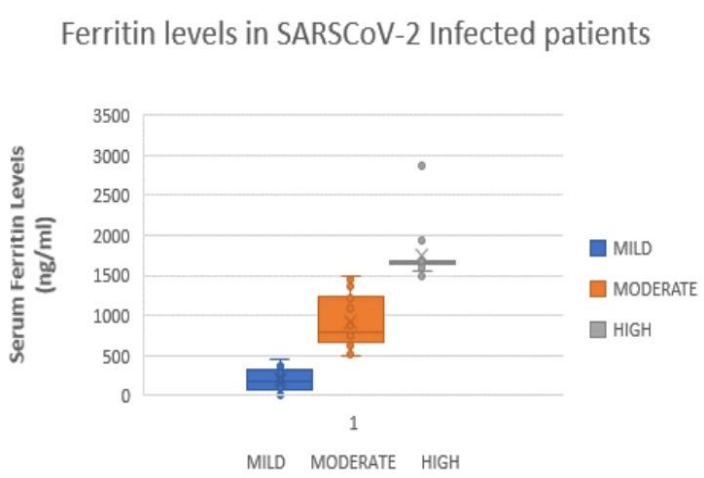


Figure 2.

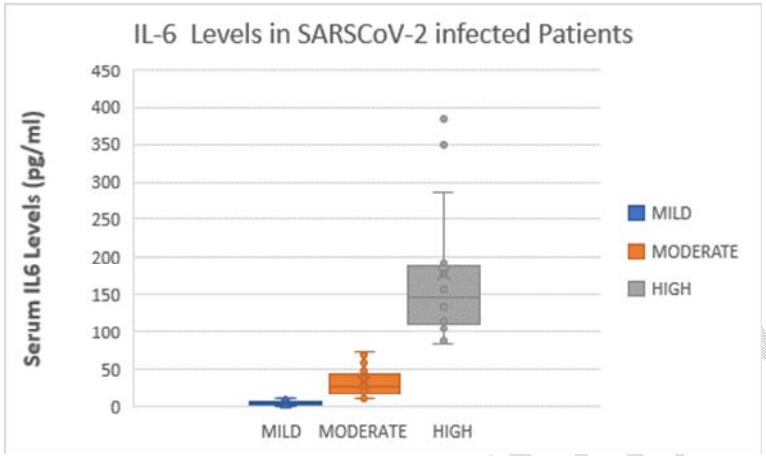

IL-6 Levels in SARSCoV-2 infected Patients
Serum IL6 Levels (pg/ml)
450
400
350
300
250
200
150
100
50
0
MILD
MODERATE
HIGH
MILD
MODERATE
HIGH

Figure 3.

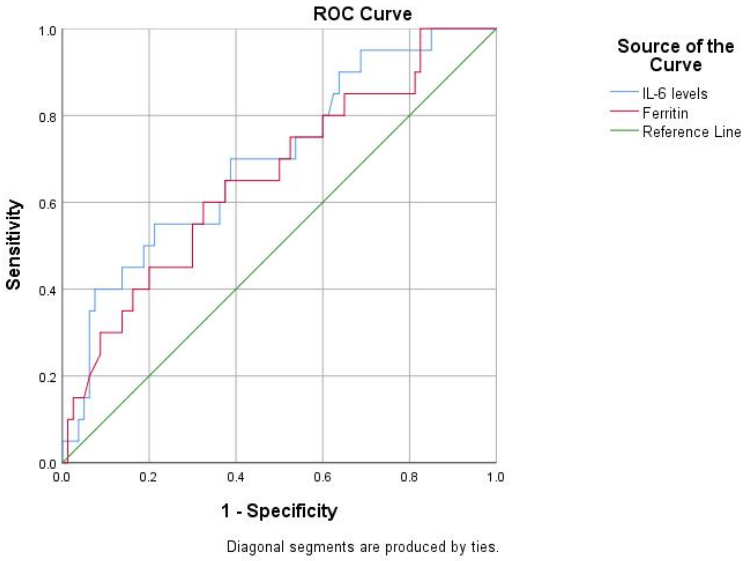
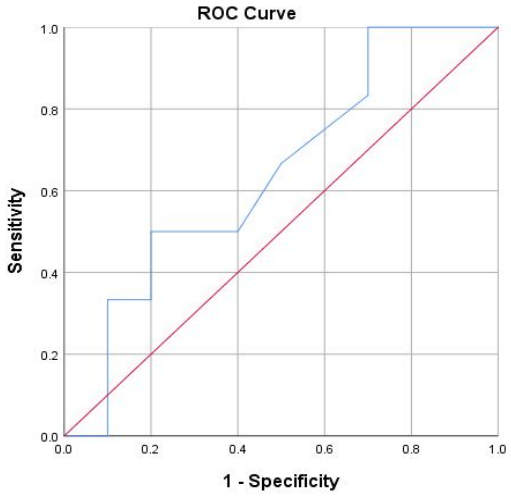

ROC Curve
Sensitivity
1 - Specificity
0.0
0.2
0.4
0.6
0.8
1.0
Source of the Curve
IL-6 levels
Ferritin
Reference Line
Diagonal segments are produced by ties.

Figure 4.

Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
IL-6 levels	.700	.066	.006	.571	.828
Ferritin	.660	.069	.028	.524	.795



Diagonal segments are produced by ties.

Area under curve	Std. Error[a]	Asymptotic Sig.[b]	Lower Bound	Upper Bound
Ferritin .642	**.143**	.357	.361	.923

FIGURE CAPTION

Figure 1. Box-whisker plot depicting the Ferritin levels of SARS- CoV-2 patients. Patients are Categorized ~~on the basis of~~based on levels into three categories. (Mild levels =<500ng/ml, Moderate levels= 500-<1500ng/ml, and Higher levels 1500ng/ml and above**).**

Figure 2: Box-Whisker Plot depicting the IL-6 levels of SARS-CoV-2 patients. Patients are categorized ~~on the basis of~~based on levels into three categories. (Mild levels = <10pg/ml, Moderate levels= ≥10pg/ml, Higher levels=≥80pg/ml).

Figure 3:Reciever operator curve of IL6 and ferritin levels with disease outcome. ~~In order t~~To find their association with the outcome of disease, we analyzed the optimal cut-off values calculated by the ROC analysis, and the ROC curves were presented in ~~fiq~~ figwith AUC of IL-6 = 0.77 with sensitivity (70%) and specificity (62%)and AUC ferritin = 0.60 with sensitivity (60%) and specificity(64%). the optimal threshold for IL6 and ferritin was 23.5, 26.5

Figure 4: ROC analysis of high levels of ferritin with disease outcome in SARS-CoV-2 infected patients.