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 understand the causes of poor disease outcome and in management of COVID-19 disease.

Objectives:

1. To estimate ferritin and IL6 levels for predicting inflammatory 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 inflammatory cytokines, 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 for a period of I month (14th and 28 th day) were included in this cohort study. The sociodemographic features and co-morbiditiesof the infected patients were recorded . Clinically patients were of 3 stages .

Results:

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One of the hallmark feature 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.Here, we estimated ferritin and IL6 levels by ELISA and fully automated immunoanalyzer 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 estimated ferritin levels, the cohort was categorized into Mild= <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 revealed percent increase in number of patients of stage 3 severity as ferritin levels increased from mild,moderate to high levels. Similarly percent increase in 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 70% & specificity 62%, is a better early predictive marker of poor disease outcome than ferritin with AUC=0.66 at sensitivity of 60% and specificity of 64% in SARS -CoV-2 infected patients from Kashmir. Further ROC analysis of patients with very high ferritin levels (>1500ng/ml) alone suggests it as early marker of patients with hyperinflammatory phenotype.

Conclusion:

Study concluded that the estimation of ferritin and IL6 levels as a simple complementary early prognostic tool that can help in clinical 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

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1. INTRODUCTION

In December 2019, Wuhan, China, reported an outbreak of a distinct coronavirus-based sickness. The WHO named this coronavirus "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) and the condition it caused "coronavirus disease 2019 (COVID-19).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 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].

The mechanism implicated in COVID-19 is the dysregulated host Immune Response (IR) reflected by excessive innate and inadequate adaptive immune response.In patients with SARS-CoV-2 infection, a rapid course of acute lung injury (ALI) and ARDS has been hypothesised to be caused by an excessively proinflammatory host response. There has been overwhelming evidences supporting the theory that not the SARS-COV-2 itself but the widespread inflammatory response that it triggers reflected by massive cytokine or chemokine release that causes the organ damage. The so called “cytokine storm” is the leading factors 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 cytokine., and ferritin is an important inflammatory marker in Covid-19. Meta-analysis of various studies conducted worldwide have recommended 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 [2].Despite the available information about predictive/prognostic

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markers in COVID-19, it has been observed people belonging to geographically diverse regions and of different ethnicity and geneticity behaves differently against SARS-CoV-2 virus. While some are able to 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.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 as markers of inflammatory and pro-inflammatory immune response in SARS-CoV-2 infected patients from Kashmir.

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2. Materials and methods

The present study was initiated after the approval obtained from theInstitutional Ethical Committee, Goverment Medical College, Srinagar (IEC-GMC-Sgr/27,19th Dec,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 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) and co-morbidities (diabetes, hypertension,COPD,CKD,hypothyroidism) of the infected patients were recorded.Clinically patients were of 3 stages viz Stage 1 patients with symptoms like myalgia, dry cough, headache;Stage2 (IIa+IIb) patientswith symptoms like high fever, cough, lymphopenia, raised CRP levels, with hypoxia in subgroup (IIb) and without hypoxia in subgroup (IIa); Stage3 patients include those with 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 IL6 Elisa kit (Diaclone) and fully automated analyzer (Siemens).

3. Results

3.1 General characteristic feature of SARS-CoV-2 infected patients (n=100) and their correlation 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 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) and co-morbidities (diabetes, hypertension,COPD,CKD,hypothyroidism) 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 urban origin, and without comrbidities. Among comorbidities, hypertension was most common finding in this cohort. Clinically patients were of 3 stages viz Stage 1 patients with symptoms like myalgia, dry cough, headache;Stage2 (IIa+IIb) patientswith symptoms like high fever, cough, lymphopenia, raised CRP levels, with hypoxia in subgroup (IIb) and without hypoxia in subgroup (IIa); Stage3 patients include those with 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/stages with sociodemographic and clinical characteristics 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 stage3 severity were of urban origin and no link between the presence of comorbidities and stage/ disease severity was observed (table 2) .

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

The inflammatory marker ferritin levels in each SARS-CoV-2 infected patient was estimated and on the basis of 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 revealed significant 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 patients with mild and moderate levels of ferritin cough was one of the symptom whereas puenmonia was usually found in patients withextremely high levels of ferritin. Further, no association was found between the varying levels of ferritin and presence absence of fever or comorbidities (table3).

Interleukin, IL-6 level in each patient was estimated and on the basis of their levels patients were categorized into three groups viz:mild=0-<10pg/ml,moderate=≥10-<80pg/mland high=≥80pg/ml, the estimated values in each is represented by box and whisker plots (fig 2). Correlationanalysis of SARS-CoV-2 infected patients with varying IL6 levels with socio-

demographic and clinical features revealed no significant association between very high IL6 levels age, gender, ororigin however moderate levels of IL6 were typically found in patients >55 years of age. Except Cough that was usually found in patients with very high IL6 levels no significant link was found between IL-6 levels and presence of symptoms (fever,hypoxia,puenmonia) 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 percent increase in number of patients of stage 3 severity as ferritin levels increased from mild,moderate to high levels (table 5). Similarly percent increase in number of SARS-CoV-2 infected patients of higher stage or increased severity was found as IL6 levels increased 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 inflammatory marker, ferritin and pro inflammatory marker, IL6, the ROC analysis of ferritin and IL-6 levels of SARS infected patients(n=100) (followed for a period of 1 month) with disease outcome (death / discharge) was done (fig 3).The cutoff value for IL6 was 23.5with sensitivity of 70% and specificity of 62%. The AUC of IL-6 was 0.70 . The cut off value of ferritin was 26.5with sensitivity of 60% and specificityof 64%. The AUC of ferritin was equivalent to 0.66 . The AUC analysis suggested both ferritin and IL-6 as early predictive markers of poor disease outcome. However

among the two markers based on 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 from kashmir. Further ROC analysis of patients with very high ferritin levels (>1500ng/ml) suggests it as early marker of patients with 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].The practical management of these individuals requires the identification of risk factors for early progression to severe disease and/or poor disease outcome.In COVID-19, a number of clinical features and laboratory data have been linked to illness severity, hospital stay, and mortality in various populations[4].According to numerous studiespro-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 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 markers of disease severity and negative disease outcome in SARS-CoV-2 patients from our region with IL-6 a better prognostic marker of negative disease outcome in comparison to ferritin and high ferritin (>1500ng/ml) as early indicator of hyper-inflammatory phenotype in SARS-CoV-2 infected patients.

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Among the clinical characteristicscough (57%) and puenmonia (55%) were dominant symptoms observed in SARS-CoV-2 infected patents of our population. In this study high levels of ferritin [≥1500 ng/ml ie > 3fold] were found in patients with puenmonia while as

cough was more common in patients with mild and moderate levels of ferritin .Further patients with high levels of ferritin usually had severedisease (table 5) and negative disease outcome (fig 4). This finding is consistent with worldwide studies that report increase in ferritin levels in SARS-CoV-2 infected patients which then began to decrease as patients began to recover [5,6].Thus Ferritin proved to be a prognosticator of disease severity and very high ferritin as 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[7]. Further the finding from this study i.e percent increase in number of SARS-CoV-2 patients of stage 3 disease severity as ferritin levels increased from mild, moderate to high level suggest relationship between disease severity and 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[7] .Iron overload, in addition to ferritin, is a factor to consider in viral infections. Viral replication, mitochondrial activity, ATP production, synthesis and repair of DNA and RNA, and cell survival/ferroptosis are all processes that require iron[8]. Iron overload has been associated to a worse prognosis in HBV and HCV infections, whereas iron supplementation has been linked to an increase in HIV patient mortality[9-12].Iron is necessary for replication of virus and function in SARS-CoV-2 infections, hence iron chelation therapy is indicated to prevent infection in these patients [13].

In SARS-CoV-2-infected patients, cytokine storm is an interesting feature. The immune response triggered by cytokines, which results in macrophage and monocyte invasion of the

alveoli, causing lung inflammation, lung damage, and ultimately multiorgan failure [14,15]. In consistent to 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 cytokines [16,17]. As a result of the hyperinflammatory response, the endothelium barrier is distrupted, 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) [18]. Taken together, our data point toCOVID-19 causes inflammatory endothelialitis, which is characterised by direct viral infection of pneumocytes, the production of inflammatory cytokines by epithelial cells, and immune-mediated damage to the surrounding tissue [18].

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 consistent to various others studies, high IL-6 levels (=>80mpg/ml) in our patients was associated to severe disease and negative disease outcome [6].Further study onother pro-inflammatory cytokine such as IL1α, IL8, IL10, VEGF, TNFα is underway in SARS-CoV-2 infected patients from our population the results of which will published in our future papers [19].

Further,the ROC analysis revealed IL6 a predictor of transition from mild to severe infection and a prognostic marker of negative disease outcome which is consistent to the meta-analysis

(9 studies) reporting > 3fold increase (≥80pg/ml)in IL6 levels as a mortality risk factor in SARS-CoV-2 infected patients[20].

In consistent 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) [21]. 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 AUC curve of 0.70 at 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 early marker of patients with hyperinflammatory phenotype. So, these patients must be closely monitored by clinician. Therefore, our results proved IL6 and ferritin are independent early prognostic biomarker of disease severity and poor disease outcome in SARS-CoV-2 infected patients from our population.

5. Conclusion

We, therefore, concludeestimation of ferritin and IL6 levels as a early prognostic tool that can help in clinical 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 and targeting the SARS-CoV-2 cytokine storm, respectively, for COVID-19 patients' treatment outcomes.

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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).

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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) Puenmonia	
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: Sociodemograhic and clinical features of SARS-CoV-2 infected patients(N=100) admitted in 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 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 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.

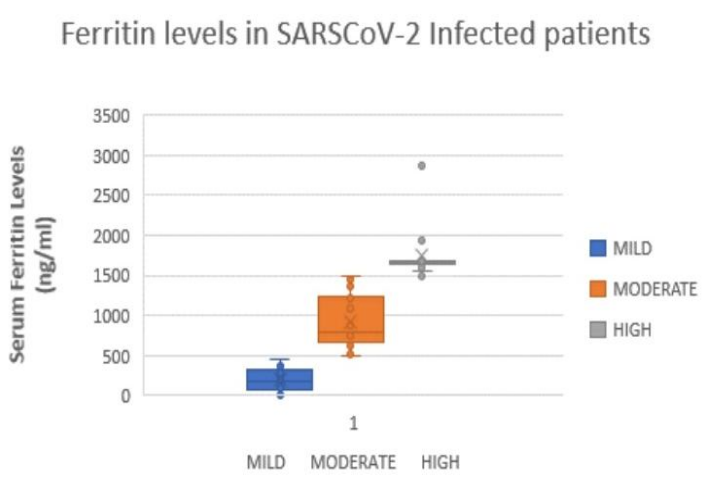


Figure 2.

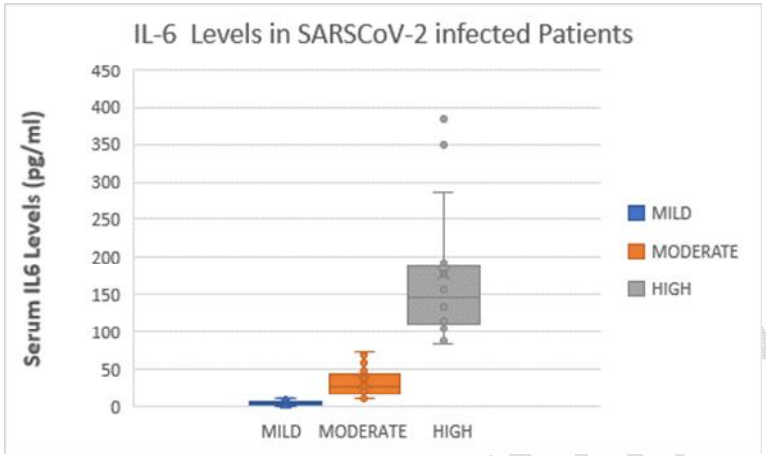

IL-6 Levels in SARSCoV-2 infected Patients
Serum IL6 Levels (pg/ml)
450
400
350
300
250
200
150
100
50
0
MILD
MODERATE
HIGH
MILD
MODERATE
HIGH

Figure 3.

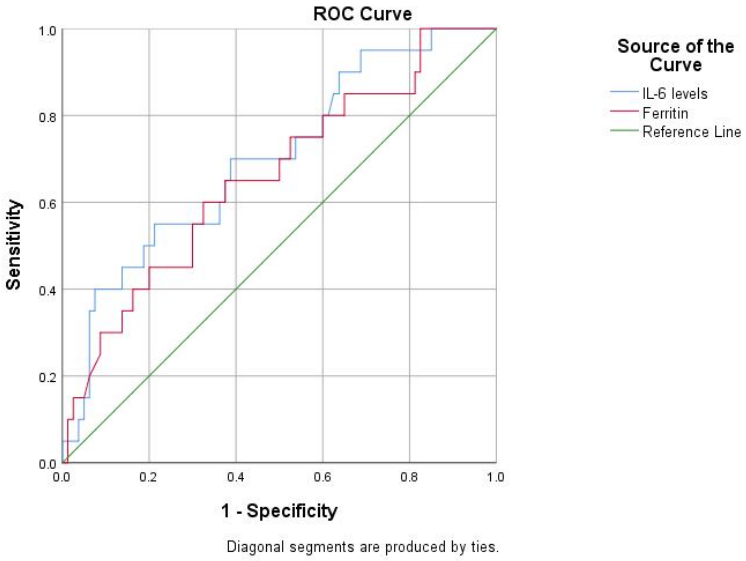
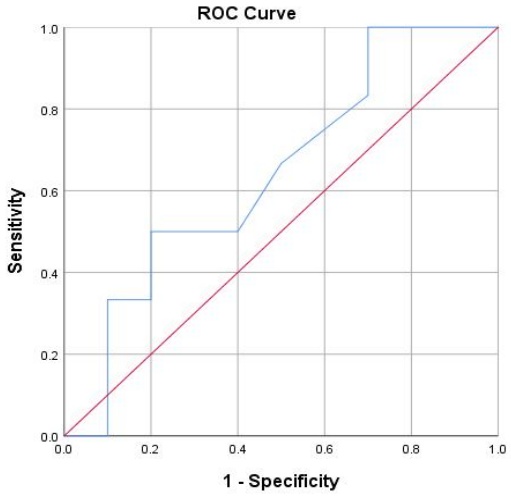

ROC Curve
Sensitivity
1 - Specificity
0.0
0.2
0.4
0.6
0.8
1.0
Source of the Curve
IL-6 levels
Ferritin
Reference Line
Diagonal segments are produced by ties.

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 Categorized on the basis of 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 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 to find their association with outcome of disease, we analyzed the optimal cut-off values calculated by the ROC analysis, and the ROC curves were presented in fiq with 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.