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Interleukin-6 and Ferritin as Prognosticators in SARS-CoV-2 Patients from Kashmir, North India

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IF and RE did conceptualization of the study. Authors MT and SH did data curation of the manuscript. Authors IF and SF performed the formal analysis. Author IJ investigated the work. Authors IF, SS, RE and SM wrote the original draft as well as reviewed and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Identifying early predictive biomarkers of disease severity and disease outcome is fundamental for the practical management of Covid -19 patients. Although prognostic value of several Pro- and inflammatory markers have been determined in different population studies, however, it remained to be determined in SARS-CoV-2 patients from Kashmir.
Aim: To evaluate the predictive value of ferritin and IL-6 levels in RT- PCR confirmed SARS-CoV-2 patients from Kashmir.
Study Design: A Cohort Study.

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Place and Duration of Study: The study was done at Government Medical College, Srinagar Kashmir, North India between October 2020 to November 2021.
Method: Here, we estimated ferritin and IL-6 levels respectively by chemiluminescent method on fully automated immune analyzer and by ELISA in a cohort of 100 RT-PCR confirmed SARS-CoV-2 patients, which were followed twice (14th and 28th day) for a period of one month.
Results: Based on 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 patients of varying ferritin levels with disease severity revealed a percent increase in the number of patients of stage 3 severity as ferritin levels increased from mild, to moderate and high levels. Similarly, a percent increase in the number of SARS-CoV-2 patients of increased severity was found as IL-6 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 IL-6 as early predictive markers of poor disease outcome. However, IL-6, with AUC =0.70 and sensitivity of 70% and specificity of 62%, is a better early predictive marker of poor disease outcome than ferritin with AUC=0.66 at sensitivity of 60% and specificity of 68% in SARS-CoV-2 patients from Kashmir. Further ROC analysis of patients with very high ferritin levels (>1500ng/ml) alone suggests it as an early predictive marker of patients with hyperinflammatory phenotype.
Conclusion: Estimation of ferritin and IL-6 levels as simple complementary early prognostic markers that are helpful in clinical decision-making and selecting appropriate treatment options in SARS-CoV-2 patients from Kashmir, North India.

Keywords: Ferritin; IL-6; cytokine storm, SARS-CoV-2; pathophysiology; RT-PCR.

1. INTRODUCTION

Globally 525,467,084 confirmed Severe Acute Respiratory Syndrome Coronavirus -2 (SARS-CoV-2) cases and 6,285,171 deaths has been reported by WHO (as of May 2022). Clinically majority of SARS-CoV-2 cases either had no symptoms or had mild to severe respiratory illnesses. In severe cases, however, multi-organ and systemic symptoms like sepsis, septic shock, and multiple organ dysfunction syndromes (MODS) [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. There have been overwhelming evidence supporting the theory that not SARS-COV-2 itself but the widespread inflammatory response that it triggers as reflected by massive cytokine or chemokine release that causes the organ damage. The so-called “cytokine storm” is the leading factor that trigger the pathological processes leading to plasma leakage, vascular permeability, and disseminated vascular coagulation as observed in SARS-CoV-2 patients accounting for life-threatening respiratory symptoms as Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS).

IL-6 is one of the important pro-inflammatory cytokines and ferritin an important inflammatory marker in Covid-19. Meta-analysis of various studies in SARS-CoV-2 patients conducted worldwide have recommended the estimation of these immune markers during hospitalization to recognize high-risk individuals that can develop ARDS and are thus clinical predictors of severe and fatal COVID-19 [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 behaves differently against the SARS-CoV-2 virus. While some can resolve it quickly others succumb to the disease. Furthermore, there is a possibility that SARS-CoV-2 could modify host innate immune responses 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 Kashmiri population. As a part of this initiative, here in, we evaluated 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.

2. MATERIALS AND METHODS

The present study was initiated after obtaining approval from the Ethical committee of Government Medical College, Srinagar (IEC-GMC-Sgr/ 27,19thDec,2020) and included SARS-

CoV-2 patients hospitalized at SHMS Hospital between Oct. 2020 and Nov 2021. In total 100 SARS-COV-2 (RT-PCR confirmed) patients followed twice (14^{th} and 28^{th} day) for a period of I month were included in this cohort study. The levels of ferritin and IL-6 were estimated respectively by chemiluminescent method on fully automated immune analyzer (Siemens) and by ELISA (using IL6 Elisa kit, Diaclone on multimode ELISA reader, Berthold) The statistical evaluation was done by SPSS software. ROC analysis was done for determining the prognostic value of each marker.

3. RESULTS

3.1 Characteristic Features of SARS-CoV-2 Infected Patients (n=100) and their Correlation to Clinical Staging

The median age of the cohort (100 SARS-CoV-2 cases) 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 SARS-CoV-2 patients were recorded and is represented in Table 1. Most of the SARS-CoV-2 patients in this cohort were >55 years of age, mostly females, of urban origin, and without co-morbidities. Among co-morbidities, hypertension was the most common finding in this cohort. Clinically patients were of 3 stages viz: Stage 1 patients with symptoms like myalgia, dry cough, headache; Stage 2(IIa+IIb) patients with symptoms like high fever, cough, lymphopenia, raised CRP levels, with hypoxia in the subgroup (IIb) and without hypoxia in the subgroup (IIa); Stage3 patients include those with the severe systemic inflammatory syndrome culminating into severe respiratory failure. The patients after following for 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 socio-demographic and clinical characteristics revealed significant number of SARS-CoV-2 patients of stage 2 severity as females compared to males mostly of stage 3 severity. Most of the patients with stage 3 severity were of urban origin and no association between the presence of co-morbidities and stage/ disease severity was observed (Table 2).

3.2 Correlation of Ferritin and IL-6 Levels with Socio-demographic and Clinical Features in SARS-CoV-2 Patients

The inflammatory marker ferritin levels in each SARS-CoV-2 patient were estimated and based on estimated ferritin levels the cohort was categorized into three groups those with Mild =<500 ng/ml, Moderate=≥500-<1500 and High = ≥1500ng/ml levels of ferritin as represented by Box and whisker plot (Fig. 1).Correlation analysis of patients with varying ferritin levels with socio-demographic and clinical features of SARS-CoV-2 patients revealed significant number of patients with very high ferritin levels (>1500ng/ml) were usually from rural areas compared to patients from urban areas that 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 the commonest symptom whereas pneumonia was usually found in patients with extremely high levels of ferritin. Further, no association was found between the varying levels of ferritin and the presence or absence of fever or co-morbidities (Table 3).

Interleukin, IL-6 level in each patient was estimated and based on their levels patients were categorized into three groups viz: mild=0-<10pg/ml, moderate=≥10-<80pg/ml and high=≥80pg/ml, the estimated values in each is represented by box and whisker plots (Fig. 2). Correlation analysis of SARS-CoV-2 infected patients with varying IL-6 levels with socio-demographic and clinical features revealed no significant association between very high IL-6 levels and age, gender, or origin however moderate levels of IL-6 were typically found in patients >55 years of age. Except Cough, which was usually found in patients with very high IL-6 levels, no significant link were found between IL-6 levels and the presence of symptoms (fever, hypoxia, pneumonia) and/ comorbidities (HTN, COPD, Diabetes, Chest disease)(Table 4).

3.3 Correlation Analysis of Varying Ferritin and IL-6 Levels and Disease Severity /Staging in SARS-CoV-2 Patients

Although not statistically significant, correlation analysis of SARS-CoV-2 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 the number of SARS-CoV-2 patients of higher stage or increased severity was found as IL-6 levels increased from mild to moderate and high levels (Table 6).

Table 1. Sociodemographic and clinical features of SARS-CoV-2 infected patients(n=100) admitted to SMHS hospital

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

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

		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%)	

		Stages		
Features	**Cases**	**2**	**3**	**Fisher exact test**
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 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

		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%)		

		Ferritin				
Features	**Cases**	**Mild <500 (ng/ml)**	**Moderate ≥500-<1500 (ng/ml)**	**High ≥1500 (ng/ml)**	**Chi square**	**P-value**
		55	**29**	**16**		
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%)		

		Ferritin				
Features	**Cases**	**Mild <500 (ng/ml)**	**Moderate ≥500-<1500 (ng/ml)**	**High ≥1500 (ng/ml)**	**Chi square**	**P-value**
		55	**29**	**16**		
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 (38%)	16 (29.1%)	15 (51.7%)	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 4. Correlation analysis of IL-6 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

		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%)		

		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**		
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%)		

		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**		
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 5. Correlation analysis of SARS-CoV-2 infected patients with varying ferritin levels with the severity of disease

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

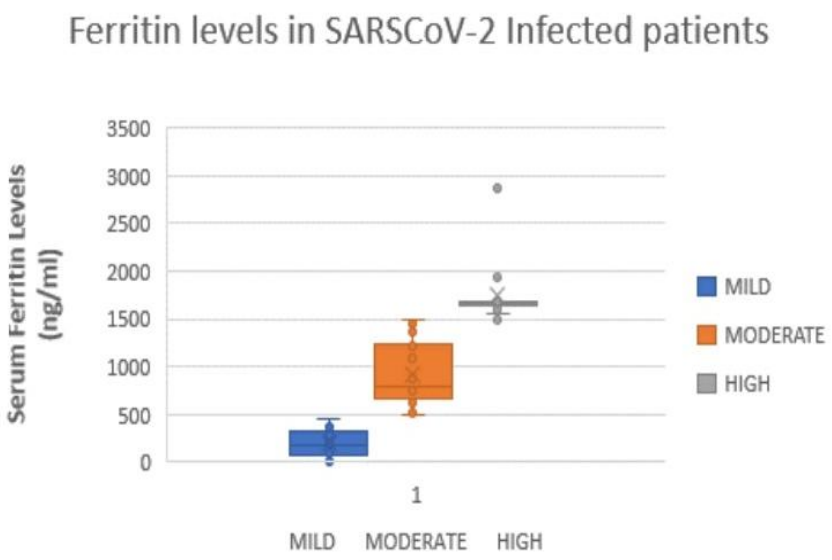


Fig. 1. Box- whisker plot depicting the Ferritin levels of SARS- CoV-2 patients. Patients Categorized on the basis of levels into three categories. (Mild levels =<500ng/ml, Moderate levels= 500-<1500ng/ml, and Higher levels 1500ng/ml and above)

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

		IL6			Total	Chi square test	P value
		Mild	Moderate	High			
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%		

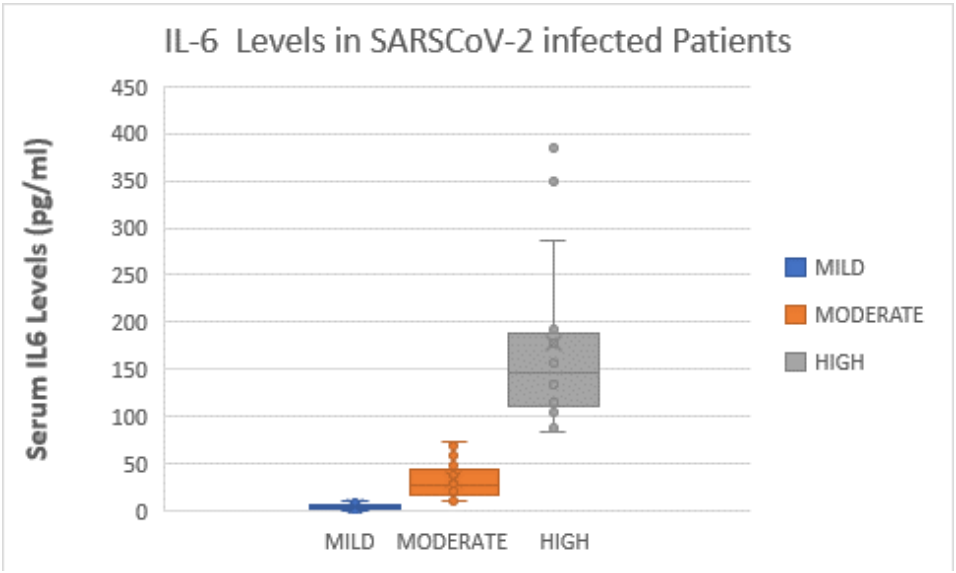
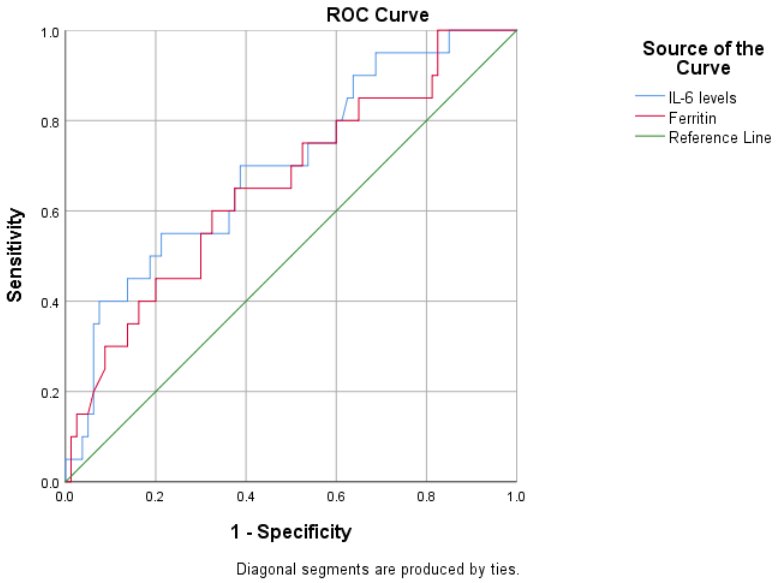
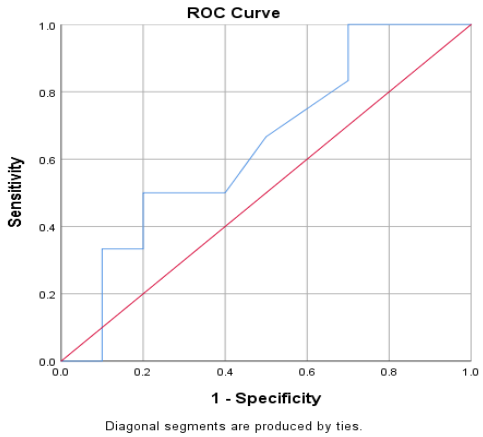


Fig. 2. Box-Whisker Plot depicting the IL-6 levels of SARS-CoV-2 patients. Patients are categorized on the basis of levels into three categories. (Mild levels = <10pg/ml, Moderate levels= ≥10pg/ml, Higher levels=≥80pg/ml)



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	.700	.066	.006	.571	.828
Ferritin	.660	.069	.028	.524	.795

Fig. 3. Reciever operator curve of IL-6 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 figure with AUC of IL-6 = 0.70 with sensitivity (70%) and specificity (62%) and AUC of ferritin = 0.66 with sensitivity (60%) and specificity (68%). the optimal threshold for IL-6 and ferritin was 23.5 and 654



Area Under the Curve					
Test Result Variable	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Ferritin	.642	.143	.357	.361	.923

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

3.4 ROC Analysis of Ferritin, IL-6 Levels and Disease Outcome in SARS-CoV-2 Patients

For determining the predictive or prognostic value of the inflammatory marker, ferritin, and pro-inflammatory marker, IL-6, the ROC analysis of ferritin and IL-6 levels of SARS-CoV-2 patients (n=100) with disease outcome (death/ discharge) (followed twice on 14th and 28th day) were performed (Fig. 3). The cutoff value for IL-6 was 23.5 and AUC = 0.70 with the sensitivity of 70% and specificity of 62%. The cut off value of ferritin was 654 and AUC equivalent to 0.66 with the sensitivity of 60% and specificity of 68%. The area under curve (AUC) suggested both ferritin and IL-6 as early predictive markers of poor disease outcomes. 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 patients from Kashmir. Further ROC analysis of patients with very high ferritin levels (>1500ng/ml) (AUC=0.64) suggests it is early marker of patients with the hyperinflammatory phenotype (Fig. 4).

4. DISCUSSION

Identifying early predictive biomarkers of disease severity and disease outcome is fundamental for the practical management of SARS-CoV-2 patients. Although several clinical features and laboratory finding and inflammatory cytokines have been associated to disease severity, hospital stay, and mortality in various studies conducted worldwide [3]. However, it remained to be determined which among them are the better prognosticators in these patients. The present

study for the first time determined IL6 and ferritin as an early predictive inflammatory bio marker of disease severity and negative disease outcome in SARS-CoV-2 patients from our region (Kashmir ,North India) with IL-6 (AUC=0.70) a better prognostic marker of negative disease outcome in comparison to ferritin (AUC=0.66).Further ROC analysis revealed very high ferritin (>1500ng/ml) (AUC=0.64) as an early indicator of patients that are likely to develop hyper-inflammatory phenotype in SARS-CoV-2 patients .

Among the clinical characteristics cough (57%) and pneumonia (55%) were dominant symptoms in SARS-CoV-2 patients from Kashmir. In consistent to various other studies here very high levels of ferritin [≥1500 ng/ml (ie > 3fold)] were found in patients with pneumonia while as cough was more common in patients with mild and moderate levels of ferritin. The possible reason could be hyperferritinemia induces inflammatory states in viral infection so in SARS-CoV-2 too as demonstrated by the presence of a high number of macrophages in the lung parenchyma of SARS-CoV-2 patients.The hyperferritinemia state supports all those processes that require iron viz: Viral replication, mitochondrial activity, ATP production, synthesis and repair of DNA and RNA, and cell survival/ferroptosis [4].This is further substantiated by finding from previous studies where Iron overload has been associated with a worse prognosis in HBV and HCV infections, and iron supplementation has been linked to an increase in HIV patient mortality [5-8]. Based on these finding iron chelation therapy has been suggested for treating of SARS-CoV-2 infection [9]. Further Ferritin also known to potentiate the production of cytokines like IL-6. Ferritin and cytokines (IL-6) have been discovered to have feedback mechanisms in the control of pro-inflammatory and anti-inflammatory responses,with cytokine triggering ferritin expression but ferritin promoting both pro-inflammatory and anti-inflammatory cytokine expression [10].

Cytokine storm is an interesting feature in SARS-CoV-2 infection. Upon infection binding the SARS-CoV-2 spike (S) protein to angiotensin converting enzyme (ACE) on type II pulmonary alveolar epithelial cells causes the production of inflammatory cytokines [11-12]. which result in macrophage and monocyte invasion of the alveoli, causing lung inflammation, lung damage [13-14]. As a result of the hyperinflammatory response, the endothelium barrier is disrupted, leading to hypercoagulability. Pulmonary hypertension increased dead space ventilation, and ultimately right heart failure. Among cytokines IL-6 is a key mediator of the inflammatory and immunological responses Interestingly, in consistent with various other studies, high IL-6 levels (=>80mpg/ml) in our patients was associated with severe disease and negative disease outcome [15]. Further studies on other pro-inflammatory cytokines such as IL-1α, IL8, IL10, VEGF, and TNF-α in SARS-CoV-2 patients has been worked out separately the results of which will be displayed in future articles [16].Further, the ROC analysis revealed IL6 as 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 IL-6 levels as a mortality risk factor in SARS-CoV-2 infection [17].

Comparative ROC analysis of IL-6 and Ferritin 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 for ferritin AUC= 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 that are likely to develop hyperinflammatory phenotype. So, these patients must be closely monitored by the clinician. Therefore, our results proved IL-6 and ferritin are independent early prognostic biomarkers of disease severity and poor disease outcome in SARS-CoV-2 patients from our population.

5. CONCLUSION

We, therefore, conclude ferritin and IL-6 levels as an early prognostic biomarkers of poor disease outcome. Estimation of these markers, therefore, can help in clinical decision-making and choosing right treatment options. Tocilizumab, an anti-IL6 drug, and iron chelation therapy, which controls iron overload are the viable therapeutic options in treatment of COVID-19.

CONSENT

Written informed consent has been obtained from the patients to publish this paper.

ETHICAL DECLARATION

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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