Journal of Advances in Medicine and Medical Research

33(13): 1-13, 2021; Article no.JAMMR.68277
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Inflammatory and Immune Biomarkers in Predicting the Severity in COVID-19

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SDP and designed the study, performed the statistical analysis. Authors SP and DS wrote the protocol and wrote the first draft of the manuscript. Authors SS and RN managed the analyses of the study. Author EM managed the literature searches and intellectual content. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2021/v33i1330950
Editor(s):
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Complete Peer review History: http://www.sdiarticle4.com/review-history/68277

Original Research Article

Received 25 March 2021
Accepted 31 May 2021
Published 05 June 2021

ABSTRACT

Aims: An early diagnosis of severity can be confidently judged by monitoring the serum biomarkers in patients with COVID-19. The study was thus aimed to explore the relationship of the inflammatory and immune biomarkers in predicting the severity of the disease.
Study design: It is a retrospective observational study.
Methodology: The study included 79 confirmed cases of COVID-19 who had complete clinical record for the analytical variables. All cases were assigned a total clinical score as per their clinical manifestations, associated co-morbidities and mortality outcome. Laboratory inflammatory and immune biomarkers at the time of admission were noted.

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Results: The mean age of the study population was 55.38 (1.69) years. The percentage of admission for males (67.1%) was twice that of females (32.9%). Serum LDH (p=0.003) and ferritin (0.019) levels were remarkably raised in severe form. Total clinical score denoted a positive correlation with the inflammatory biomarkers (p<0.001). IgM exhibited a significant negative trend with increasing clinical score (p<0.001) and CRP levels (p=0.022) of the patients. The multivariate analysis reflected that the total clinical score was significantly influenced by initial SpO_2 values (0.011), serum ferritin (0.027), IgM (0.001) and C3 levels (0.044) in the COVID-19 patients. Lower serum C3 values significantly influenced the hospitalization duration in moderate cases (p=0.034) and total clinical score in severe cases (p=0.01).

Conclusion: The findings of the study signified that besides serum ferritin, a serial and close monitoring of serum IgM with complement factor C3 would aid in early prediction of clinical severity and thus guide physicians to start effective management strategy.

Keywords: Inflammation; total clinical score; immunoglobulins; complement C3.

ABBREVIATIONS

ANOVA	:	*analysis of variance*
ARDS	:	*acute respiratory distress syndrome*
C3 and C4	:	*complement factors 3 and 4*
COPD	:	*chronic obstructive pulmonary disease*
COVID-19	:	*coronavirus infection disease 2019*
CRP	:	*C-reactive protein*
CVD	:	*cardiovascular disease*
DM	:	*diabetes mellitus*
DOH	:	*duration of hospitalization*
LDH	:	*lactate dehydrogenase*
ICU	:	*intensive care unit*
IgG and IgM	:	*immunoglobulin G and M*
RT-PCR	:	*reverse transcriptase-polymerase chain reaction*
SAA	:	*serum amyloid protein*
SARS-CoV-2	:	*severe acute respiratory syndrome coronavirus 2*
SE	:	*standard error of mean*
SpO_2	:	*percentage saturation of oxygen*
TCS	:	*total clinical score*

1. INTRODUCTION

Recent studies have proposed that uncontrolled and altered immune response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been the major immunopathogenic mechanism for coronavirus infection disease 2019 (COVID-19) that leads to acute respiratory distress syndrome (ARDS) and multi-organ failure [1–3]. The co-existence of co-morbid conditions like diabetes mellitus (DM), hypertension, cardiovascular diseases (CVD), underlying pulmonary diseases, cancer therapy highly makes the individual prone for severe grade of the disease and increased probability of death. The unregulated immune response led to hyperinflammation and cytokine storm that begets more admissions in intensive care units (ICU) and high mortality [1,4]. Early recognition of the shift from milder grade to severe grade of the disease would aid physicians to intervene in timely therapy to control inflammation. Besides, there are no specific signs and symptoms that could clearly define the progression of the disease course.

Inflammation activates production of various acute-phase reactants. One such marker, C-reactive protein (CRP) has been linked to severe form of COVID-19 [5,6]. Significant higher levels of severe lactate dehydrogenases (LDH) and ferritin also have been associated with poor prognosis and failure to normalize of the biomarkers was considered as a significant predictor for mortality [7,8]. Coronavirus mediated complement activation has a critical role in immune pathogenesis of disease severity in SARS-CoV-2 infection. Over activation resulting in immune dysregulation has been implicated for poorer clinical outcome [9]. These immune mediated inflammatory changes are quite early to the clinical manifestations. Hence,

by the time a clinician diagnosed the severity changes, the underlying inflammation would have been extensive with poorer outcomes [10]. Therefore, changes in inflammatory and immune biomarkers would provide a more reliable determinant to predict the clinical worsening of COVID-19 patients. The study was thus aimed to explore the relationship of the inflammatory and immune biomarkers in predicting the severity of the disease.

2. MATERIALS AND METHODS

2.1 Study Design

The study was a retrospective observational study approved by Institute Ethics Committee along with the waiver of informed consent. The study was conducted in our institute at Raipur, Chhattisgarh, India.

2.2 Data Collection

Laboratory details of all COVID-19 positive cases evaluated for inflammatory markers and immune markers under study were screened from Central Service Lab. The case records of those were traced retrospectively for clinical details from the Medical Record section of the hospital. Only those with a confirmatory report by reverse transcriptase-polymerase chain reaction (RT-PCR) in their throat/nasopharyngeal swab samples were considered for inclusion. During the two months study period, a total of 79 medical case records with complete demographic, clinical and laboratory data were included. Patients with incomplete medical records with respect to clinical, and laboratory data and those with a history of autoimmune disorders and other chronic and acute infective diseases were excluded from the study. A total of 30 mild, 23 moderate and 26 severe cases were included for the study. They were diagnosed as mild, moderate and severe cases primarily on the basis of percentage saturation of oxygen (SpO_2) measured by pulse oximetry [11].

Data including personal details, demographic, clinical details (co-morbidities, signs and symptoms on admission) were entered into the pre-designed questionnaire.

2.3 Analytical Variables

Taking into account the co-morbid condition, clinical features associated and mortality, a clinical score was assigned to each case which was analyzed statistically. The SpO_2 value at the time of admission, duration of stay and clinical scores were considered as the dependent variables during regression analysis.

Laboratory reports for inflammatory markers, lactate dehydrogenase (LDH), C-reactive protein (CRP) and ferritin levels at the time of admission were noted. Similarly, the biomarkers for immune response immunoglobulins (IgG, IgM) and complements (C3 and C4) reported simultaneously were entered in case record form for analysis.

Serum CRP and LDH were analyzed in fully automated clinical chemistry analyzer AU680 Beckman Coulter Inc. Serum levels of IgG, IgM, C3 and C4 were measured by nephelometry method in Mispa i3 Agappe diagnostics. In addition, serum ferritin assay was performed in Advia Centaur XP immunoassay system from Siemens Healthineers using direct chemiluminescence principle. The biological reference range as per kit insert is provided in (Supplementary Table 1).

2.4 Statistical Analysis

Data analysis was done by SPSS software version 20. The data were transformed to normalized data following which the statistical analysis was performed. The results were reported as frequency percentage, mean and standard error of the mean (SE). Mean values within the group were compared using one–way ANOVA. Correlation between the parameters was performed by Pearson correlation coefficient. Univariate Logistic regression was applied to evaluate of the relationship between the variables with SpO_2, duration of hospitalization and total clinical score. Multivariate Logistic regression was applied for the establishment factor responsible for the outcome variables. Results with ap-value less than 0.05 were considered significant.

3. RESULTS

3.1 General Characteristics of the Variables in the Study Population

The mean (SE) values and distribution of study variables are delineated in Table 1. The mean age of the study population was 55.38 (1.69) years and it was significantly different within the study groups(p=0.93). The percentage of males admitted for COVID-19 (67.1%) was found to be

more than double the percentage of females (32.9%). However, the gender distribution within the study groups was relatively uniform (p=0.12). The mean (SE) days for the duration of hospitalization of the study population was 8.73 (0.47) days with range of 26 days that did not differ significantly among the three groups (p=0.15). 46.8% of the cases had no associated co-morbidities. Diabetes was the most common co-morbidity with a frequency percentage of 40.5% followed by hypertension in 30.4%. The presence of co-morbidities did not reflect any significant association with mild, moderate and severe forms of COVID-19 (p=0.82). However, the frequency percentage of COPD and sickle cell disease in severe cases was nearly three times that of the mild cases. Almost 62% cases had cough followed by fever in 51.9% cases and breathlessness in nearly 48%. Out of the six deaths in the study period, five were admitted with the severe forms of the disease (p=0.02). The total clinical score was significantly higher in severe cases (p<0.001).

3.2 Mean (SE) for the Laboratory Variables in the Study Groups

Serum CRP, LDH, ferritin and IgG levels, were significantly higher in severe cases than in the milder group (p<0.001) (Fig. 1). Except for serum CRP values (p=0.003), none of the biochemical variables showed a significant difference between mild and moderate forms of the disease. When compared to moderate grade, serum LDH (p=0.003) and ferritin (0.019) levels were remarkably raised in severe form.

The levels of inflammatory markers (CRP, LDH, ferritin) were found elevated and that of immunoglobulins and complements were found lowered in severe COVID-19 cases when compared to the mean values of the whole study population (data not shown).

3.3 Correlation between the Study Variables in the Whole Population

The correlation matrix in Table 2 revealed a significant positive correlation of age factor with that of IgG levels (p=0.02). SpO_2 recorded significant negative correlation with the clinical score, CRP, LDH and ferritin levels (p<0.001). On the contrary, clinical score denoted a positive correlation with these inflammatory factors (p<0.001). IgM exhibited a significant negative trend with increasing clinical score (p<0.001) and CRP levels (p=0.022) of the patients. Both complements, C3 and C4 showed a significant positive relationship with each other (p=<0.001)

3.4 Univariate and Multivariate Regression Analysis in the Study Population

Univariate regression analysis in the study population (Supplementary Table 2) reflected significant dependency of SpO_2values with serum CRP levels (p=0.044). The total clinical score was found to be significantly associated with SpO_2 values (p=0.011) and IgM levels (p=0.001). The multivariate analysis reflected that the total clinical score was significantly influenced by initial SpO_2 values (0.011), serum ferritin (0.027), IgM (0.001) and C3 levels (0.044) in the COVID-19 patients

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3.5 Univariate and Multivariate Regression Analysis in the three Study Groups

As illustrated in Table 3, in mild cases (Table 3a), SpO_2 values at the time of admission were found to be significantly related to the patient's serum LDH (p=0.012), ferritin (p=0.005) and IgM (p=<0.001) levels. Age depicted a significant impact on the duration of hospitalization (p=0.037) and total clinical score (p=0.049) in the milder form of the disease. Serum ferritin was significantly influenced initial SpO_2, duration of stay and the total clinical score. Besides serum ferritin, multivariate analysis reflected a strong effect of age (p=0.049), serum CRP (0.002) and serum IgG (<0.001) levels on the clinical score in milder grade of COVID-19. Whereas, reducing trend in serum IgM significantly influenced the duration of stay in them.

Unlike the mild COVID-19 group, in moderate and severe cases, both serum ferritin and C3 levels depicted significant impact on SpO_2 values at the time of admission. Duration of hospitalization was highly influenced by serum C3 values in moderate cases (p=0.034) (Table 3b). Lowered complement factor C3 was found to affect the total clinical score in severe cases (p=0.01) with ahigh probability of death (Table 3c).

4. DISCUSSIONS

The study included analysis of inflammatory and immune biomarkers with clinical variables in COVID-19 confirmed cases admitted in our institute. Nearly 50% of the cases admitted under

moderate and severe cases had associated co-morbidity like diabetes and hypertension. Usually, with increasing age, individuals are associated with one or the other co-morbid conditions. Nearly, 71.4% of cases in this study with one or more co-morbidity were at or above 55 years of age. These individuals are considered to more under effect of chronic inflammation due to associated co-morbidity. Hence, IgG showed a positive trend with age (p=0.02) (Table 2) as a marker of chronic inflammation.

Male patients showed 3.98 times higher risk for severity (95%CI:1.04-15.27; p=0.044) when compared to females (data not shown). Jin et al in their study observed higher severity risk in men (p=0.035) [12]. The odds for men was shown to be 2.84 times for admission to the intensive care unit in Peckham et al study [13]. The probability of death in males was 25% times to that of females enrolled in the present study. In presence of co-morbidity, the risk for severe form of the disease was increased by about 14%. With age, the incidences of co-morbidities also increase both of which have been associated with poorer outcomes. In addition, slow immune response and intake of medications for co-morbid conditions impair immune protection and further accelerate the adverse clinical outcome [14,15].

86.1% of the study population presented with one or more clinical signs and symptoms of which breathlessness with and without other features (Table 1), was most common (48.1%) followed by fever with cough (13.9%) only. Most of the mild cases presented with fever and cough whereas nearly 96% of severe cases had breathlessness.

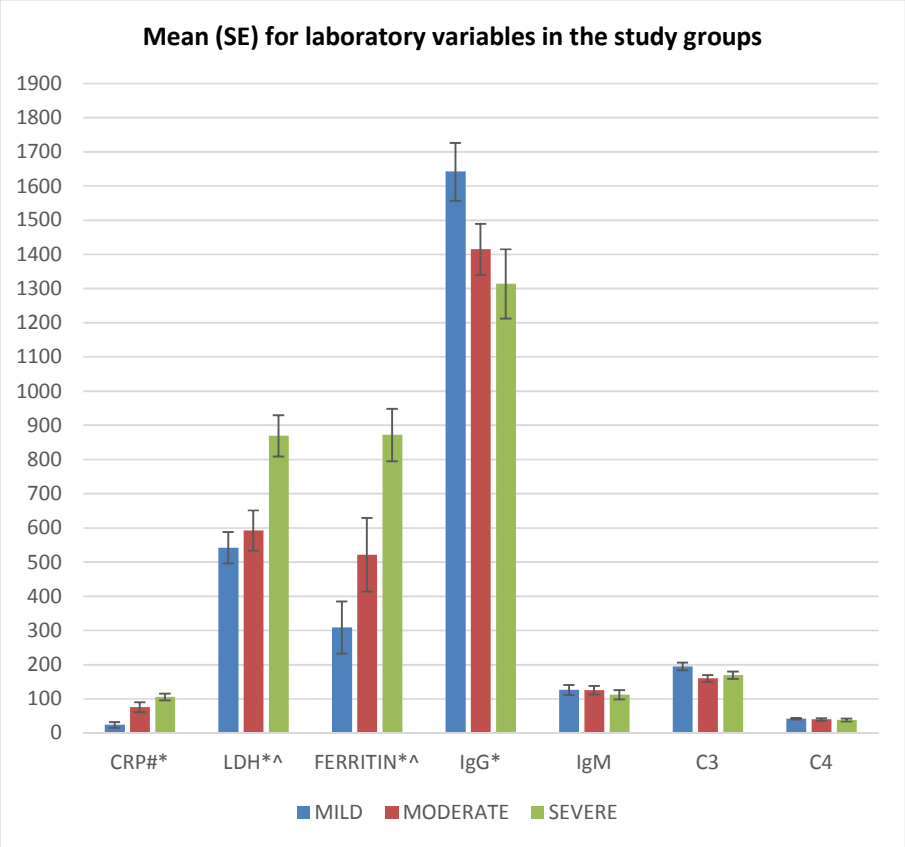

Fig. 1. Mean (SE) comparison of laboratory variables in the three study groups (N=79)

*Mean(SE): mean with standard error of mean; CRP: serum C-reactive protein in mg/L; LDH: serum lactate dehydrogenase deficiency in U/L; Ferritin: serum ferritin in ng/ml; IgG: serum immunoglobulin G in mg/dl; IgM: serum immunoglobulin M in mg/dl; C3: serum complement C3 in mg/dl; C4: serum complement C4 mg/dl; #:denotes significance at p<0.05 between mild and moderate cases; *:denotes significance at p<0.05 between mild and severe cases; ^:denotes significance at p<0.05 between moderate and severe cases*

Table 1. General characteristics of the variables in the study population

Demographic and Clinical Variables	Study Population (N=79)	Mild (N=30)	Moderate (N=23)	Severe (N=26)	F-value	p-value
			Mean (SE)			
Age (years)	55.38 (1.69)	55.39 (3.3)	56.23 (2.7)	54.59 (2.6)	0.07	0.93
Duration of hospitalization (DOH)	8.73 (0.47)	8.57 (0.57)	7.58 (0.94)	9.89 (0.91)	1.96	0.15
SpO_2 (%)	89.65 (1.24)	97.47 (0.19)	93.22 (0.21)	77.46 (2.31)	64.72	<0.001*
Total clinical score (TCS)	3.87 (0.19)	2.93 (0.22)	3.7 (0.33)	5.12 (0.31)	16.055	<0.001*
	n(%)				**p-value**	
Gender						
Female	26 (32.9)	14 (46.7)	6 (26.1)	6 (23.1)	0.12	
Male	53 (67.1)	16 (53.3)	17 (73.9)	20 (76.9)		
Co-morbidities					0.82	
No associated co-morbidities	37 (46.8)	14 (46.7)	10 (43.5)	13 (50)		
DM	32(40.5)	8 (26.6)	13 (56.52)	11 (42.3)		
Hypertension	24(30.4)	12 (40)	6 (26.08)	6 (23.07)		
CVS	3(3.8)	2 (6.66)	1 (4.34)			
COPD	1 (1.3)	0		1 (3.8)		
Sickle cell anemia	1 (1.3)	0		1 (3.8)		
Hypothyroidism	2 (2.5)	2(6.66)				
Clinical Features						
No symptoms	11 (13.9)	8 (26.7)	3 (13)	0		
Fever	41 (51.9)	14 (46.7)	12 (52.17)	16 (61.53)		
Cough	49 (62)	14 (46.7)	14 (60.86)	21 (80.76)		
Breathlessness	38 (48.1)	4 (13.3)	9 (39.13)	25 (96.15)		
Sore throat	22 (27.8)	2 (6.66)	6 (26.08)	14 (53.84)		
Dyspnea	14 (17.7)	2 (6.7)	5 (21.7)	7 (26.9)		
Diarrhea	9 (11.3)	4 (13.3)	4 (17.4)	1 (3.8)		
Death					0.02	
No	73 (92.4)	30 (100)	22(95.7)	21 (80.8)		
Yes	6 (7.6)	0	1(4.3)	5 (19.2)		

SpO_2: percentage saturation of oxygen by pulse oximetry; DM: diabetes mellitus; CVS: cardio-vascular disease; COPD: chronic obstructive pulmonary disease; p-value: statistical significance level at p<0.05 for Chi-square analysis

Table 2. Correlation between study variables in the study population (N=79)

	Age	SpO_2	TCS	DOH	CRP	LDH	FERRITIN	IgG	IgM	C3	C4
Age	1										
SpO_2	-0.05	1									
	0.68										
TCS	0.123	-0.526	1								
	0.28	<0.001*									
DOH	0.15	0.085	0.129	1							
	0.19	0.46	0.26								
CRP	0.184	-0.49	0.413	0.067	1						
	0.11	<0.001*	<0.001*	0.56							
LDH	0.018	-0.42	0.41	0.038	0.544	1					
	0.87	<0.001*	<0.001*	0.74	<0.001*						
FERRITIN	0.112	-0.52	0.444	0.089	0.602	0.54	1				
	0.33	<0.001*	<0.001*	0.44	<0.001*	<0.001*					
IgG	0.26	0.17	-0.085	-0.04	-0.014	-0.06	-0.124	1			
	0.02*	0.14	0.46	0.72	0.9	0.61	0.28				
IgM	-0.19	0.048	-0.401	-0.16	-0.26	-0.18	-0.113	0.005	1		
	0.09	0.68	<0.001*	0.16	0.022*	0.12	0.33	0.96			
C3	-0.01	0.202	-0.172	0.06	0.09	-0.047	0.089	0.03	-0.02	1	
	0.93	0.074	0.13	0.59	0.45	0.68	0.44	0.79	0.87		
C4	-0.06	0.05	0.009	0.068	0.13	-0.15	0.002	-0.35	-0.14	0.44	1
	0.62	0.66	0.94	0.56	0.25	0.18	0.98	0.002*	0.22	<0.001*	

*SpO_2: percentage saturation of oxygen by pulse oximetry; TCS: total clinical score; DOH: duration of hospitalization; CRP: serum C-reactive protein in mg/L; LDH: serum lactate dehydrogenase deficiency in U/L; Ferritin: serum ferritin in ng/ml; IgG: serum immunoglobulin G in mg/dl; IgM: serum immunoglobulin M in mg/dl; C3: serum complement C3 in mg/dl; C4: serum complement C4 mg/dl; *:denotes significance level at p<0.05*

Table 3. Univariate and multivariate regression analysis in study groups

Table 3a. MILD CASES (n=30)

	UNIVARIATE						MULTIVARIATE		
Variables	**SpO_2**		**DOH**		**TCS**		**SpO_2**	**DOH**	**TCS**
	F-score	***p-Value***	***F-score***	***p-Value***	***F-score***	***p-Value***		***p-value***	
Age	2.431	0.136	4.363	0.045*	0.065	0.8	-----	0.037*	0.049*
Gender	3.132	0.094	0.445	0.51	1.235	0.28	-----	-----	-----
SpO_2	-----	-----	10.448	0.005*	19.431	<0.001*	-----	-----	-----
DOH	10.448	0.005*	-----	-----	9.876	0.006*	-----	-----	-----
TCS	19.431	<0.001*	9.876	0.006*	-----	-----	-----	-----	-----
CRP	0.299	0.59	3.941	0.063	7.955	0.011*	0.008*	-----	0.002*
LDH	7.853	0.012*	0.624	0.44	3.566	0.075	0.032*	-----	-----
Ferritin	10.143	0.005*	5.192	0.035*	20.27	<0.001*	-----	-----	0.015*
IgG	0.724	0.41	2.429	0.14	12.118	0.003*	0.019*		<0.001
IgM	18.838	<0.001*	10.452	0.005	24.829	<0.001*	-----	0.031*	-----
C3	0.343	0.57	0.056	0.815	0.073	0.79	-----	-----	-----
C4	0.101	0.754	0.13	0.72	0.695	0.415	-----	-----	-----

Table 3b. Moderate cases (n=23)

	UNIVARIATE						MULTIVARIATE		
Variables	**SpO_2**		**DOH**		**TCS**		**SpO_2**	**DOH**	**TCS**
	F-score	***p-Value***	***F-score***	***p-Value***	***F-score***	***p-Value***		***p-value***	
Age	1.731	0.225	0.693	0.43	1.512	0.254	-----	-----	-----
Gender	6.623	0.033*	0.018	0.895	2.776	0.134	-----	-----	-----
SpO_2	-----	-----	0.089	0.773	4.4	0.07	-----	-----	-----
DOH	0.089	0.77	-----	-----	0.682	0.433	-----	-----	-----
TCS	4.401	0.069	0.682	0.433	-----	-----	-----	-----	-----
CRP	1.747	0.223	1.41	0.27	1.033	0.34	-----	-----	-----
LDH	1.949	0.2	0.94	0.36	0.906	0.37	-----	-----	-----
Ferritin	16.262	0.004*	0.017	0.9	2.561	0.15	0.004*	-----	-----
IgG	1.917	0.204	0.082	0.78	0.227	0.65	-----	-----	-----
IgM	0.121	0.737	0.301	0.59	1.512	0.25	-----	-----	-----
C3	5.615	0.045*	1.873	0.21	3.926	0.083	0.019*	0.034*	
C4	3.794	0.087	1.825	0.214	4.015	0.08	-----	-----	-----

Table 3c. Severe cases (n=26)

Variables	UNIVARIATE						MULTIVARIATE		
	SpO_2		DOH		TCS		SpO_2	DOH	TCS
	F-score	*p-Value*	*F-score*	*p-Value*	*F-score*	*p-Value*		*p-value*	
Age	0.018	0.896	0.096	0.76	1.534	0.25	-----	-----	-----
Gender	0.225	0.646	1.336	0.278	0.087	0.77	-----	-----	-----
SpO_2	-----	-----	2.612	0.14	0.734	0.41	-----	-----	-----
DOH	2.612	0.14	-----	-----	0.374	0.556	-----	-----	-----
TCS	0.734	0.414	0.374	0.556	-----	-----	-----	-----	-----
CRP	0.62	0.45	0.619	0.452	0.073	0.79	-----	-----	-----
LDH	2.136	0.18	0.003	0.96	1.815	0.21	-----	-----	-----
Ferritin	2.631	0.139	0.033	0.86	2.956	0.12	0.048*	-----	-----
IgG	0.174	0.686	0.101	0.76	0.051	0.83	-----	-----	-----
IgM	0.218	0.652	0.764	0.4	1.971	0.19	-----	-----	-----
C3	5.494	0.044	0.671	0.434	7.835	0.021*	0.03*	-----	0.01*
C4	1.135	0.314	3.126	0.111	0.755	0.41	-----	-----	-----

*SpO_2: percentage saturation of oxygen by pulse oximetry; TCS: total clinical score; DOH: duration of hospitalization; CRP: serum C-reactive protein in mg/L; LDH: serum lactate dehydrogenase deficiency in U/L; Ferritin: serum ferritin in ng/ml; IgG: serum immunoglobulin G in mg/dl; IgM: serum immunoglobulin M in mg/dl; C3: serum complement C3 in mg/dl; C4: serum complement C4 mg/dl; *:denotes significance level at p<0.05*

With disease progression from mild to severe form, the inflammatory markers depicted increasing trend while immunoglobulin IgG and complement levels showed downward trend (Fig. 1). Jimenez et al study published a similar trend in 276 COVID-19 cases varying from mild to critical form. Complement factors, C3 and C4 were found lowered in severe cases. Although IgA and IgM did not change but IgG was significantly reduced in severe grade of the disease [16].Unlike serum CRP levels, serum LDH and ferritin levels in present study reported remarkable rise in severe cases when compared to mild and moderate forms of the disease (Fig. 1). A significant positive correlation of these inflammatory biomarkers with clinical score (Table 2) indicates that serial monitoring these markers, especially LDH and ferritin, hence would be beneficial to predict the shift of milder form towards severity at an early stage. Kermali et al analysis suggested that biomarkers like CRP, LDH, interleukin-6, serum amyloid protein (SAA), D-dimer and white cell count are significantly elevated in severe form of COVID-19 and these markers need to be monitored to predict the clinical course of the disease in admitted patients [17]. Similarly, Cheng et al study also derived the conclusion that serum ferritin could be a useful biomarker in predicting worsening condition in this disease [1].

None, but serum immune marker, IgM, depicted significant inverse correlation with CRP levels and clinical score in the study population (Table 2). Thus, a reducing trend of serum acute phase immunoglobulin, IgM with rise in inflammatory marker, ferritin (Supplementary Table 2), together would be alarming for clinical deterioration of the patient. Wang et al study on 114 hospitalized cases observed higher IgM levels in cases with severe form of the disease [18]. Similarly, another study by Ma Huan et al, on 87 COVID-19 subjects documented that serum IgG and IgM were significantly higher in moderate and severe cases when compared to milder form [19]. In disagreement to the above findings, the present study recorded lowered IgG ($p=0.024$) and IgM ($p=0.75$) levels in severe cases than milder group (Fig. 1). However, the presented findings need to be cautiously interpretated and recommend further larger scale studies on immunoglobulin modulation in COVID-19 clinical course. Sequential and close monitoring of the cases with milder form of the disease would essentially prevent critical conversion of the patient and thus reduce the probability of death. Serum inflammatory markers, CRP and ferritin along with immunoglobulins, IgG and IgM exhibited significant influence on total clinical score and hospitalization stay (Table 3a). The downward trend of serum immunoglobulins recorded in the present study was in agreement to Showers et al case description that reported negative correlation of immunoglobulins IgG, IgM and IgA, with duration of hospitalization [20]. This might be an effect of weak immune response or over utilization of immunoglobulins in the process of opsonization and neutralization of viral particles. The negative correlation with inflammatory markers like CRP, ferritin, LDH, also explains the resulting effects of either of the two above mechanisms that had resulted in higher grade inflammation in tissues.

On contrary to milder grade of the disease, serum complement C3 recorded significant impact on duration of hospitalization in moderate cases (Table 2b) whereas it influenced the clinical score in severe form of the disease (Table 3c). Fang et al study also provided a supportive evidence for reduced complements C3 in non-survivors and suggested complement C3 as a potential marker for predicting the prognosis of severity in COVID-19 [21]. Reduced levels of C3 significantly affected the severity outcome in the patients enrolled for the present study (Supplementary Table 2).

Immune dysregulation leading to uncontrolled systemic inflammatory response during the course of the SARS-CoV-2 infection as it courses from mild to severe form have been proposed by many researchers [2,10]. Dheir et al study did observe a lowered value of C3 and C4 on first day of admission in 29 patients admitted in ICU when compared to those 28 admitted in non-ICU [22]. Similarly, Diaz etal study also agreed reduction in C3a with increasing time span in severe cases [23]. Complement activation and immune complex mediated pathophysiology of deranged coagulopathy have major contribution towards poor COVID-19 clinical outcome [24].In contrary, Holter et al observed raised C4 in 23 COVID-19 cases with respiratory failure than those with no features of respiratory failure ($p=0.034$) [25]. In the present study, serum C3 did show a significant association with the duration of stay ($p=0.034$) in moderate cases and total severity score in severe cases ($p=0.01$) which is explained by the mechanism of hyperactivation of complement pathways leading to higher inflammatory response in various tissues begetting adverse outcome.

Complement imparts a role of double-edged sword on immune response. Studies have documented that complement C3 activation required for protection for viral pathogen. In knocked-out C3-/- mice, failed to activate C3 showed higher viral load post-infection than the wild-type mice and higher macrophage infiltration into lung parenchyma and other inflammatory features [26,27]. Few studies have reported that mannose-binding lectin (MBL) binding to SARSCoV-2 and C3-C4 deposition on virions as possible mechanism of antigen neutralization. However, unrestrained complement activation also has been reported for causal association towards acute and chronic inflammation and aggravation of immune response resulting in poor outcomes [27,28]. The classical pathway is mainly an effect due to IgM, IgG1 and IgG3 activity with complement C1q. Serum C3 levels were reported to be reduced in SARS-Cov-2 infection in severe form when compared to mild group. This could be an effect on protein activation and cleavage into C3a components [29]. This might explain the inverse trend observed for values of serum C3 total clinical score. Estimation of C3 fractions would have provided an accurate insight regarding the response. Hence, in addition to serum ferritin, serial monitoring of serum IgM with serum C3 could be useful as potential immune biomarker for early prediction of worsening condition in patients diagnosed with COVID-19.

5. CONCLUSION

The present study documented a changing pattern of inflammatory markers and immune biomarkers in different grades of COVID-19 cases. Besides serum ferritin, a serial and close monitoring of serum IgM with complement factor C3 would aid in the early prediction of clinical severity and thus guide physicians to start an effective management strategy. Clinicians should consider low IgM and C3 and higher values of serum ferritin should be used as risk stratification to predict disease severity. Though, the molecular mechanism regarding the role of immune markers in COVID-19 is not fully understood, yet, this study would provide a foundation for further large-scale, well-designed studies.

6. LIMITATIONS

Besides being a retrospective observational study, the small sample size was the major limitation of the study which was due to limited number of testing facilities available for the patients during the study period. First, with the sudden surge in in-patient admissions, the lab faced a constraint in testing facilities due to delayed delivery of specialized items (not used as a part of routine investigations) owing to prevailing partial lockdown conditions during the study period. Secondly, the study included the laboratory reports of the patients at the time of admission only. Subsequent reports were not analyzed for this study. However, minimal studies are available regarding the inflammatory markers and immune markers, the present study would provide a valuable insight regarding the immunomodulation mechanism COVID-19 patients.

CONSENT AND ETHICAL APPROVAL

The study was a retrospective observational study approved by Institute Ethics Committee along with the waiver of informed consent. The study was conducted in our institute at Raipur, Chhattisgarh, India

SUPPLYMENTARY MATERIALS

Supplementary materials is showing in following link:
https://www.journaljammr.com/index.php/JAMMR/libraryFiles/downloadPublic/17

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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