Association of Inflammatory Markers with the Severity of COVID-19 in a Tertiary Care Hospital in Bagalkot, India - A Cross-Sectional Study

Sunil Baragi[1], Pavani Mallikarjun Dyavannavar[2]

[1, 2] Department of General Medicine, S. Nijalingappa Medical College, Bagalkot, Karnataka, India.

ABSTRACT

BACKGROUND

A novel coronavirus was identified as being responsible for a cluster of pneumonia cases worldwide. With an upward trajectory of (corona virus disease-19) COVID-19 cases and its numerous presentations, there is an urgent requirement of identifying initial signs of decline of quality and an adequate response in order to shift a patient to specialized intensive care units (ICU) for those who progress morbidly to severe or critical categories. It has been reported that various acute phase reactants like erythrocyte sedimentation rate (ESR), ferritin, C-reactive protein (CRP), lactate dehydrogenase (LDH) and D-dimer are raised much more in severe and critical patients than in the mild cases. These markers might have a role to predict mortality. The present study was done to assess the relationship of serum ferritin, and CRP levels at admission with in-hospital mortality among patients with COVID-19 infection and to determine cut-off values of the best prediction of mortality.

METHODS

A cross-sectional study of 109 reverse transcription polymerase chain reaction (RTPCR) confirmed COVID-19 patients admitted in our hospital was done. The outcome of cases was categorized into mild, moderate and severe grades.

RESULTS

Out of 109, 80(73.4 %) were males and 29 (26.6 %) were females. Majority patients of both genders were having severe disease with 30 males and females 10 (P - value = 0.066). Among 109 patients, mild cases (33), moderate case (36), severe case (40). Serum ferritin value severe group (n = 49) 422.45 ng/mL, moderate group (n = 33) 563.64 ng/mL, mild group (n = 27) 529.63 ng/mL. Mean ESR value in severe group 98.37, moderate group 100, mild group 100. Mean CRP in severe group 242.86 mg/L, moderate group 248.48 mg/L, mild group 307.41 mg/L. Mean d-dimer in severe group 971.43 ng/mL, moderate group 803.03 ng/mL, mild group 811.11 ng/mL.

CONCLUSIONS

Our study showed higher levels of markers like ferritin, D-dimer, CRP and ESR in severe patients as compared to mild and helped in forecasting the advancement of mild cases to severe. Also, the blood levels of CRP and ferritin and the duration to complete symptomatic relief all demonstrated a substantial statistical link thus aiding for monitoring of patients at home and in hospitals.

KEYWORDS

Covid-19, Inflammatory Markers

Corresponding Author:
Dr. Pavani Mallikarjun Dyavannavar,
SNMC PG Hostel Room No-122,
S. Nijalingappa Medical College,
Bagalkot – 587102, Karnataka, India.
E-mail: pavanidyavannavar@gmail.com

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BACKGROUND

World health organization (WHO) declared the COVID-19 as an outbreak global pandemic in March 2020, which can affect the individuals with diverse clinical profile, progression and severity of the disease. The WHO classified infection of SARS CoV 2, a pandemic in March 2020, and it continues to create havoc leading to various presentations and consequences. The clinical spectrum of SARS-CoV-2 is wide, ranging from asymptomatic illnesses to pneumonia and C-ARDS (Covid ARDS). The outbreak most probably originated as a zoonosis and ultimately led to human infections. Person to person infections spread as droplets through cough. Studies also suggest transmission from asymptomatic carriers.

Patients usually present with the following symptoms pyrexia, cough with or without sputum, headaches, myalgias, diarrhoea, shortness of breath, or even leading to potentially serious conditions like acute respiratory distress syndrome (ARDS) and cardiac myocyte injuries. Biological markers provide an adequate help in interpreting the above conditions. "Symptomatic disease is frequently associated with transmissibility of a pathogen".[1] We can define patients as mild, moderate, severe or critical, and this is useful in predicting the further management. The mean incubation duration, is in most cases of four to five days, with more than 95 percent having symptoms within first 11.5 days. As of 20 October 2020, India has crossed the 7.6 million mark for COVID-19 cases, with a case fatality rate of 1.9 %.[2] According to the COVID-19 situation report by WHO, 80 % of infections are mild or asymptomatic and 15 % are severe infection requiring oxygen and nearly 5 % are critical infections, requiring ventilation.[3] The chance of transmission being more during pre-symptomatic period. Diagnosis of these cases at early stage with biomarkers may help in timely detection, implementation of therapy as delay may lead to its progression to severe stage of the disease. In India because of COVID-19 care centres at all district hospitals and sub-divisional hospitals, this information will bring awareness among health care workers.[2]

Many patients show wavering complete haemogram variables, liver and kidney function examinations, and also in troponin I and T, inflammatory markers and also disseminated intravascular coagulation (DIC) profiles. The hallmark of the COVID-19 infection might include precarious host immune response, which leads to development of acute respiratory distress syndrome. In critically ill COVID-19 cases has shown the involvement of both humoral and cellular host immune response resulting in serious outcome of the patients. Acute phase reactants like IL-6, procalcitonin, ferritin, C-reactive protein, etc., released from the proinflammatory cytokines cause severe lung injury, along with many other clinical manifestations of COVID-19 infection. Resultant systemic inflammation also affects circulating blood cells like neutrophils, lymphocytes along with neutrophil to lymphocyte ratio. The macrophages clear the path for the release of various inflammatory cytokines, causing polymorphonuclear cells and monocytes to invade lungs and cause injuries to endothelium as well as alveolar cells. This causes ARDS and hypoxemia.

IL-6 and other inflammatory markers cause hepatocytes to make proteins like CRP. This marker is used in various conditions and can indicate severity. C-reactive proteins attach to a molecule phosphocholine on diseased cells. This then stimulates the classical complement pathways and ultimately leads to removal of diseased cells and also the SARS-CoV-2 viruses.

D-dimer originate from the lysis of cross-linked fibrin with rising levels indicating the activation of coagulation and fibrinolysis. The mechanism of the association of ferritin with COVID-19 is not well delineated but it is suggested to increase due to the influence of pro-inflammatory cytokines.[4] Three studies revealed a high level of ferritin at the time of admission, with the severe group of patients having more comorbidities.[5,6,7] The various suggested mechanisms of raised ferritin level are the pro-inflammatory cytokines like interleukin (IL-16) and tumour necrosis factor (TNF-α) promoting synthesis of ferritin, leakage of intracellular ferritin by cellular damage.[8] Raised ferritin leads to thymo suppression with lowering of absolute lymphocyte count affecting CD4+ and CD8+ count adding to the state of inflammation.[9] Thus hyperferritinaemia is also associated with systemic inflammation due to co-morbidities and can correlate with the severity of the disease. One of the major causes of mortalities in severe and critical diseases is cytokine storm, in which there is elevated levels of IFN-gamma and IL-6. Pulmonary affliction is known to cause increased respiratory failure and need for artificial ventilation, often leading to death within as early as 3 days.

Both lab as well as clinical parameters are of paramount importance in classifying cases. Many have studied the role of various inflammatory markers of COVID-19 infection for prognostication. Many tests like serum PCT (procalcitonin) and IL-6 are either not readily available or expensive in developing countries. Blood ferritin levels and C-reactive protein levels could be assessed in almost all labs, and they require limited resources too. The results could be of use in making tailored treatment on patient basis and also to review them later. COVID-19 could be divided on the basis of clinical criteria as mild, moderate and severe infection. Mild cases of upper respiratory tract infections (URTI) symptoms without shortness of breath or hypoxia, moderate cases any one of respiratory rate more then 24/min and spo2 ≤ 93 % on room air, severe cases any one of respiratory rate > 30/min and spo2 < 90% at room air. With an upward course of COVID-19 cases and its numerous presentations, there is an immediate need for identifying early signs of decline of quality and an adequate response in order to shift a deteriorating patient to specialized intensive care units. It has been reported that various acute phase reactants like ESR, ferritin, CRP and D-dimer are raised much more in severe and critical patients than in the mild cases. These markers might have a role to predict mortality.

Cardiac muscle damage has been reported earlier. However, the present study could not correlate a significantly raised levels of cardiac enzymes in severe cases than in non-severe ones. We believe more studies on the pathophysiology of such an injury might help, however a possibility might be infiltration of interstitium by mononuclear cells than the direct damage of cardiac

myocytes. The literature on COVID-19 is still expanding rapidly, however there is still a dearth in local data correlating the lab markers and clinical outcomes of patients with different severities. The present study was done to assess the relationship of serum ferritin, and CRP levels at admission with in-hospital mortality among patients with COVID-19 infection and to determine cut-off values of the best prediction of mortality.

So far as we know till date the complete inflammatory profile picture of the COVID-19 infection is not well apprehended due to limited sample size. Our study focuses on the association of inflammatory markers with the severity of COVID-19 infection and will help in monitoring and evaluating the severity and prognosis of infection.

Objectives

1. To estimate proportion of severe COVID19 patients.
2. To determine the association of inflammatory markers with the severity of Covid-19.

METHODS

This is a cross-sectional study of 109 RTPCR confirmed COVID-19 patients admitted in S. Nijalingappa Medical College and Hospital from July 2020 to October 2020.

Inclusion Criteria

Patients diagnosed with COVID-19 and who had positive results of SARS–CoV-2 RNA

Exclusion Criteria

RTPCR negative for COVID-19, admitted as severe acute respiratory illness (SARI).

Methods of Data Collection

1. We collected data from 109 patients with laboratory-confirmed COVID-19 infection who were admitted in S. Nijalingappa Medical College and Hospital from July 2020 to October 2020.
2. A confirmed case of COVID-19 was defined by a positive result on a reverse-transcriptase–polymerase-chain-reaction assay of a specimen collected on a nasopharyngeal swab.
3. Clinical specimens for COVID-19 were obtained in accordance with Centers for Disease Control and Prevention (CDC) guidelines.[4] We included only laboratory-confirmed cases.
4. Inflammatory markers like S. ferritin, C-reactive proteins, ESR and D-dimer was taken at the time of hospital admission until day of 14.
5. Outcome of the cases were graded as,
   a) Mild [(Influenza like illness (ILI)]
   b) Moderate (SARI: sat 90 - 94 %)
   c) Severe [SARI: sat < 90 %/ non-invasive ventilation (NIV)/ 02 support: high flow nasal cannula (HFNC)]

Investigations

Serum ferritin, erythrocyte sedimentation rate, C-reactive protein, COVID-19 RTPCR.

Statistical Analysis

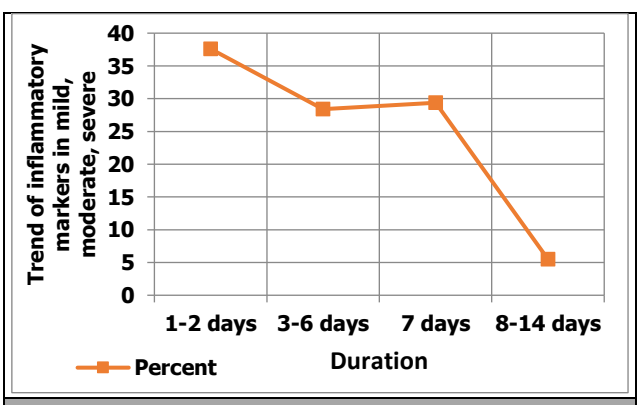
Data was entered in MS-Excel and analysed in statistical package for social sciences (SPSS V25). Descriptive statistics were represented with percentages and mean with standard deviation (SD). Shapiro-wilk test was applied to find normality. Chi-square, analysis of variance (ANOVA), post hoc Tuckey test were applied to find significance. $P < 0.05$ was considered as statistically significant.

RESULTS

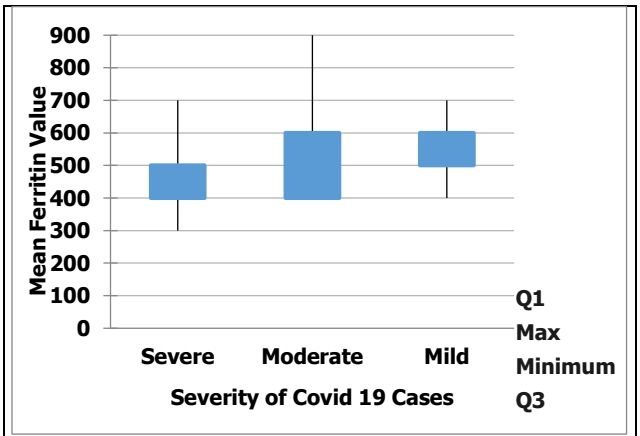
All of the enrolled 109 patients infected with SARS-COV-2 were confirmed with RT-PCR. Out of 109, 80 (73.4 %) were males and 29 (26.6 %) were females. Majority patients of both genders were having severe disease with 30 males and females 10 (P - value = 0.066). Among 109 patients, mild cases (33), moderate case (36) and severe case (40). Trend of inflammatory markers increased from day of admission until 14 days.

Status		N	Minimum	Maximum	Mean	SD	P - Value
Age	Severe	49	32	88	55.31	13.21	0.25
	Moderate	33	35	89	59.76	14.12	
	Mild	27	32	89	59.63	13.45	
Raised CRP	Severe	49	100	400	242.86	70.71	0.003
	Moderate	33	200	300	248.48	50.75	
	Mild	27	200	600	307.41	120.66	
Ferritin ng/ml	Severe	49	300	700	422.45	96.32	< 0.001
	Moderate	33	400	900	563.64	136.52	
	Mild	27	400	700	529.63	77.53	
LDH U/L	Severe	49	240	4000	491.22	519.74	0.89
	Moderate	33	400	600	463.64	69.90	
	Mild	27	400	650	507.63	76.68	
D-DIMER	Severe	49	700	7000	971.43	885.53	0.36
	Moderate	33	700	900	803.03	76.99	
	Mild	27	700	900	811.11	69.80	
ESR	Severe	49	60	100	98.37	6.88	0.19
	Moderate	33	100	100	100.00	0.00	
	Mild	27	100	100	100.00	0.00	

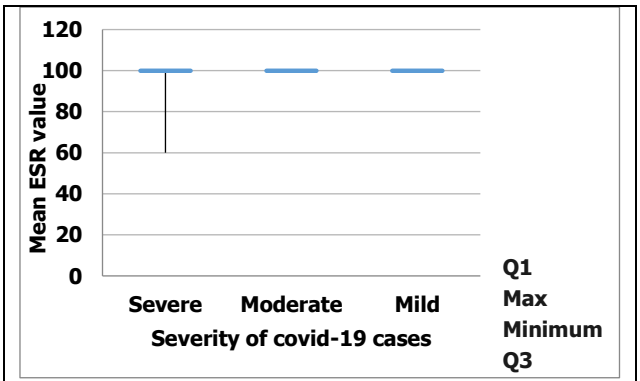
Table 1. Association of Laboratory Parameters with Standard Deviation and P-Value in Severity of Covid 19 Patients



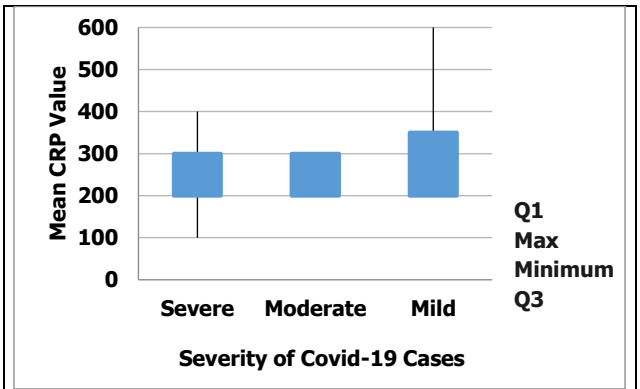
Graph 1. Trend of Inflammatory Markers throughout the Course of Disease Until Day of 14



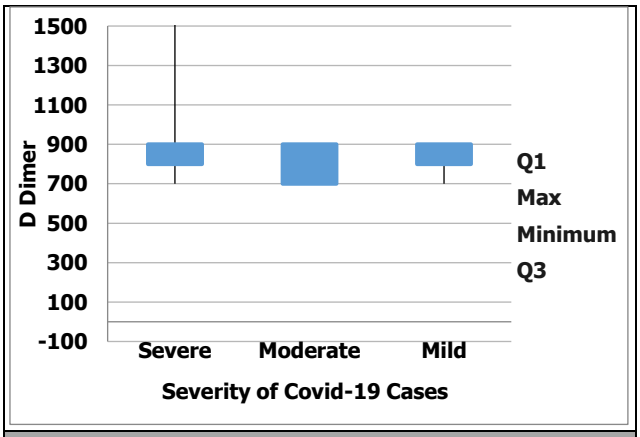
Graph 2. Comparison of Mean Value of Serum Ferritin with Severity of Covid-19 Cases



Graph 3. Comparison of Mean Value of ESR with Severity of Covid-19 Cases



Graph 4. Comparison of Mean Value of CRP with Severity of Covid-19 Cases



Graph 5. Comparison of D-Dimer with Severity of Covid-19 Cases

Group 15 moderate group 10, mild case 5.10, moderate group 6, mild case 5. *Normal value of CRP = (6 - 8) mg/L *Normal value of D–dimer = < 0.5 mg/L. Graph 1 shows the trend of overall inflammatory throughout the course of the disease until the day 14 which shows the decreasing graphical representation. Graph 2 shows the mean ferritin values in mild, moderate, and severe COVID-19 cases which shows there is increasing level of serum ferritin from mild, moderate, and severe case. Graph 3 shows the mean ESR values in mild, moderate, and severe COVID-19 cases showing the increase trend of ESR from mild, moderate, and severe cases. Graph 4 shows the mean CRP values in mild, moderate, and severe COVID-19 cases showing the greater increase of the CRP value from mild to moderate to severe cases. Graph 5 shows the mean D-dimer values in mild, moderate, and severe COVID-19 cases showing minimal increase in D-dimer value from mild to moderate cases and greater increase in D-dimer value in severe cases.

DISCUSSION

COVID-19 is a raging pandemic overwhelming the medical facilities. A wide spectrum of mild infection to ARDS or multiple organ dysfunction syndrome (MODS) often causing difficulties in diagnosis, prognosis, and monitoring treatment. Thus, it is essential to ascertain a patient's condition in a time. Biomarkers are quantitative tools used clinically for many conditions reflecting pathology. Excessive pro-inflammatory cytokines responsible for the lymphopenia associated with COVID-19 also promotes expression of proteins like programmed cell death protein and mucin domain 3 and other markers of T cell exhaustion in CD4 + and CD8 + cells.[10]

Certain genes related to T cell activation are down regulated in severe COVID-19 infection which return to normal on recovery.[10] Since lymphocytes carry angiotensin converting enzyme (ACE2) receptors they can also get infected by the virus leading to death.[10] Understanding the mechanism of lymphopenia in COVID-19 infection can provide an effective strategy for treatment. Biomarkers might better prognosis and outcomes of patients. Our results highlight more levels of inflammatory markers among patients with moderate and severe diseases, when compared to milder cases. In this study we concluded that inflammatory markers especially ferritin, CRP, ESR, D-dimer were positively correlated with severity of disease.

Our study found that while most individuals recovered without any complications, some did progress to moderate illness, and inflammatory markers such as ferritin and ESR predicted this progression. Ferritin and ESR levels done at admission for our patients were significantly higher in severe group. Those individuals who had mild disease had minimal raised values of these biomarkers. C-reactive protein was high in severe and critically ill patients.[11,12,13,14,15,16,17,18,19,20]

The mean difference in CRP in severe 242.86, moderate 248.48, mild 307.41 with I2 of 94%. Increased CRP is mostly indicative of bacterial infections, unlike a study, showing evidence of increased CRP with respiratory virus infection and also a predictive factor for hospitalization in ICU and for

mechanical ventilation.[21] Further raised CRP levels have also been found in individuals infected with virulent influenza A virus being associated with its severity and mortality.[22] Therefore, the observations of CRP from our meta-analysis can be used as a biomarker to categorize COVID-19 infections as per the severity. Blood sampling was done within half an hour of admission. And those with missing data from day of admission were not included. We only studied the role of inflammatory markers measured at the time of admission. It might also benefit to see how changes during hospitalization affect the outcomes.

Mortality rate in our study group was a little higher than the entire cohort treated at the hospital. Despite this, the results provide a very useful insight into the ability of the three inflammatory markers to predict in-hospital death. We would like to suggest multi-centric studies incorporating larger number of patients to refine the results. COVID-19 is a multisystem disorder afflicting multiple organs. Deranged renal and liver function test results and elevated serum glucose might happen due to hypoxemia, hypoperfusion, ARDS, shock, and DIC. Elderly patients with underlying comorbidities and COVID-19 infection found to be more susceptible and likely to be admitted in ICU care with higher mortality rate. One of the other inducible factor is drug induced hepatitis. Covid-19 may induce inflammation which may develop into hemophagocytic lymphocytosis which is characterized by hypercytokinemia which later progresses to multi organ failure in elderly patients with underlying comorbidities is another possible explanation for the above stated laboratory findings.

Meta-analysis published by Zhang et al. reported that the most common laboratory findings were increased CRP (73.6 %), decreased albumin (62.9 %), increased ESR (61.2 %), decreased eosinophils (58.4 %), increased IL-6 (53.1 %), lymphopenia (47.9 %), and increased LDH (46.2 %).[23] CRP, ferritin, D-dimer, LDH and erythrocyte sedimentation rate to obtain a reliable and easily accessible diagnostic tool, as an alternative to polymerase chain reaction and chest CT scans and to predict disease severity. In our study D-dimer which is the marker of hypercoagulability, and its elevated levels has been a part of disease severity confirms clinical utility of laboratory findings as indicator of severe and progressive inflammation, and there is a significant association of laboratory findings with severe disease.

Limitations

- The limitations of this study include its cross-section design which made the study prone to missing data.
- Limited duration of study and smaller sample size.
- Lack of information on analysis and different units of measurements, which lead to heterogenicity in studies.

CONCLUSIONS

This study has shown similar results with respect to increased levels of inflammatory markers like ferritin, ESR, D-dimer and CRP predicting progression of disease from mild to moderate illness. At the early stage of COVID-19, CRP levels were directly correlated with lung involvement. Raised CRP levels emulate the disease progression and severity, and can be used as a salient indicator for disease progression. Furthermore, serum ferritin, ESR, D-Dimer and LDH also had remarkable statistical correlation with complete symptom resolution of these patients to duration of the disease and can be used in home and hospital as an important indicator.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

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