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Retrospective Cohort Study of Correlation Between Inflammatory Markers, its Severity and Prognosis in COVID 19 (SARS) Patients

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ABSTRACT
Coronavirus disease-2019(COVID-19) caused by SARS Cov-2. Studies have shown that COVID 19 is a progressive inflammatory process in which clinical deterioration occurs 7-10 days after onset of disease associated with declining viral titers suggesting pathology is driven by inflammation rather than direct viral injury. Present study was aimed to study correlation between inflammatory markers, its severity and prognosis in COVID 19 (SARS) patients. Present study was Retrospective observational cohort study, conducted in adult hospitalized patients of SARS COV2, nasopharyngeal swab RTPCR positive patients, imaging suggestive of COVID pneumonia. Medical record information including clinical, laboratory as well as outcome data were extracted by using data collection forms. The study consisted of 174 patients, mean age of selected patients was 53.55, most of the patients were in age group of 61-70 years. About 57.5% patients had mild, 28.7% moderate and 13.4% severe disease based on clinical symptoms and radiological findings. patients 56.9% had mild, 20.1% moderate and 11.5% severe involvement of lungs. patients 10.9% required ICU care during hospital stay. Rest of the patients were under ward care. About 10 patients expired and rest 164 patients recovered from illness. Levels of CRP, ferritin, LDH, ESR, neutrophil to lymphocyte ratio were found to be statistically higher in patients with moderate to severe disease as compared to mild disease. Levels of IL-6 and PCT were found to be statistically insignificant in this study. Higher levels of IL-6, LDH, PCT, ESR at the time of admission predicted a worsened outcome. Levels of CRP, ferritin, neutrophil to lymphocyte were found to be statistically insignificant. Immune-inflammatory parameters such as IL-6, Ferritin, LDH, WBC, lymphocyte, NLR, PCT and CRP were correlated with disease severity and could be used as potentially important risk factors for disease progression.

INTRODUCTION

Coronavirus disease-2019(COVID-19) caused by SARS Cov-2 emerged in late 2019 in city of Wuhan, China and caused cluster of pneumonia cases. It resulted in rapid rise of cases and uncontrolled worldwide spread despite control and quarantine measures becoming a big challenge to health care professionals globally[1]. Coronavirus is single stranded RNA beta coronavirus SARS COV2.it is member of coronavirus family along with SARS COV and MERS COV but it's transmission speed and infectivity are stronger than both SARS COV 2. It can be transmitted through respiratory tract, mainly causing respiratory infections and developing severe pneumonia respiratory failure and even death infected patients[2]. Inflammatory response central role in manifestation of disease. There's release of cytokines and chemokines resulting in cytokine storm which is self-perpetuating and tissue damaging inflammatory activity[3,4].

Studies have shown that COVID 19 is a progressive inflammatory process in which clinical deterioration occurs 7-10 days after onset of disease associated with declining viral titers suggesting pathology is driven by inflammation rather than direct viral injury Raised Inflammatory markers are significantly Associated with high risk of development of severe COVID 19. Acute Respiratory Distress Syndrome (ARDS) characterized by rapid onset of generalized inflammation is leading cause of mortality in patients with COVID 19[3,4]. Present study was aimed to study correlation between inflammatory markers, its severity and prognosis in COVID 19 (SARS) patients.

MATERIAL AND METHODS

Present study was Retrospective observational cohort study, conducted in department of General Medicine, at Holy Spirit hospital, Mumbai, India. Study duration was of 1 year (April 2020 to April 2021). The detailed plan of the study was submitted to ethical committee of the institute and after the approval of this committee, the study was started.

Inclusion Criteria:

- Adult hospitalized patients of SARS COV2, nasopharyngeal swab RTPCR positive patients imaging suggestive of COVID pneumonia

Exclusion Criteria:

- Patients with previous inflammatory condition like Connective tissue disease, Inflammatory bowel disease, co-existing systemic or local infection

Medical record information including clinical, laboratory as well as outcome data were extracted by using data collection forms. Nasopharyngeal swab specimens were collected for extracting COVID-19 RNA from patients. All patients with SARS-CoV-2 were confirmed using quantitative real-time reverse transcription polymerase chain reaction (RT- PCR) assay. Pulmonary involvement was assessed by serial Chest X-ray and CT chest imaging. Imaging suggestive of COVID pneumonia grouped into mild, moderate and severe category based on clinical symptoms

Chest x ray was assessed using RALE scoring system identifying the area of involvement[5]. Each lung was assessed individually and depending on the extent of involvement by consolidation or ground-glass opacity a score of 0-4 points was given (0-no involvement 1-less than 25%, 2-25-50%, 3-50-75%, 4 more than 75% involvement). The overall score was the sum of points from both lungs. The CORADS (COVID 19 Reporting and Data System) categorization of given non enhanced chest CT scan into groups related to likelihood of patient having confirmed COVID 19 with lung involvement. It is further classified based on involvement. Each of the five pulmonary lobes was visually scored from 0-5 as

- no involvement
- <5% involvement
- 5-25% involvement
- 26-50% involvement
- 51-75% involvement and
- 76-100% involvement

The scores were added together to provide a total CT severity score ranging from 0 (no involvement) to 25 (maximum involvement). In a qualitative evaluation, CT severity scores of 1-5, 6-14, and 15-25 were categorized as mild, moderate and severe involvement, respectively Patients were classified as follows based on WHO guidelines (at the of start of the study):

Mild Disease: Symptomatic individuals without evidence of viral pneumonia or hypoxia Symptoms include: Fever, cough, fatigue, anorexia, sore throat, nasal congestion, headache, diarrhoea, nausea, vomiting, anosmia and ageusia.

Moderate Disease: Symptomatic individuals with evidence of viral pneumonia i.e., hypoxia along with abnormal chest imaging (X-ray/HRCT chest-mild-moderate severity).

Severe Disease: Symptomatic individuals requiring non-invasive or invasive ventilation support with chest imaging suggestive of severe form or ARD. All the data collected were numerically encoded and collated in

Microsoft Excel sheet which was re-checked and analyzed using SPSS statistical software version 22. Independent sample test and ANOVA were used in case of normally distributed data. Mann Whitney test and Kruskal Wallis test were used in case of data not normally distributed to test statistical significance of difference between means of variables among different independent groups. A $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS

The study consisted of 174 patients admitted in Holy Spirit hospital between April 2020 to March 2021.The mean age of selected patients was 53.55, most of the patients were in age group of 61-70 years. The average period for which the patients stayed in hospital was around 8-14 days. Minimum period for which all the patients under study stayed in hospital was 8 days and went serial evaluation of inflammatory markers. The mean saturation of oxygen at room air was 93.49% on admission, 91% during stay and 97% on discharge. About 57.5% patients had mild, 28.7% moderate and 13.4% severe disease based on clinical symptoms and radiological findings. 11.5% patients had normal HRCT chest scan, 56.9% patients had mild 20.1% moderate and 11.5% severe involvement of lungs. 10.9% patients required ICU care during hospital stay. Rest of the patients were under ward care. About 10 patients expired and rest 164 patients recovered from illness. At the time of admission, the mean level of inflammatory markers was elevated.

On serial evaluation the inflammatory marker levels reduced during illness in patient who recovered and increased in patients who expired. Ferritin level on admission in patients with mild disease (558.54±1685.77), was comparatively less than patients with moderate (919.59±1573.28) and severe disease (616.75±596.24) and was statistically significant ($p<0.001$)Ferritin level measured after 5 days in patients showed a falling trend in mild and moderate disease compared to on admission, however there is rising trend in severe disease i.e. mild disease (369.54±1061.51), moderate (543.50±455.27) severe -625.84±539.64.12 was statistically significant ($p<0.001$) Ferritin on admission in recovered patients (672.87±1591.14) was comparatively found to be more than deceased (628.54±530.20) and was statistically insignificant ($p = 0.292$). Ferritin after 5 days of admission in recovered patients (432.84±880.79) was comparatively lesser than deceased (811.30±520.20) and was statistically significant ($p=0.003$*). IL-6 level on admission and 3 days after admission in patients with mild disease (155.26±250.32) moderate (153.96±29.70) and severe (388.74±501.56) was statistically insignificant ($p<0.097$). IL-6 after 3 days mild (83.09±144.80) moderate (84.07±159.73) severe (469.64±969.78) with $p=0.109$. IL-6 on admission in patients who recovered (56.63±259.68) and deceased (686.66±634) was statistically significant ($p = 0.023$).

LDH on admission in mild disease (176.04±69.26), moderate (205.62±62.2), severe (377.88±201.33) was statistically significant on comparison ($p<0.001$). LDH level on admission in patients who recovered (266.16±144.33) compared to deceased (456.80±89.95) was statistically significant ($p<0.001$). N/L ratio on admission in patients with mild disease (5.64±3.07), moderate disease (7.82±4.23) and severe (19.85±37.22) was statistically significant ($p<0.001$*). N/L ratio on admission in patients who recovered (6.71±3.86) compared to deceased (33.15±56.34) was statistically significant ($p<0.001$). PCT on admission in patients with mild disease (1.34±2.58), moderate (1.27±2.11) severe -2.18±3.33 was statistically insignificant ($p<0.407$.)

PCT on admission in recovered patients (1.28±2.42) compared to deceased (3.96±3.71) was statistically significant ($p<0.001$). CRP level on admission in patients with mild disease (4.05±5.33), moderate (9.95±7.58) Severe (10.97±7.01) was statistically significant ($p<0.001$). CRP level after 3 days in patients with mild disease (2.40±4.23) moderate (4.04±4.68) severe (7.54±6.38) was statistically significant ($p<0.001$). CRP after 5 days in mild disease (0.68±1.35), moderate (0.88±1.68) severe (6.55±7.12) was statistically significant ($p<0.001$). As per the study, CRP level on admission in recovered patients (6.51±7.00) compared to deceased (9.84±6.03) was statistically insignificant ($p = 0.065$). CRP level after 3 days of admission in recovered patients (3.23±4.62) compared to Deceased (9.48±7.13) was significant (p-0.003). ESR on admission in patients with mild disease (50.69± 31.35), moderate (53.90±33.53), severe (74.48±35.43) was higher in severe disease and statistically significant ($p<0.0019$). ESR on admission in patients who recovered (52.251±31.96) and deceased (96.184±28.03) was statistically significant.

COVID-19 has an ambiguous presentation, and it becomes difficult to assess which patients will worsen during the course of illness. The majority of patients have relatively mild symptoms, but a considerable number of patients progress to severe pneumonia and even develop septic shock and/or multiple organ failure. Therefore, it is of great importance to study inflammatory markers for timely diagnosis and initiation of treatment. At right time, taking necessary steps will help in halting the progression of disease and reducing mortality[6,7]. Serial evaluation of inflammatory markers helped in assessing the management. In patients who got better i.e, reduced symptoms, decreased oxygen requirement, decreased lesion or no fresh lesion on imaging, there was reduction in level of markers.

Table 1- General characteristics

	No. of patients/ Mean ± SD	Percentage / Median(IQR)
Mean age	53.55±10.92	
Duration of hospital stay-days	10.63±4.69	10(8-14)
SpO2		
On admission	93.49±8.30	97 (90-98)
During stay	91.06±9.94	96 (87-98)
On discharge	97.24±1.81	98 (97-98)
Based on radiological findings		
Mild	100	57.5
Moderate	50	28.7
Severe	24	13.8
HRCT findings		
Mild	99	56.9
Moderate	35	20.1
Severe	20	11.5
Normal	20	11.5
ICU admission required	19	10.9
Outcome		
Recovered	164	94.3
Death	10	5.7

Table 2: Association of different factors with clinical severity

	Clinical severity			
Variable	**Mild**	**Moderate**	**Severe**	**p-value**
CRP				
On admission	4.05±5.33	9.95±7.58	10.97±7.01	<0.001*
After 3 days	2.40±4.23	4.04±4.68	7.54±6.38	<0.001*
After 5 days	0.68±1.35	0.88±1.68	6.55±7.12	<0.001*
IL6				
On admission	155.26±250.32	153.96±290.70	388.74±501.56	0.097
After 3 days	83.09±144.80	84.07±159.73	469.64±969.78	0.109
Ferritin				
On admission	558.54±1685.77	919.59±1573.28	616.75±596.24	<0.001*
After 5 days	369.54±1061.51	543.50±455.27	625.84±539.64	<0.001*
LDH				
On admission	240.68±154.10	283.74±62.20	377.88±201.33	<0.001*
After 5 days	176.04±69.26	205.62±62.20	377.88±201.33	<0.001*
PCT	1.34±2.58	1.27±2.11	2.18±3.33	0.407
ESR	50.69±31.35	53.90±33.53	74.48±35.43	0.019*
WBC				
On admission	6368.39±2660.74	7324.38±2882.34	8795.42±4613.83	0.023*
After 5 days	8089.22±3687.06	10771.20±4514.04	16356.25±26313.08	<0.001*
Neutrophil	74.45±9.10	80.09±7.93	85.11±7.52	<0.001*
Lymphocyte	16.96±8.22	13.22±6.76	8.49±4.67	<0.001*
N/L ratio	5.64±3.07	7.82±4.23	19.85±37.22	<0.001*

*Statistically significant

Table 3: Association of different factors with outcome

	Outcome		
Variable	**Recovered**	**Death**	**p-value**
CRP			
On admission	6.51±7.00	9.84±6.03	0.065
After 3 days	3.23±4.62	9.48±7.13	0.003*
After 5 days	0.82±1.61	13.44±5.41	<0.001*
IL6			
On admission	156.63±259.68	686.66±634.46	0.023*
After 3 days	85.00±149.58	1204.94±1461.50	0.001*
Ferritin			
On admission	672.87±1591.14	628.54±530.20	0.292
After 5 days	432.84±880.79	811.30±520.20	0.003*
LDH			
On admission	266.16±144.33	456.80±89.95	<0.001*
After 5 days	191.62±71.22	552.80±192.09	<0.001*
PCT	1.28±2.42	3.96±3.71	<0.001*
ESR	52.25±31.96	96.10±28.02	<0.001*
WBC			
On admission	6789.56±2875.67	10066.00±5486.31	0.09
After 5 days	9066.05±4142.93	25320±39924.52	0.027*
Neutrophil	76.92±9.16	87.65±7.17	<0.001*
Lymphocyte	15.18±7.86	7.09±5.61	<0.001*
N/L ratio	6.71±3.86		<0.001*

Patients who worsened during illness with increased oxygen requirement or new lesions of imaging showed rising trend of inflammatory markers. Elevated pro-in ammatory cytokine or chemokine responses induced immunopathology, described as a cytokine storm, has been involved in the pathogenesis of human

coronavirus[8,9]. It is hypothesized that SARS-CoV-2 rst binds to alveolar epithelial cells and then the virus triggers the innate immune system and the adaptive immune system, leading to the release of a substantial number of cytokines, including IL-6, which is a pleiotropic cytokine important in regulating immunological and in ammatory responses. Abnormally increased levels of such cytokines or chemokines can cause tissue damage, resulting in respiratory failure or multiple organ failure. In addition to its strong pro-in ammatory function, IL-6 induces various acute- phase proteins, such as CRP, SAA, brinogen, antitrypsin, hepcidin and components of complement to deteriorate in ammatory reactions and activate coagulation pathway with resultant disruption of procoagulant–anticoagulant homeostasis, induction of disseminated intravascular coagulation, and multi-organ failure[6,7].

Among various cytokines and chemokines (IL-2, IL-8, IL-17, GCSF, IP-10 and TNF-a) recognized, IL-6 has been considered as the most signi cant cytokines, which was found increased in both SARS and MERS, as well as in COVID-19[6,7,8]. Ferritin level in this study revealed higher levels in moderate and severe cases compared to mild cases throughout the course of illness. As per the study ferritin level on admission doesn't predict the outcome of disease. However, during the course of illness rise in ferritin from baseline predicts worsening of disease and poor outcome.

Interleukin 6 (IL-6) is an interleukin that acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine[10,11]. In humans, it is encoded by the IL6 gene. IL-6 is a pleiotropic cytokine important in regulating immunological and inflammatory responses. IL-6 level in this study revealed insignificant difference between moderate and severe cases compared to mild cases throughout the course of illness. As per the study IL-6 level on admission is good predictor of outcome of disease. Patients who have higher IL-6 level have increased mortality risk. LDH level in this study revealed higher levels in moderate and severe cases compared to mild cases throughout the course of illness. As per the study LDH level on admission is good predictor of outcome of disease.

The neutrophil-lymphocyte ratio (NLR), derived from the absolute neutrophil and absolute lymphocyte counts of a full blood count, is a potential marker of the systemic inflammatory response[12,13]. A rising neutrophil count and a falling lymphocyte count indicate the intensity of the inflammatory response and damage to the immune system, respectively. In this study, we observed a significant increase in the number of neutrophils and a significant decrease in the lymphocyte count during the severe phase. Therefore, higher NLR could be a potential maker for predicting the disease progression. At the same time, elevated NLR was significantly associated with increased disease severity or demonstrating that elevated NLR could be a predictor of disease progression in COVID-19 patients.

Procalcitonin (PCT), used as a marker of severe inflammation, is released during infections caused by bacteria, fungi, and parasites but is normal or only slightly elevated in viral infections[14]. PCT level in this study revealed insignificant difference between moderate and severe cases compared to mild cases throughout the course of illness. As per the study IL-6 level on admission is good predictor of outcome of disease. C-reactive protein is an acute-phase in ammatory protein produced by the liver and regulated at the transcriptional level by the cytokine IL- 6[15,16]. CRP level in this study revealed higher levels in moderate and severe cases compared to mild cases throughout the course of illness. As per the study CRP level on admission doesn't predict the outcome of disease However, during the course of illness rise in CRP from baseline predicts worsening of disease and poor outcome.

The Erythrocyte Sedimentation Rate (ESR or sed rate) level in this study revealed higher levels in moderate and severe cases compared to mild cases throughout the course of illness. As per the study ESR level on admission is good predictor of outcome of disease. Levels of CRP, ferritin, LDH, ESR, neutrophil to lymphocyte ratio were found to be statistically higher in patients with moderate to severe disease as compared to mild disease. Levels of IL-6 and PCT were found to be statistically insignificant in this study. Higher levels of IL-6, LDH, PCT, ESR at the time of admission predicted a worsened outcome. Levels of CRP, ferritin, neutrophil to lymphocyte were found to be statistically insignificant. Inflammatory markers help in identifying the severity of disease, evaluation of line of management and assessing the progression of disease. Rising trend of inflammatory markers is worrisome and requires re-evaluation of line of treatment.

CONCLUSION

Immune-inflammatory parameters such as IL-6, Ferritin, LDH, WBC, lymphocyte, NLR, PCT and CRP were correlated with disease severity and could be used as potentially important risk factors for disease progression. In addition, high levels of IL-6, PCT, ESR, LDH at the time of admission reflect an enhanced inflammatory process may also suggest a poor prognosis. Therefore, surveillance of immune-inflammatory parameters, may be helpful in the diagnosis, early screening and predicting of severe illness, and treatment of COVID-19.

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