Original Research Article

Inflammatory Markers and Their Use in Covid-19 Patients

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Abstract

Background: COVID-19 infections are increasing worldwide and treating these patients has become a major challenge for the clinicians. Inflammatory markers are very helpful in this scenario to partially classify patients into mild, moderate and severe disease and also to prioritize their clinical needs. This study is aimed to investigate serum levels of interleukin 6 (IL-6), C-reactive protein (CRP), lactate dehydrogenase (LDH), D-dimer and procalcitonin in COVID-19 patients, and to find the correlation of these parameters with disease severity and progression.
Materials and Methods: This was a one year prospective study done between January 2020 and December 2020 and the sample size was 4308 COVID-19 infection positive cases. Inflammatory markers such as Lactate Dehydrogenase(LDH), C-reactive protein (CRP),D-Dimer, Procacitonin and Interleukin-6 were used to correlate with the disease severity of COVID -19 patients.**Results:** All the inflammatory markers were elevated in most of the COVID-19 patients and maximum elevation of these markers was seen in patients with co-morbid conditions. Patients with higher levels of these inflammatory markers required oxygen , ICU admissions and non-invasive or invasive ventilation. **Conclusion:** Inflammatory markers are very useful biomarkers which help clinicians in identifying severe disease earlier and subsequently improve prognosis.

Keywords: COVID-19, Corona Virus, Inflammatory Markers , C-reactive Protein, D-Dimer, Procalcitonin.

Introduction

COVID-19 Infections are increasing rapidly in various countries since its first appearance in Wuhan city of China in December 2019. Initial treatment of even mild to moderate cases were very challenging as there was no specific treatment to arrest the replication of the virus in-vivo in the affected patients; however many supportive treatments were tried and few countries were successful in treating the COVID-19 cases effectively. But still treatment in intensive care units (ICUs) has become a great challenge for intensivists, physicians and emergency physicians , early recognition of severe forms of COVID-19 is utmost important for timely triaging of patients. SARS-CoV-2 infection, especially in elderly patients and in older patients with pre-existing illness or con-morbid conditions as they have higher chances of progressing into a severe form of disease marked by critical respiratory symptoms and significant pulmonary changes such as ground glass opacities , patchy consolidation , alveolar exudates and septal involvement visible by imaging techniques [1]. Due to the limitations in imaging techniques and exposure to unnecessary radiation in patients with pre-existing diseases several biochemical markers have been identified that give a basic picture of the COVID-19 case. Patients admitted to ICUs have higher concentrations of pro-inflammatory cytokines leading to respiratory complications and mortality.

In various studies, various inflammatory cytokines and chemokines, tumour necrosis factor alpha (TNF-α), interferon-g-induced protein 10(IP-10),monocyte chemo-attractant protein 1(MCP-1), chemo-kines (C-C motif), ligand 3 (CCL-3), and interleukins (IL) (IL-2, IL-6, IL-7, IL-10) were remarkably associated with disease severity and particularly observed among cases admitted to ICUs. Other biological markers such as C - reactive protein (CRP), Lactate dehydrogenase (LDH), Serum Ferritin, Procalcitonin and D-Dimer are also used for assessing the severity of the disease.

Aim Of the Study

This study is aimed to investigate serum levels of interleukin 6 (IL-6), C-reactive protein (CRP), lactate dehydrogenase (LDH), D-dimer and procalcitonin in COVID-19 patients, and to find the correlation of these parameters with disease severity and progression.

Materials and Methods

This was a one year prospective study done between January 2020 and December 2020 and the sample size was 4308 COVID-19 infection positive cases. This study was done at Aakashpathlab, Aakash Healthcare super-specialty hospital, Dwarka. Informed consent for the sample testing was taken from the patient and this study was approved by the institutional ethics committee (IEC). Tests included for this study are C-reactive protein (CRP),D-Dimer , Interleukin-6 (IL-6), Procalcitonin and Lactate Dehydrogenase

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(LDH).Minimum of two milliliters (2 ml) of sample was collected in ne EDTA (Ethylene Diamine Tetra-acetic acid) violet or yellow top tube Acid-Citrate-Dextrose-A Anticoagulant)for IL-6 , 2ml blood sample in 3 separate clot activator tubes (red top tube) for LDH,CRP AND Procalcitonin. One sample collected in blue-top tube with 3.2% of sodium citrate for D-Dimer assay. All collected blood samples per patient were received in the lab with proper details of the patient such as name with surname, IP or OP number, unique health identity number (UHID) etc along with the proper requisition. For the red top tubes (clot activator) samples were centrifuged at 2000 rotation per minute (rpm) for 5 minutes and serum was taken for the tests. IL-6, CRP and procalcitonin tests were done by using Roche Cobas 6000 equipment and the method was Electro-chemiluminescence immunoassay(**ECLIA**).

D-Dimer test was done by immunoturbidometry method by ACL Elite Pro equipment and LDH UV assay was done by IFCC. All COVID-19 positive patients with mild, moderate and severe disease who were admitted in the hospital was included. Patients who tested positive and took treatment at home or on out-patient basis were excluded from this study. Above mentioned tests were repeated whenever there was a need.

Statistical Analysis

Continuous variables are expressed as median, mean±standard deviation and categorical variables are expressed as numbers (percentages).A Chi square test were performed for the p-value. P value of <0.05 was considered significant.

Results

A total 4308 cases were included in this study. Tests were done for the markers CRP, LDH, IL-6, D-dimer and procalcitonin. Males were 69.21 % and females 30.79 %.Most of the cases reported were in the age group of 36- 45 years (1468 cases-35.95%) followed by 26-35 years (996 cases-24.34 %)and 46-55 years(924 cases- 22.63%) , in age group 56-65 years (407 cases-9.97%), in age group 66-80 years (184 cases-4.10 %) least cases were noted in 16-25 age group (329 cases-8.05%.) . Median age was 39.4 years. Inflammatory markers tests results for this study are tabulated in Table No: 1

Table 1: CRP, LDH, D-Dimer, IL-6 and Procalcitonin levels with Mean and standard deviation

CRP Levels (Mean ± SD) Normal <6mg/L	Number of Patients	Percentage
≤ 6 mg/L (5.3 ± 1.08 mg/L)	1833	42.55
7-10 mg/L (9.4 ± 1.93 mg/L)	594	13.79
11-20 mg/L (18.1 ± 3.08 mg/L)	795	18.46
>20 mg/L (26.9± 4.12 mg/L)	1086	25.20
Total	4308	100
LDH Levels (Mean ± SD) Normal Range 85-230 IU		
< 230 IU (199 ± 15.4 IU)	2679	62.19
231-450 IU (402 ± 44.2 IU)	893	20.73
460-600 IU (543 ± 62.3 IU)	482	11.19
>600 IU (794 ± 71.4 IU)	254	05.89
Total	4308	100
D-Dimer (Mean ± SD) Normal Range <500 ng/mL		
<500 (301 ± 6.2)	2218	51.48
500-2500 (1980 ± 38.2)	701	16.27
2500-5000 (2645 ± 44.6)	686	15.93
5000-10000 (6278 ± 69.1)	497	11.54
>10000 (10988 ± 148.6)	206	04.78
Total	4308	100
IL-6 (Mean ± SD) Normal Range <9.4 pg/mL		
<9.4 (8.6 ± 1.2)	2062	47.86
10-250 (198 ± 9.2)	782	18.15
250-500 (431 ± 11.6)	678	15.74
500-1000 (831 ± 16.4)	502	11.65
>1000 (1245 ± 22.9)	284	06.60
Total	4308	100
Procalcitonin (Mean ± SD) Normal Range <0.05 ng/mL		
<0.05	2684	62.30
0.05-2 (1.05 ± 0.06)	1249	28.99
2-5 (3.15 ± 0.85)	267	06.20
>5 (5.42 ± 1.04)	108	02.51
Total	4308	100

Total number of patients with co-morbidities like obesity, diabetes mellitus, hypertension, heart disease, renal disease and cancers were 603 cases. The levels of inflammatory markers (Median) were compared with the patients with co-morbid factors and those without any pre-existing disease and the results are tabulated in Table 2.

Table 2:Comparison of Inflammatory markers in patients with co-morbidities

Markers	Co-Morbidities (Median)	No Co-morbidities(Median)	p Value
CRP	24.8 mg/L	18.4 mg/L	<0.05
LDH	584 IU	309 IU	<0.05
D-DIMER	6426 ng/mL	4129 ng/mL	<0.05
IL-6	879 pg/mL	644 pg/mL	<0.05
PROCALCITONIN	3.46 ng/mL	2.69 ng/mL	<0.05

*p Value was significant <0.05

It was clearly evident that the inflammatory markers were high in the patients with co-morbid conditions and had a moderate to severe grade of the disease when compared the patients with no co-morbid factors.

Comparison of disease course in the hospital was made and it is tabulated in Table no: 3.

Table 3: Comparison of disease course in the hospital

	Not Required Oxygen	Oxygen Required	ICU Transfer	Non-Invasive or Invasive Ventilation	Death
No Comorbidities (n=3705)	2481(67%)	1224 (33%)	21(0.056%)	14 (0.003%)	16 (0.004)
Comorbidities (n= 603)					
Diabetes (n=189)	189 (31.34%)	72 (11.95 %)	14 (2.32%)	5 (0.82 %)	4 (0.66 %)
Hypertension(n=93)	93 (15.41 %)	41 (6.80 %)	12 (1.99 %)	4 (0.66 %)	6 (0.99 %)
Coronary Artery Disease(n=62)	03 (4.83%)	59 (95.16 %)	59 (95.16%)	07 (11.30 %)	3 (4.83%)
Congestive Heart Failure(n=23)	00 (00%)	23 (100%)	19 (82.6 %)	03 (13.04%)	02 (8.69%)
Asthma(n=44)	00 (00%)	44 (100%)	41 (100%)	05 (11.36 %)	04 (9.09%)
Allergic Bronchitis(n=35)	00 (00%)	35 (100%)	33 (100%)	04 (11.42 %)	02 (5.71%)
Emphysema(n=29)	00 (00%)	29 (100%)	27 (100%)	04 (13.79 %)	05(17.24%)
Chronic Bronchitis(n=24)	00 (00%)	24 (100%)	22 (100%)	05 (20.83 %)	02 (8.33 %)
Chronic Kidney Disease(n=19)	01 (1.61%)	18 (94.73%)	18 (94.73%)	06 (31.57 %)	03(15.79%)
End Stage Renal Disease(n=15)	00 (00 %)	15 (100%)	13 (100%)	06 (40 %)	06 (40 %)
Cancer(n=14)	00 (00 %)	14 (100%)	13 (100%)	05 (35.71 %)	07 (50%)
Obesity (BMI >30) (n=39)	03 (4.83%)	36 (92.30%)	13 %	01 (2.56 %)	01 (2.56%)
Morbid Obesity (BMI>35) (n=17)	01 (1.61%)	34 (87.17%)	19 %	03(8.57 %)	06(35.29%)

From the above table it is clearly evident that the patients with co-morbid and pre-existing diseases were more prone to oxygen support, Intensive Care Unit (ICU) requirement, and Non-Invasive ventilation support such as BiPAP or C-PAP or Invasive ventilation. More number of deaths was recorded in the people admitted to ICU and with co-morbid conditions.

When duration of the hospital stay was assessed, it was noted that patients with higher levels of inflammatory markers and co-morbid conditions required increased stay in the hospital when compared to the non-co-morbid factors (Table No. 4).

Table 4: Duration of hospital stay

	< 7 days	8-14 days	>14 days
No Co-Morbidities (n=3705)	3108 cases	411 cases	186 cases
Co-Morbidities (n=603)	169 cases	230 cases	204 cases

Discussion

Inflammatory markers and its responses play a major role in the progression and severity of COVID-19 infection [2, 3].Inflammatory responses occur due to and aggravate at times because of rapid viral replication of SARS-CoV-2 and host cellular destruction which leads to recruitment of macrophages and monocytes and induce the release of chemical mediators like cytokines and chemokines [4]. These cytokines and chemokines then attract immune cells by a process named chemotaxis and activate immune responses, leading to cytokine storms and aggravations [5]. Several inflammatory markers have some tracing and detecting accuracy for disease severity and fatality [6]. Various inflammatory markers such as procalcitonin (PCT), serum ferritin, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), LDH, and interleukin-6 (IL-6) have been reported and their association is established, these inflammatory markers are significantly associated with the high risks of the development of severe COVID- 19 if not taken care of in the initial stage of the infection [7,8] .

C-Reactive Protein (CRP)

CRP is a plasma protein produced by the liver cells and induced by other inflammatory mediators such as IL-6. Despite being non-specific, this acute phase reactant is used clinically as a biomarker for various inflammatory conditions; a rise in CRP levels are associated with an increase in disease severity in various infections including COVID-19 [9]. The application of CRP in COVID-19 has been highlighted by a retrospective single-centre study in Wuhan, China, where the maximum number of patients in the severe cohort showed significantly higher levels compared to the non-severe cohort (57.9 mg/L vs 33.2 mg/L, P<0.001) [10]. A second retrospective cohort study found the likelihood of progressing to severe COVID-19 disease increased in patients with CRP levels>41.8 mg/L [11]. Both studies suggest CRP levels are a strong indicator to reflect the presence and severity of COVID-19 infection.

Interleukin-6 (Il-6)

Cytokine release syndrome (CRS) is an over-exaggerated immune response involving an overwhelming release of pro-inflammatory mediators. This mechanism underlies several pathological processes including acute respiratory distress syndrome (ARDS) [12]. Studies investigating the role of cytokines in SARS have had also found a link between CRS and disease severity [13]. Understanding their role in COVID-19 disease may help facilitate the design of novel immunotherapies. Studies have revealed that levels of IL-6, the most common type of cytokine released by activated macrophages, rise sharply in severe manifestations of COVID-19 [14]. One meta-analysis reviewing six studies show mean IL-6 concentrations were 2.9-fold higher in patients with complicated COVID-19 compared to

those with non-complicated disease (n = 1302; 95% CI 1.17–7.19) [15].

Lactate Dehydrogenase (LDH)

LDH is a part of glucose metabolism, the enzyme LDH converts pyruvic acid to lactate or lactic acid. LDH secretion is triggered by necrosis of the cell membrane, hinting to viral infection or lung damage, such as the pneumonia induced by SARS-CoV-2. There is convincing evidence linking LDH levels to the development of COVID-19 disease. A study found significantly higher levels of LDH in ICU patients than non-ICU patients (248 U/L vs 151 U/L, p=0.002). Since high levels of LDH continued in the ICU patients number of days post-admission (160 U/L vs 218 U/L, p=0.002), LDH may be a predictive biomarker of severe disease. A multi-centre study involving 1099 patients reported supporting evidence correlating extent of tissue damage and inflammation with increasing levels of LDH [16]. Furthermore, when LDH levels were correlated with CT scans, significantly higher levels reflected the severity of pneumonia [17]. There is increasing confidence in using LDH as a biomarker to measure severity of COVID-19 infection.

D-Dimer

D-dimer originates from the lysis of cross-linked fibrin with rising levels indicating the activation of coagulation and fibrinolysis [18]. Early studies have associated COVID-19 with haemostatic abnormalities with one study observing elevated levels of D-dimer, the measure of coagulation, in non-survivors compared to survivors [19]. A retrospective cohort study composed of 191 patients found that Ddimer levels>1.0 μg/mL(p=0.0033) were associated with increased mortality among COVID-19 patients.

Procalcitonin (PCT)

Procalcitonin is a peptide precursor of the hormone calcitonin and is produced by parafollicularceels of the thyroid and by the neuroendocrine cells of the lung and the intestine. Calcitonin is involved with calcium hemostasis. It increases in circulation when precalcitonin is cleaved by endopeptidase. The normal level of procalcitonin in the blood stream of healthy individuals is below the limit of detection (0.01 μg/L) of clinical assays.The level of procalcitonin rises in a response to a pro-inflammatory stimulus, especially of bacterial and viral origin. It is therefore often classed as an acute phase reactant.

In this study, it is evident that higher levels of CRP, LDH, D-Dimer, Procalcitonin and IL-6 are significantly associated with severity of the disease and high levels lead to rapid disease progression in COVID-19 patients. It was also found that higher levels (1.5 to 2 times higher) are detected much earlier during the disease in patients with co-morbid conditions than patients with no pre-existing disease. This study is in sync with the findings of the studies done by authors Mostaghim et al , MuhammedKermali et al , Thirumalaisamy P. Velavan et al, Rui Hu et al , ShantAyanian and Kadhim AS et al where all these authors also reported that increased levels of inflammatory markers are directly proportional to the disease severity of COVID-19 infection [22-25].

Conclusion

Infection of COVID-19 can lead to increase in the inflammatory markers which are more marked in the cases of pre-existing disease or patients with co-morbidities. Laboratory markers of inflammation and coagulopathy can help clinicians identify patients who are prone for clinical deterioration and require more clinical attention.

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