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Original Research Article

Study of various laboratory parameters in COVID-19 patients at a tertiary care center

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ABSTRACT

Introduction: In China, Wuhan City became the epicentre of unexplained cases of pneumonia in December 2019 and was temporarily labelled as, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). World Health Organization renamed it to coronavirus disease 2019 (COVID-19) in February 2020. In COVID-19 patients hematological profile showed increased total leukocyte count (TLC) and neutrophilic count, while decrease in other hematological parameters. The mild increase in TLC was found in severe disease patients; however significant increase was associated with clinical worsening and poor outcome. Significant elevation of various biochemical and inflammatory markers was noticed among COVID-19 patients

Materials and Methods: We conducted a retrospective study on RT-PCR confirmed 239 COVID-19 patients who were hospitalized in various COVID-19 facilities from Sept 2020 to Dec 2020. The laboratory data is used in de-identified form and doesn't reveal identity of any subjects.

Results: A total of 239 cases were examined, out of which 176(73.7%) were male and 63(26.3%) were female (M:F ratio= 2.79:1). We found that 104 cases had Hb level \geq 12gm/dl. We found that maximum patients (175) had TLC level $>$ 11000/cumm, 187 patients had ANC level $>$ 7000/cumm. We found that 160 cases had serum urea of $>$ 43mg/dl, 134 case with serum creatinine of $>$ 1.4gm/dl. 134 cases had ALT level of $>$ 40IU/L and 143 cases had AST levels of $>$ 42IU/L. In this study out of 239 cases 126 cases were found to have increased level of all inflammatory markers levels.

Conclusion: We found a significant association of various inflammatory markers with haematological and biochemical parameters in COVID-19 patients.

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1. Introduction

In China, Wuhan city became the epicentre of unexplained cases of pneumonia in December 2019 and was temporarily labelled as, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by Chinese scientists after identification of a novel coronavirus in January 2020.^{1,2} The illness spread rapidly around the country and the world reaching a pandemic level. On 30 January 2020, the WHO declared

the outbreak of COVID-19 to be a “public health emergency of international concern”.³

In COVID 19 patients hematological profile showed increased WBC count and neutrophil count, while decrease in lymphocyte, platelet, eosinophil count, and hemoglobin level.⁴ The research data of last few months revealed the slightly increase in levels of ALT, AST, ALP and bilirubin along with significant increase in levels of renal biomarkers such as serum urea; creatinine and markers of glomerular filtration rate in critically ill patients.^{2,5-9}

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The higher levels of D-dimer in non-survivors as compared to survivors associated with hemostatic abnormalities in COVID-19 patients were observed by one study.¹⁰ Acute phase reactant such as CRP induced by various inflammatory mediators such as IL-6 is used clinically as a biomarker and elevated level indicate disease severity.¹¹ Circulating PCT levels are generally within the normal range for a viral infection as observed in COVID-19 patients.¹² LDH may be a predictive biomarker of severity in COVID-19 patients, as persistent high levels are seen in the ICU patients leads to post admission prolonged stay.¹³ High serum ferritin level was seen in severe and very severe COVID-19 patients.¹⁴

2. Material and Methods

2.1. Study design and data collection

We conducted a retrospective study on RT-PCR confirmed 239 COVID-19 patients who were hospitalized in COVID-19 facilities of our institute from Sept 2020 to Dec 2020. The data is used in de-identified form and doesn't reveal identity of any subject and all medical records were obtained, including epidemiology, demographics, clinical manifestations, comorbidity, and laboratory data. For inpatients, laboratory data included complete blood count, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen and creatinine, high-sensitivity C-reactive protein, Lactate dehydrogenase, Procalcitonin, serum Ferritin, and D-dimer.

This study was reviewed and approved by the institutional ethics committee (ethical clearance certificate no. IEC/2021/59).

3. Results

In this study a total of 239 cases were studied, out of which 176(73.7%) were male and 63(26.3%) were female (M:F ratio= 2.79:1). Maximum numbers of patients were found in age group of 51-60 years followed by 61-70.[Table 1]

In this study, we correlated inflammatory markers with hematological and biochemical parameters. We found that 104 cases had Hb level \geq 12gm/dl followed by 97 cases had 10-11.9 gm/dl. We found that maximum patients (175) had TLC level $>$ 11000/cumm followed by 62 cases had TLC within normal limit. We found that 187 patients had ANC level $>$ 7000/cumm followed by 50 patients who had ANC within normal limits. We found majority of patients had ALC within normal limit followed by 81 cases was found had ALC $<$ 1500/cumm. We found that majority of patients (166) had platelet count within normal range, followed by 71 patients with platelet count $<$ 1.5lac/cumm.[Table 2]

We found that 160 cases had serum urea of $>$ 43mg/dl, 134 case with serum creatinine of $>$ 1.4gm/dl. 134 cases had ALT level of $>$ 40IU/L and 143 cases had AST levels of $>$ 42IU/L. Serum electrolytes were within normal range in

majority of patients.[Table 3]

In this study out of 239 cases 126 cases were found to have increased level of all inflammatory markers levels, while increase D-dimer in 192 cases, Procalcitonin in 132 cases, LDH in 179 cases, CRP in 192 cases and ferritin in 137 cases, respectively.[Table 4]

Out of 126 cases with elevated inflammatory markers, 120 cases had TLC $>$ 11000, 31 cases with low Hb level (7 to 9.9 gm/dl) followed by 03 case with Hb $<$ 7.0 gm/dl, 120 cases with ANC $>$ 7000, 78 cases with ALC $<$ 1500, 71 cases with platelet count $<$ 1.5 lacs/dl, 115 cases with urea $>$ 43 mg/dl, 110 cases with creatinine $>$ 1.4 mg/dl, 110 cases with ALT $>$ 40 IU/L and 110 cases with AST $>$ 42 IU/L.

Table 1:

Age group (years)	Male	Female	Total number
1-10	00	00	00
11-20	01	00	01
21-30	01	09	10
31-40	25	05	30
41-50	39	09	48
51-60	43	19	62
61-70	40	15	55
$>$ 70	27	06	33
Total (%)	176(73.7)	63(26.3)	239(M:F=2.79:1)

4. Discussion

The epidemic of SARS-CoV-2 infection has spread globally, posing a great threat to public health.¹⁵ As the disease spread worldwide, World Health Organization renamed it to coronavirus disease 2019 (COVID-19) in February 2020.² The severe acute respiratory syndrome Coronavirus-2(SARS-CoV-2) belongs to the Coronaviridae family. The Coronaviridae family consists of single stranded positive RNA enveloped viruses, categorized into four sub-groups: 1) α - coronavirus (α -COV), 2) β -coronavirus (β -COV), 3) δ -coronavirus (δ -COV) and 4) γ -coronavirus (γ -COV), in which SARS-CoV-2 fall into β - COV sub-group.¹⁶ The β -COV sub-group has two highly pathogenic viruses, SARS-CoV and the Middle East Respiratory Syndrome Corona Virus (MERS-CoV). The SARS-CoV (now named SARS-CoV-1) was identified in November 2002 in Guangdong, China, and subsequently spread rapidly all over the world affecting 29 countries and only a decade later in Middle Eastern countries MERS-CoV caused an endemic in June 2012.^{17,18} It has been hypothesized that SARS-CoV-2 might have been transmitted by bats, snakes, or pangolins; however the virus is highly contagious from human to human through respiratory droplets and aerosols.¹⁹⁻²² In COVID-19, the incubation period varies from 1 to 14 days and manifest as respiratory tract infection with a different degree of severity, from asymptomatic

Table 2: Showing variation in hematological parameters in covid-19 patients

Hematological parameters		Total number of patients	Percentage (%)
Hemoglobin (gm/dl)	<7.0	03	1.24%
	7.0-9.9	35	14.64%
	10.0-11.9	97	40.60%
	>12	104	43.52%
Total Leukocyte Count (/cumm)	<4000	02	0.84%
	4000-11000	62	25.94%
	>11000	175	73.22%
Absolute Neutrophil Counts (/cumm)	<3000	02	0.84%
	3000-7000	50	20.92%
	>7000	187	78.24%
Absolute Lymphocyte Counts (/cmm)	<1500	78	32.64%
	1500-4000	150	62.76%
	>4000	11	4.60%
Platelets Counts (/cumm)	<1.50	71	29.70%
	1.50-4.50	166	69.46%
	>4.50	02	0.84%

Table 3: Showing variation in biochemical parameter in covid-19 patients

Biochemical parameters		Total number of patients	Percentage
Urea (mg/dl)	<13	02	0.84%
	13-43	77	32.21%
	>43	160	67.51%
Creatinine (mg/dl)	<0.4	03	1.26%
	0.4-1.4	109	45.60%
	>1.4	127	53.14%
ALT (IU/L)	<5	02	0.84%
	5-40	103	43.10%
	>40	134	56.10%
AST (IU/L)	<5	03	1.26%
	5-42	93	38.91%
	>42	143	59.83%

Table 4: Showing variation in inflammatory marker in covid-19 patients

Inflammatory markers		Total number of patients	Percentage
D-DIMER	<0.5	44	18.41%
	0.5-5.0	101	42.30%
	>5.0	94	39.33%
Procalcitonin	<0.05	107	44.80%
	0.05-2.00	97	40.59%
	>2.00	35	14.61%
C-Reactive Protein	<6.0	47	19.66%
	6-50	72	30.13%
	>50	120	50.21%
LDH	<240	60	25.10%
	240-480	52	21.76%
	>480	127	53.14%
Ferritin	<30	39	16.32%
	30-220	63	26.36%
	>220	137	57.32%

patients to pneumonia progressing to acute respiratory distress syndrome (ARDS) and multiple organ failure (MOF) and ultimately death. The patients associated with comorbidities, like diabetes, hypertension, chronic obstructive pulmonary disease and elderly (>65 years) are vulnerable to severe disease.²²

In COVID 19 patients hematological profile showed increased WBC count and neutrophil count, while decrease in lymphocyte, platelet, eosinophil count, and hemoglobin levels. The mild increase in WBC count was found in severe disease patients, significant increase was associated with clinical worsening and poor outcome. The critical illness in hospitalized COVID-19 patients can be monitored by WBCs, lymphocyte and platelet count, along with serum ferritin level.⁴ The evidence that lymphocytes express the ACE2 receptor on their cellular membrane supporting the hypothesis that the virus might directly infect lymphocytes, inducing depletion of CD4+ and CD8+ T cells leading to lymphopenia as the most common laboratory finding followed by neutrophilia.^{5,6,23} Additionally, the virus might directly destroy lymphatic organs. The pro-inflammatory cytokines, such as IL-6 and TNF-alpha, can lead to lymphocyte deficiency.²⁴ Finally, thrombocytopenia has been associated with the progression and prognosis of the disease.²⁵ Some authors suggested neutrophil-to-lymphocyte ratio (NLR) as an independent risk factor for severe disease.³ Multiple causes can induce platelet deficiency such as SARS-CoV2 directly infecting the hematopoietic cells or bone marrow stromal cells, leading to inhibition of hematopoiesis and their increased consumption due to the activation, aggregation, and retention of platelets in the lung injury, and the formation of thrombus at the injured site.^{26,27}

Liver function tests constitutes liver enzymes, proteins, bilirubin and some biochemical parameters such as ALT, AST, ALP, LDH, albumin, and total bilirubin are screening tool for several medical conditions not only biochemical markers of liver dysfunction.⁷ Without baseline liver impairment in COVID-19 patients, higher levels of ALT, AST, ALP and bilirubin were at higher risk of admitting to ICU/CCU indicating that elevated enzymes are due to systemic inflammation and predict disease progression.^{7,28} In serious COVID-19 patients albumin level was reported significantly low, underlying causes include decreased biosynthesis due to poor intake of protein and excessive loss of albumin.²¹ The gradual monitoring of liver enzymes and protein, especially LDH and albumin indicate progression, severity and ultimate clinical outcome hence their monitoring is helpful in COVID-19 progression.⁷

Patients with significant increase in renal bio-markers were more likely to needed mechanical ventilation or be placed in intensive care and correlating with abnormalities in the coagulation pathway.^{2,8,9}

The rising levels of D-dimer indicate the activation of coagulation and fibrinolysis.²⁹ A retrospective cohort study 191 COVID-19 patients found that D-dimer levels >1.0 $\mu\text{g/mL}$ were associated with increased mortality. Furthermore, it was found that levels equal to/or more than 2.0 $\mu\text{g/mL}$ on admission was the minimum cut-off to predict outcome in hospital mortality.³⁰ Huang et al. found that levels of D-dimer on admission may be used in triage patients for critical care.⁶ The researchers found higher median D-dimer levels in ICU patients as compared to non-ICU patients suggesting that D-dimer levels can be used as a prognostic marker and helps in monitoring the patients, those who are likely to deteriorate earlier.² D-dimer levels are associated with a poor outcome & with increased risk of ARDS, ICU admission, and mortality reflecting the coagulation alterations.^{6,31,32} The elevated levels of fibrinogen, fibrin degradation products (FDP), prothrombin time (PT) and activated partial thromboplastin time (aPTT) during the early phase of COVID-19 have been associated with severe disease.¹⁰

A significantly higher level of CRP was observed in severe cohort as compared to the non-severe cohort in the retrospective single-center study conducted in Wuhan, China.³³ Patients with CRP levels >41.8 mg/L are more likely to progress to severe COVID-19 according to another retrospective cohort study.³⁴ Both studies observed that CRP levels are a strong indicator of severity of COVID-19 infection.³⁵ Studies have recommended that CRP levels can be a useful biomarker for the disease progression as it is independent of the factors such as age, physical conditions and gender.³⁶ During infections, CRP levels increase as a defense mechanism because it activates the immune complement system, increasing phagocytosis to remove the pathogens.³⁷

However, 5-times higher risk of evolution of severe disease and a bacterial co-infection have been associated with significantly raised levels of PCT.¹²

Lung damage due to viral infection is associated with cell membrane damage which triggers the LDH secretion.³⁸ The LDH levels have been found to be associated in development of COVID-19 disease.³⁹ The LDH level was found significantly higher in ICU patients as compared to non-ICU patients (248 U/L vs 151 U/L, $p=0.002$) in a study. The evidence of increasing levels of LDH and inflammation along with extent of tissue damage was supported by a multi-center study of 1099 patients.¹³ The LDH as a biomarker is used confidently to measure the severity of COVID-19 infection which was also supported by correlation with CT scans finding with significantly higher levels reflected the severity of pneumonia.²

A study revealed that high levels of ferritin were seen at the time of hospital admission and throughout the hospital stay in patients who died of COVID-19. Chen et al. analyzed the clinical characteristics of 99 patients observed that

out of 99 patients, 63 of them had serum ferritin way above of the normal range.²³ Autopsies of 12 patients who died due to SARS-CoV-2 infection revealed high serum ferritin level. Peripheral blood of 69 patients with severe COVID-19 revealed elevated levels of ferritin as compared to patients with non-severe disease hence, concluding that serum ferritin levels were closely related to the severity of COVID-19.⁴⁰

COVID-19 is a rapidly spreading pandemic; patients present with wide range of symptoms varying from mild fever to ARDS complicating diagnosis, prognosis, and monitoring. Chen D et al.²⁹ found leukocytosis in 62 cases and lymphopenia in 179 cases out of 274 cases, while in this study leukocytosis was found in 163 cases out of 234 cases and lymphopenia in 81 cases out of 234 cases. Lai CC. et al.,¹⁰ Qin C. et al.,³⁰ and Lippi G et al.³¹ also found leukocytosis, especially neutrophilia. Agarwal A et al.³² found lymphopenia in 19 patients out of 102 cases. Lippi G et al.¹¹ found significantly lower platelet count in 375 cases out of 1725 cases. Cai Q et al.³³ found abnormal liver function tests in 318 cases out of 417, while we found abnormal liver function tests in 143 cases out of 239 cases. Guan et al.³⁴ found elevated AST in 18.2% case of non-severe and 39.4% cases of severe cases, however we found elevated ALT in 134 cases and elevated AST in 143 cases out of 239 cases. Pei G. et al.³⁵ found abnormal renal function in 251 cases out of 333 cases, while we found 160 cases out of 234 cases. F Liu et al.²⁶ found CRP level > 41.8 mg/L in severe COVID 19 patients indicating the disease progression, however we found increase CRP level in 192 cases and significantly high level >50.0 mg/L was found in 115 cases. Due to weak ACE2 receptor expression on hepatocytes the liver function is not severely impaired. Luo et al.³⁶ found significantly higher levels of LDH in severe patients, while in this study 127 cases out of 239 had high LDH levels. L. Zhang et al.³⁷ found high levels of D-dimer in non-survivors as compared to survivors of COVID-19 infection and E.R. Milbrandt et al.¹² found high D-dimer levels in 191 patients and was associated with increased mortality among COVID-19 patients.

In present study, we found a significant association of various inflammatory markers with haematological and biochemical parameters in COVID-19 patients, but variation among patients could affect the findings of studies. Therefore, further research on accuracy of these biomarkers correlation and understanding their relation with disease prognosis and outcome is required.

COVID-19 is a rapidly spreading pandemic; patients presents with wide range of symptoms varying from mild fever to ARDS complicating diagnosis, prognosis, and monitoring. The quantitative measurement of biomarkers used clinically for many conditions reflecting underlying pathological changes and clinician in diagnosis, monitoring and predicting prognosis and outcome of a disease. White cell counts (WCC) are influenced by many factors

such as glucocorticoid treatment which increases it, as compared to other biomarkers WCC is less reliable used. Although WCC encompasses many cell types, neutrophil and lymphocyte counts are most clinically relevant biomarkers. Many studies on COVID-19 found high NLR in severe cases as compared to non-severe cases. Therefore, further research on WCC accuracy for assessing disease progression is necessary. In present study, we found a significant association of various biochemical parameters and inflammatory markers with haematological parameters in COVID-19 patients, but variation among patients could affect the findings of studies. Therefore, further research on accuracy of these biomarkers correlation and understanding their relation with disease prognosis and outcome is required.

5. Conflict of Interest

The authors declare no relevant conflicts of interest.

6. Source of Funding

None.

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