ORIGINAL ARTICLE

Laboratory Parameters in Patients with Moderate and Severe COVID-19 in 2020 and 2021: A Comparative Study

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Abstract:

Background: Coronavirus Disease (COVID-19) is caused by the SARS-CoV 2 virus. Understanding the common laboratory features of COVID-19 in more severe cases versus non-severe patients and its variability over time could be quite useful for clinicians to predict the model of disease progression. Aim and Objectives: To assess the laboratory parameters in patients with COVID-19 and to compare the laboratory parameters of patients presenting in 2020 to 2021. Material and Methods: This was be a single centre, observational, comparative study done on patients with COVID-19 in 571 patients presenting with moderate to severe severity in a tertiary care hospital. This study was conducted over period of one year from July 2020 to June 2021. All enrolled patients underwent inflammation markers C-reactive protein (CRP), D-Dimer, Sr. Ferritin, Interleukin-6 (IL-6), haematological markers (neutrophil, lymphocyte count) Neutrophil to Lymphocyte Ratio (NLR) and High-resolution Computed Tomography (HRCT) scan of the thorax. Data were analysed for mean, percentage, standard deviation and chi square test for quantitative data by using SPSS software version 21 (trial version) for analysis and 'p' value < 0.05 was considered as statistically significant. Results: The present study was conducted on 571 patients presenting with moderate to severe COVID-19 infection. A total 279 moderate to severe COVID-19 patients of year 2020 and 292 of 2021 were included and compared in the present study. The HRCT score, IL-6 level, D-Dimer, serum ferritin, neutrophil lymphocyte ratio, total leucocyte count and age had a positive correlation with the severity of the disease in both the year 2020 and 2021. CRP and Erythrocyte Sedimentation Rate (ESR) had a positive correlation in 2021. Except NLR, all other parameters had a positive correlation with severity of disease. *Conclusion:* In the year 2021 there was a trend of relatively younger population being infected often. The correlation of various inflammatory markers with the severity of disease was positive with majority of them demonstrated significant correlation, further supporting their use for disease progression monitoring.

Keywords: COVID-19, Inflammatory Markers, Creactive protein, total leucocyte count, neutrophil lymphocyte ratio, ferritin, IL-6, D-dimer, Highresolution computed tomography

Introduction:

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has been indicated the virus responsible of SARS-CoV-2 disease (COVID-19), which has spread worldwide since December 2019 [1]. There has had been a more severe second wave of the COVID-19 pandemic than the first [2]. The clinical spectrum of the disease varies from asymptomatic infection, mild upper respiratory symptoms to severe pneumonia with respiratory failure and even death. The most common symptoms at onset of SARS-CoV 2 illness are fever, cough, ache, dyspnoea, haemoptysis and diarrhoea. The severe symptoms of SARS-CoV 2 are associated with an increase rate of fatalities [3].

The older population continues to be more vulnerable to COVID-19 in the second wave while a marginal increase is recorded in the number of young people testing positive for the virus. A higher proportion of asymptomatic patients have been recorded. Triage of the COVID-19 patients is based on clinical and laboratory parameters, whilst chest imaging might be required for second-level triage by means of chest radiography as the first step and supplementary Computed Tomography (CT) in more severe cases or in case of discrepancy between clinical and radiographic findings [5]. Laboratory testing is often done in patients with COVID-19. Each of the laboratory parameters do have a potential value for risk stratification and the prediction of COVID-19 outcomes [5].

Inflammatory markers such as serum ferritin, Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP) and Interleukin-6 (IL-6) have been reported to be of significant association with the increased risk of the development of severe COVID-19 [6, 7]. This study is designed to investigate the differences in the clinical, radiological and laboratory parameters amongst the patients who had COVID-19 in 2020 and 2021.

Material and Methods:

Aim and Objectives: To assess the laboratory parameters in patients with COVID-19 and to compare the laboratory parameters of patients presenting in 2020 to 2021.

Study design: This was be a single centre, observational, comparative study done on patients with COVID-19 infection.

This study was conducted in KIMS Hospital, over period of one year from April 2020 to April 2021. The Institutional Ethics Committee approval was taken (protocol number: 376/2020-2021) Date: 08/06/21.

Study sample: The study was conducted in 571 patients presenting with moderate to severe COVID-19 infection in a tertiary care hospital.

Study setting: This study was conducted in KIMS Hospital, over period of one year from July 2020 to June 2021. The Institutional Ethical committee approval was taken (protocol number: 376/2020-2021).

Inclusion criteria: were patients with moderate and severe COVID-19 pneumonia with at least one positive RT-PCR test for COVID-19 requiring oxygen support with at least 5 lit/min, high flow nasal cannula, non-invasive ventilation or invasive ventilation and with a known endpoint of the disease.

Exclusion criteria: The patient with a mild infection, hepatic and renal dysfunction and those with history of ischemic heart disease and ischemic stroke were excluded from this study. All enrolled patients underwent inflammation markers (CRP, D-Dimer, Sr. Ferritin, IL-6), haematological markers (Neutrophil, Lymphocyte, Leucocyte Count) and High Resolution Computed Tomography (HRCT) scan of the thorax. Raw data was collected from medical records by the trained medical staff. Data were double checked for correctness, subsequently anonymized and transferred to the first authors for data analysis. We have aimed to study and compare the laboratory parameters and the radiological parameters of moderate to severe COVID-19 patients.

Statistical Analysis:

Collected data were analysed for mean, percentage, standard deviation and chi square test for

quantitative data by using SPSS software version 21 (trial version) for analysis and 'p' value < 0.05 was considered as statistically significant.

Results:

The present study was conducted on 571 [2020 year 279(48.86%); 2021 292(51.13%)] patients presenting with moderate to severe COVID-19 infection in a tertiary care hospital. A total 279 moderate to severe COVID-19 patients of year 2020 and 292 of 2021 were included and compared in the present study. In the year 2020 mean severity was 2.19 ± 0.45 . The mean age was 55.24 ± 16.29 , mean haemoglobin was 12.78 ± 2.25 , mean Total Leukocyte Count (TLC) was 7870.35 ± 4144.92 and the mean NLR was 4.12 ± 4.57 . The mean ESR was 39.69 ± 31.57 , mean

CRP level was 1.79 ± 2.58 , mean Sr. Ferritin was 285.09 ± 336.67 , mean D-Dimer was 0.95 ± 2.52 and the mean IL-6 was 39.99 ± 133.20 . The mean HRCT score was $7.56/25 \pm 5.85$. In the year 2021 mean severity was 2.19 ± 0.56 . The mean age was 50.63 ± 14.41 , mean haemoglobin was $12.38 \pm$ 1.68, mean TLC was 8644.76 ± 5824.10 and the mean NLR was 4.85 ± 2.28 . The mean ESR was 46.70 ± 26.76 , mean CRP level was 1.86 ± 6.21 , mean Sr. Ferritin was 426.37 ± 423.85 , mean D-Dimer was 0.77 ± 1.49 and the mean IL-6 was 35.84 ± 97.48 . The mean HRCT score was 9.48/25 \pm 4.27. The mean age in year 2021 year group was significantly lower compared to year 2020 ('p' <0.05), while all other variables were had no statistical significance (Table 1).

Table 1: The Mean and Standard Deviation of Numerical Variable in COVID-19 Patients

Parameter	Mean ± SD (Year 2020)	Mean ± SD (Year 2021)	р
Age	55.24 ± 16.29	50.63 ± 14.41	< 0.05
Haemoglobin (Hb)	12.78 ± 2.25	12.38 ± 1.68	
Total Leukocyte Count (TLC)	7870.35 ± 4144.92	8644.76 ± 5824.10	
Neutrophil to Leukocyte Ratio (NLR)	4.12 ± 4.57	4.85 ± 2.28	cant
Erythrocyte Sedimentation Rate (ESR)	39.69 ± 31.57	46.70 ± 26.76	mific
C-reactive Protein (CRP)	1.79 ± 2.58	1.86 ± 6.21	insig
Ferritin	285.09 ± 336.67	426.37 ± 423.85	ally
D Dimer	0.95 ± 2.52	0.77 ± 1.49	istic
Interleukin-6 (IL-6)	39.99 ± 133.20	35.84 ± 97.48	Stat
High Resolution Computed Tomography (HRCT) Score	7.56 ± 5.85	9.48 ± 4.27	
Group (severity)	2.19 ± 0.45	2.19 ± 0.56	

We compared the correlation coefficient among year 2020 group and year 2021 group, and it was observed that the correlation coefficient (r) of CT severity in HRCT of patients belonging to a moderate to severe group in 2021was higher than those belonging to the same group in 2020 (2020: r=0.3; 2021: r=0.39) ['p'<0.01].The correlation coefficient (r) of IL-6 of patients belonging to a moderate to severe group in 2021was lower than those belonging to the same group in 2020 (2020: r=0.28; 2021: r=0.09) ['p' <0.09]. The correlation coefficient (r) of D-Dimer of patients belonging to a moderate to severe group in 2020 was lower than those belonging to the same group in 2021 (2020: r=0.09; 2021: r=0.11) [statistically insignificant]. The correlation coefficient (r) of Sr. Ferritin of patients belonging to a moderate to severe group in 2020 was lower than those belonging to the same group in 2021 (2020: r=0.21; 2021: r=0.29) ['p' =0.156]. The correlation coefficient (r) of CRP level of patients belonging to a moderate to severe group in 2020 was lower than those belonging to the same group in 2021 (2020: r=-0.03; 2021:

r=0.43) ['p' >0.01]. The correlation coefficient (r) of ESR of patients with moderate to severe group in 2020 was lower than those in 2021 (2020: r=-0.01; 2021: r=0.22) ['p'<0.01]. The correlation coefficient (r) of NLR of patients belonging to a moderate to severe group in 2020 was higher than those belonging to the same group in 2021 (2020: r=0.26; 2021: r=0.11) ['p' <0.03]. The correlation coefficient (r) of TLC of patients belonging to a moderate to severe group in 2020 was lower than those belonging to the same group in 2021 (2020: r=0.07; 2021: r=0.24). The correlation coefficient (r) of age of patients belonging to a moderate to severe group in 2020 was lower than those belonging to the same group in 2021 (2020: r=0.19; 2021: r=0.21) ['p'<0.4]. On comparison of correlation coefficient of year 2020 and 2021, ESR, TLC, CRP and HRCT Score correlation coefficient (r) with severity of COVID-19 was higher and positively correlated in year 2021 than 2020. NLR and IL-6 correlation coefficient (r) with severity of COVID-19 was higher and positively correlated in year 2020 than 2021 (Table 2, Figs. 1 and 2).

Variables	Year 2020	Year 2021	p
Age	0.19	0.21	0.401
Erythrocyte Sedimentation Rate (ESR)	-0.01	0.22	0.01
Total Leukocyte Count (TLC)	0.07	0.24	0.01
Neutrophil to Leukocyte Ratio (NLR)	0.26	0.11	0.03
Interleukin-6 (IL-6)	0.28	0.09	0.09
D-Dimer	0.09	0.11	0.4
Ferritin	0.21	0.29	0.156
C-reactive Protein (CRP)	-0.03	0.43	0.01
High Resolution Computed Tomography (HRCT) Score	0.20	0.39	0.01

Table 2: Comparison of Correlation Coefficient between Year 2020 and 2021



Fig. 1: Correlation and Clinical and Laboratory Parameters with Group of COVID 19 in Year 2020



Fig. 2: Correlation of Clinical and Laboratory Parameters with Group of COVID 19 in Year 2021

Discussion:

In the present observational study, we observed that in the year 2020the mean age of the cohort with COVID-19 was 55.24 ± 16.29 whereas in 2021, the mean age in the 2021 was 50.63 \pm 14.41(p<0.05) indicating a lower mean age group in the year 2021. It was also noted that the age in the year 2021 (r=0.21) was higher than those in 2020 (r=0.19) in relation to severity of the disease. In the present study, it was observed that HRCT severity scores in the year 2021 (r=0.39) were higher than those in 2020 (r=0.30) in relation to severity of the disease with a positive correlation in both the years (severity of disease was more with increasing HRCT score). The mean IL-6 levels in the year 2021 (r=0.28) were lower compared to 2020 (r=0.09) in relation to severity of the disease with a positive correlation in both the years. The D-Dimer levels in the year 2021 (r=0.11) were higher than in 2020 (r=0.09) in relation to severity of the disease with a positive correlation in both the years. Sr. Ferritin levels in the year 2021 (r=0.29) were higher than those in 2020 (r=0.21) in relation to severity of the disease with a positive correlation in both the years. The CRP levels in the year 2021 (r=0.43) were higher than in 2020 (r=-0.03) in relation to severity of the disease with a positive correlation in 2021 but not in 2020. The ESR levels in the year 2021 (r=0.22) were higher than those in 2020 (r=-0.01) in relation to severity of the disease with a positive correlation in 2021 but not in 2020. The NLR in the year 2021 (r=0.11) were lower than those in 2020 (r=0.26) in relation to severity of the disease with a positive correlation in both the years. There was no statistical difference among the two groups (year 2020 and year 2021).

Kumar et al. (2021) quoted that the second wave of COVID-19 in India was slightly different in presentation than the first wave, with a younger demography, lesser comorbidities, and presentation with breathlessness in greater frequency [8]. Jain et al. (2021) quoted that the age profile in 2020 consisted older population whereas in 2021 it consisted younger population [9]. Asrani et al. (2021) quoted patients in the first wave infected with SARS-CoV-2 were predominantly older than 60 and younger adults appear to be prone to infection during this latest cycle and many patients have died at a young age, including patients aged between 25 and 50 years [10]. Muhsen *et al.* (2021) quoted that the highest mortality rate was found amongst the elderly [11]. Asghar et al. (2021) quoted that increased age and length of ICU stay

was observed during the first wave [12]. Iftimie et al. (2021) quoted those in the second wave were significantly younger (58 ± 26 vs. 67 ± 18 years; p <0.001) [13]. Fluck et al. (2021) quoted that the mean \pm SD age of patients with COVID-19 in wave 1 was 69.4 years \pm 18.0 and wave 2 was 66.2 years \pm 18.4 [14]. The abovementioned studies reported a younger population being affected more commonly, similar to the findings of present study. Zeng et al. (2020) quoted those patients with COVID-19 in the non-severe group had lower levels for CRP, IL-6, ESR, and serum ferritin, compared with those in the severe group similar to the present study. Moreover, survivors had a lower level of IL-6 than non-survivors. The meta-analysis highlights the association of inflammatory markers with the severity of COVID-19. Measurement of inflammatory markers might assist clinicians to monitor and evaluate the severity and prognosis of

COVID-19 [15]. Francone et al. (2020) guoted CT score is positively correlated with age, inflammatory biomarkers, severity of clinical categories, and disease phases [16]. Elshazli et al. (2020) reported that ICU admission was associated with higher levels of WBCs, neutrophils, D-dimer, and prolonged PT; patients with high IL-6, CRP, Ddimer, and neutrophils had the highest likelihood of mortality [17]. Ghahramani et al. (2020) quoted ESR,CRP, D-dimer, and NLR in the severe group compared with the non-severe group similar to this study [18]. Jain et al. (2021) guoted that CRP, D-Dimer and CT severity score levels only can predict final outcome of death even in the patients in 2021 [19]. Laguna-Goya et al.(2020) quoted high IL-6 level, CRP level, Lactate Dehydrogenase (LDH) level, ferritin level, D-dimer level, neutrophil count, and NLR were all predictive of mortality [20]. Ata et al. (2021) quoted that lymphocyte count, ferritin, ESR, and CRP values. The CRP and ferritin were moderately and positively correlated with CT-SS, while NLR and ESR were mildly and positively correlated with chest Computerized Tomography Severity Score (CT-SS) [21]. Marimuthu et al. (2021) guoted IL-6 levels >60.5

pg/mL and D-dimer levels >0.5 mcg/ml predicted in-hospital mortality with sensitivities of 80% and 76.7%, respectively. NLR and CRP levels had good correlation with the need for oxygen supplementation and/or invasive ventilation. The TLCs in the year 2021 (r=0.24) were higher than those in 2020 (r=0.07) in relation to severity of the disease with a positive correlation in both the years [22]. Selim (2020) quoted those patients with COVID-19 pneumonia may have normal $(4-11 \times 109/l)$, low, or high leukocyte count and lymphocytopenia is a reliable indicator of early SARS CoV-2 infection and helps in tracing of contacts besides assessment of disease progression along the course of COVID-19 pneumonia [23]. Zhu et al. (2021) quoted WBC count at admission is significantly correlated with death in COVID-19 patients and that a higher level of WBC count should be given more attention in the treatment of COVID-19 [24]. In contrast to the present study, Asghar et al. (2021) reported that, the inflammatory markers were less severe during second wave [12]. We compared present study with various studies (Table 3).

Author name	Type of study	Sample size	Findings	Conclusion
Zeng <i>et al.</i> (2020) [15]	Meta-analysis	3962	Non-severe group had lower levels for CRP, procalcitonin, ESR, serum ferritin compared to severe group.	There is an association of inflammatory markers with the severity of COVID-19.
Francone <i>et</i> <i>al.</i> (2020) [16]	Retrospective study	130	CT score was significantly correlated with CRP ($r = 0.6204$) and D-dimer ($r = 0.6625$). ($p < 0.0001$)	CT score is highly correlated with inflammatory markers and disease severity.

Fable 3:	Comparison	of Various	Studies	with	Present	Study

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Author name	Type of study	Sample size	Findings	Conclusion
Elshazli <i>et</i> <i>al.</i> (2020) [17]	Meta-analysis	6320	Elevated WBC, D-dimer, ESR, IL-6 had high odds of progression to severe. Patients with high IL-6, CRP, D-dimer, & neutrophils had the likelihood of mortality.	Several laboratory parameters were associated with the severity and mortality of COVID-19.
Ghahraman i <i>et al.</i> (2020) [18]	Meta-analysis	3396	There is an increase in ESR, C- reactive protein (CRP), D-dimer and neutrophil to lymphocyte ratio (NLR) in the severe group.	Inflammatory/infection markers, were different between severe & non-severe cases of COVID-19.
Jain <i>et al.</i> (2021) [19]	Retrospective observational cohort study	735	CRP and D-Dimer on admission positively predicts final outcome of in hospital mortality. Whereas, CT severity score had AUROC 0.73 (p= 0.014)	CRP level of 45 mg/l, D-dimer level of 1000 μ g/L and CT severity level at the time of admission can be more accu- rately predicting final outcome
Laguna- Goya <i>et al.</i> (2020) [20]	Prospective cohort study	611	High IL-6 level, CRP level, LDH level, ferritin level, d-dimer level, neutrophil count, and NLR were predictive of mortality.	This mortality risk model allows early risk stratification of hospitalized patients with COVID-19.
Ata <i>et al.</i> (2021) [21]	Observational study	242	The CRP (P<0.001), ferritin (P<0.001) were moderately and positively correlated with CT-SS,	Elevation in CRP and ferritin on admission to the hospital was significantly correlated with CT-SS.
Marimuthu <i>et al.</i> (2021) [22]	Retrospective cross- sectional	221	N: L ratio and CRP levels had good correlation with the need for oxygen.	IL-6 and D-dimer were predictors of in-hospital mortality.
Zhu <i>et al.</i> (2021) [24]	Retrospective cross- sectional	163	Significant association was found between WBC count and death ($p < 0.001$).	WBC count at admission is correlated with death in COVID-19.
Asghar <i>et</i> <i>al.</i> (2021) [12]	Retrospective, observational study	160	During second wave neutrophils and lymphocytes were found to peak higher.	Most of the severity markers were less intense during the second wave
Present study	Observational study		In the year 2021 there was a trend of relatively younger population often infected.	TLC, CRP, D-Dimer, serum ferritin, severity in HRCT, ESR of patients belonging to a moderate to severe group in year 2021 was relatively higher than in 2020.

Conclusion:

To conclude we observed that in the year 2021 there was a trend of relatively younger population often infected. The age, TLC, CRP level, D-Dimer, serum ferritin, severity in computed tomography of the chest and erythrocyte sedimentation rate of patients belonging to a moderate to severe group with COVID-19 in 2021 was relatively higher than those belonging to the same group in 2020. The IL- 6 and NLR of patients belonging to a moderate to severe group with COVID-19 in 2021 was significantly lower than those belonging to the same group in 2020. The correlation of various inflammatory markers with the severity of disease was positive over time with a majority of them showing correlation over time, further supporting their use for disease progression and monitoring.

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