Assessment of Severity and Outcome of COVID-19 Cases by Haematological and Biochemical Markers at Tertiary Care Centre in India

Amresh Kumar Singh[1*], Jayesh Pandey[1], Indra Prasad Adhikari[2], Vivek Gaur[3], Ankur Kumar[3], Satya Prakash[2] and Rajju Tiwari[4]

[1]*Department of Microbiology, Baba Raghav Das Medical College, Gorakhpur, Uttar Pradesh-273013, India.*
[2]*Department of Biochemistry, Baba Raghav Das Medical College, Gorakhpur, Uttar Pradesh-273013, India.*
[3]*Viral Diagnostic Research Laboratory, Department of Microbiology, Baba Raghav Das Medical College, Gorakhpur, Uttar Pradesh-273013, India.*
[4]*Department of Biochemistry, Government Medical College, Datia, MP-475661, India.*

Authors' contributions

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*Corresponding author: E-mail: amresh.sgpgi@gmail.com;

ABSTRACT

Background: In December 2019, a cluster of pneumonia cases caused by a novel corona virus (2019-nCov), later named as severe acute respiratory syndrome corona virus-2 (SARS-CoV-2) was detected in China. In India, 30th January 2020 first positive case ofSARS-CoV-2was reported from Kerala. The reverse transcriptase polymerase chain reaction (RT-PCR) is the standard method of choice for diagnosis of SARS-CoV-2 infection. Certain biomarker molecules that are being evaluated for assessment of severity and prognosis are; D-dimer, C reactive protein (CRP), lactate dehydrogenase (LDH), complete blood counts (CBC) and serum ferritin. The elevated levels of these biomarkers were associated with extent of inflammation.

Objective: This prospective study was designed to assess the severity and prognosis of pneumonia cases caused by SARS-CoV-2 using different haematological and biochemical biomarkers.

Materials and Methods: This study was conducted among 242 participants attending covid-19 facility of BRD Medical College Gorakhpur**,** after confirmation by RT-PCR. Different haematological and biochemical biomarkers were analyzed using 5 ml fasting venous blood samples and these were analysed in auto analysers using standard protocol as per manufacturer's instructions. Finally result was analyzed using standard statistical calculation by %positivity, confidence interval, p values and ≤ 0.05 is considered as statistically significant.

Results: Among a total of 242 COVID-19 cases based on different haematological and biomarkers assessment; 92 were critically ill and 150 non-critically ill. The mean ±SD of various haematological parameters among critically ill cases were; haemoglobin (13.0±1.8),TLC (13846.13±3903.76),PLT (92213.48±61415.07),NLR(36.5±30.4).The mean ±SD of the biochemical parameters of critically ill participant was; CRP 44.7±35.4 (95%CI 25.06,2.93),D. dimer 2.9±2.6 (95%CI 1.69,0.10), serum ferritin 1204.7±750.7 (95%CI 581.8,60.1),LDH 397.2±180.8 (95% CI 133.01,0.0163). The level of different haematological and biochemical parameters was raised also in non-critically ill cases but at lower side. Out of these 242 cases; 106 (43.8%) were died and 136 (56.2%) survived but the mortality was high in critically ill cases.

Conclusion: Our findings show that level of D-dimer, LDH, CRP, NLR and serum ferritin,can be used to assess the severity and prognosis of COVID-19 cases. Among these biomarkers; D-dimer levels correlate more precisely with severity and can be considered as a reliable prognostic marker.

1. INTRODUCTION

At the end of 2019, a cluster of pneumonia cases caused by a novel coronavirus (2019-nCov) were detected in Wuhan, China [1]. Later the term "severe acute respiratory syndrome corona virus-2" (SARS-CoV-2) was coined. Because of its contagious nature it causes rapid spread, progression and due to lack of specific therapeutic strategy resulted in an epidemic [1]. In India on 30th January 2020 first positive case in a student from Kerala of the SARS-CoV-2 infection, who was studying in Wuhan University and had travelled to India, tested positive by reverse transcriptase polymerase chain reaction (RT-PCR). Soon after, this it became a global concern and on January 30th 2020, the World Health Organization (WHO) declared that the epidemic of SARS-CoV-2 was a public health emergency of international concern (PHEIC). n February 11th, 2020, the WHO designated the coronavirus disease 2019 (COVID-19). On dated, 27thApril 2020, first case of confirm corona virus infection was reported in Gorakhpur district, who has travelled from Mumbai to Gorakhpur.

Based on the phylogenetic analysis, COVID-19 belongs to a distinct clade of beta-coronavirus similar to human SARS-CoV-1 and Middle East Respiratory Syndrome Coronavirus (MERS-CoV), two species out breaking in the latest decade and causing severe human deaths [2]. Despite the estimated case fatality rate of COVID-19 is lower than that of SARS or MERS, the scale of the COVID-19 contagion has caused more casualties than either of them as of this writing [3]. In addition, human to human transmission of COVID-19 has been confirmed [4].

The SARS-CoV-2infection has led to a pandemic that has affected millions worldwide. In general,

corona viruses can cause various conditions, including respiratory, enteric, neurological and hepatic diseases [5]. The SARS-CoV-2 can cause serious clinical complications, especially in elderly patients and in those with co-morbidities, especially diabetes [6]; cardio and cerebrovascular diseases [7,8]; obesity; cancer and digestive, endocrine, nervous and respiratory systems pathologies [9], constituting 50% to 75% of deaths [10] pertaining to COVID-19 infection.

The RT-PCR is the standard method for SARS-CoV-2 detection and it is the laboratory test of choice for the diagnosis of symptomatic patients in the acute phase [11]. The diagnosis made by real time RT-PCR uses the RNA extracted from samples of the respiratory tract, such as nasopharyngeal swab/oropharyngeal, tracheal aspirate, sputum and bronchoalveolar lavage [10,12,13]. Blood tests have a vital role in early diagnosis of the disease, considering the information they provide to physicians regarding the inflammatory process. This information includes leukocyte count and characteristics such as neutrophil or lymphocyte-dominance, inflammatory markers like C-reactive protein (CRP), collateral organ damage (acute renal failure, acute liver failure) and the severity of the disease [14]. Furthermore, biomarkers provide information regarding the nature and extent of pneumonia, meaning that physicians can determine whether a disease is bacterial or due to other etiologist by analysing blood test results [14].

There is several evidences that in critically ill patients, there are characteristics of hyperinflammation and cytokine storm due to SARS-CoV-2 infection, which consist of elevated serum C-reactive protein (CRP), LDH, D-dimer, and hyperferritinemia. These findings suggest a possibly crucial role of a cytokine storm in pathophysiology of COVID-19 infection [15]. The main biomarker molecules that are being evaluated are; D-dimer, CRP, LDH and serum ferritin. The elevated levels of these biomarkers were associated with severity of inflammation and bleeding associated with Covid-19 infection, showing an independent increased risk for admission in the Intensive Care Unit (ICU), invasive ventilatory support, and death. The highest odds of death occurred when levels of the serum LDH level was greater than 1200 units per litre, and the D-dimer level was greater than 3 μg/ml. Additionally increased level of ALT,

AST, and creatinine level in severely infected patients suggests; that SARS-CoV-2 carries an increased risk of impaired/deranged liver and kidney function.

Complete blood counts (CBC) are easily performed and inexpensive method for assessment of COVID-19 cases. In the CBC different parameters were assessed such as white blood count, neutrophil, lymphocyte and platelet count (PLT), mean platelet volume and certain ratios of these values. Some of these can be used as inflammatory markers. Neutrophils are the most characteristic cell type among the white blood cells and are an important component of the immune system. Regulated by mast cells epithelial cells and macrophages, neutrophils also take part in inflammatory processes. Additionally, thrombocytes also have importance in the regulation of various inflammatory processes.

While these parameters may be used as inflammatory markers by themselves, their ratios to one another may also be indicators of early inflammation [16,17]. Currently, the scientists have determined different risk factors for SARS-CoV-2 deterioration and death based on age and certain underlying medical conditions, like having a compromised immune system, obesity, and heart disease. So it is now advisable that performing a simple blood test for patients admitted to the ICU, and also making decisions based on the biomarkers present. In this study we aim to describe the changes and correlation the haematological and biochemical changes in critically and non-critically SARS-CoV-2 infection patient.

This was prospective observational study to assess the severity of pneumonia cases caused by SARS-CoV-2 using haematological and biochemical biomarkers for Corona virus disease attended at tertiary care centre.

2. MATERIALS AND METHODS

This was hospital based cross-sectional study carried out among corona virus disease was conducted in the Department of Microbiology & Central pathology, Baba Raghav Das Medical College Hospital and research centre from April 2020 to August 2020. A total of 242 patients with confirmed corona virus disease by RT-PCR were enrolled in this study.

2.1 Inclusion Criteria

All RT-PCR positive, non-duplicate sequential cases enrolled in defined study period were enrolled. Criteria for enrollment of cases are-

1. The recommended criteria established by the scientific committee of the Indian Council of Medical Research (ICMR), ministry of health and family welfare, Govt. of India were used for the selection of "suspected COVID-19" patients
2. At least one sign or symptom of either fever, altered smell or taste or acute respiratory disease (cough and respiratory distress).
3. The presence of clinical features that are unexplainable by any other disease and or suggested by computed tomography scan.
4. History of travel to another country in the previous14 days before onset of symptoms suggesting COVID-19 infection.
5. Positive RT-PCR case for SARS-CoV-2 or close contact with a patient confirmed positive by real-time PCR (RT-PCR).
6. The other complaints, epidemiological features and, radiological features suggestive for atypical pneumonia and blood test results of patients were obtained from the patient files.

2.2 Exclusion Criteria

1) All RT-PCR negative cases for SARS-CoV-2 were excluded.
2) Patient or their attendants (in case of ICU/unconsciousness) refusal to be enrolled in the study.
3) Allergic bronchitis/pneumonia (acute or chronic eosinophilic pneumonia).
4) COVID-19 positive case with congestive heart failure (CHF)

2.3 SARS-CoV-2 RT-PCR Sample

The real-time RT-PCR test is based on the qualitative detection of nucleic acid from the SARS-CoV-2 from upper respiratory specimens (nasopharyngeal swabs, oropharyngeal swabs). Swabs should be placed immediately after collection into a sterile tube containing 2-3mL of either viral transport medium (VTM). The assay is composed of two principal steps: (1) extraction of RNA from patient specimens, (2) one-step reverse transcription and PCR amplification with SARS-CoV-2 specific primers and real-time detection with 2019-nCoV specific probes.

2.4 Blood Sample

Overnight fasting blood sample of 5ml was collected from the each participant's in plain vial for serum testing, EDTA (k3EDTA) vial for estimation of hematological parameters citrated vial for D-dimer assessment.

2.5 Methods of Estimation of Covid-19 RNA

The estimation of corona virus disease was done by RT-PCR with the help of QuantStudio™ 5 Real-Time PCR System (Thermofisher, USA) and result was calculated on the basis on cycle threshold (CT) value and graphical analysis. We have used multiplex RT-PCR kit for the detection of different region of RNA virus primer sequence by the AllPlex[TM] 2019-nCoV Assay (Seegene, South Korea).

2.5.1 Specimen

The qualitative detection of SARS-CoV-2 viral RNA was done in human nasopharyngeal swab, oropharyngeal swab, anterior nasal swab, mid-turbinate and sputum specimens from individuals who are "suspected of COVID-19" by health care provider.

2.5.2 Estimation of hematological and biochemical parameters

Blood sample was taken by the hospital nurse. Blood sample was obtained from the ante-cubital vein using disposable syringes with steel needles and discharged into lithium heparin specimen tubes as per standard methodology. Serum was separated from cells by centrifuging blood at 2500 RPM for 5 minutes. It was done by using the fasting blood sample based upon the spectrophotometric principle with the help of Selectra PRO-M and Elitech auto analyzer machine.

The blood sample was studied in the hospital laboratory by the laboratory technician using the Medonic (BOULe) auto analyzer (Sweden). The CBC results obtained from this analysis were studied and approved by a pathologist. Laboratory abnormalities, particularly haematological and biochemical changes, among SARS-CoV-2 cases offer significant impacts during the evolution and management of COVID-19 [18]. The measurement principles of the Medonic (BOULe) based on impedance for cell counts and spectrophotometry for

hemoglobin (HGB). Medonic has three main components to identify blood cell, Electrical impedance: Accurate identification of red blood cells and platelets. Laser-based flow cytometry: white blood cells identification. Colorimetric: determination of hemoglobin.

2.5.3 Diagnostic criteria based on clinical and or laboratory findings for assessment of severity of disease-

2.5.3.1 Non-critically ill

Criteria of patients with Non-critically ill [19]

1. Fever and cough and other respiratory symptoms
2. <50% of lung involvement on either chest X-ray or HRCT
3. Lung imaging showed viral pneumonia.
4. No oxygen support is required
5. Discharge after 10 days of symptom onset.
6. Blood oxygen saturation between 94% to 84%
7. CRP=5-10 mg/L, D-dimer=450-1000ng/mL, serum ferritin=500-1000ng/mL.

2.5.3.2 Critically ill

1. >50% of lung involvement on either chest X-ray or HRCT
2. Blood oxygen saturation <84%
3. Shock
4. Acute Respiratory Distress Syndrome (ARDS)
5. Cardiac injury
6. Multi-organ dysfunction

Shock: Persistent hypotension despite volume resuscitation, requiring vasopressor to maintain mean arterial pressure (MAP)±65 mmHg and serum lactate level > 2 mmol/L [20].

ARDS: As per the literature definition of ARDS [21]:

1. Onset: Within one week of a known clinical consultation.
2. Respiratory failure not fully explained by cardiac failure or uid overload.
3. Bilateral opacities not fully explained by uid overload, lobar or lung collapse or nodules.
4. Oxygen impairment with PF ratio < 300 mmHg.

2.5.4 Multi-organ dysfunction

Acute life-threatening organ dysfunction with any of the following signs:

1. Altered mental status
2. Reduced urine output
3. Shortness of breath or increased respiratory failure
4. Signs of impending shock or circulatory failure
5. Decrease oxygen saturation
6. Acidosis
7. Raised lactate level
8. Deranged liver function and renal function

2.6 Statistical Analysis

SPSS v20 (IBM[©,]Chicago, IL, USA) conducted. Quantitative results have been provided as a mean and standard deviation (SD) and have been measured, if appropriate, by the ANOVA(F) method. Test parameters were tabulated as per the master chart.Qualitative results is provided as numbers and percentages and contrasted, if possible, with the Chi-square (X2) method. Statistically significant was a P value <0.05.

3. RESULTS

In the present study, disease manifestation is defined on the basis of different clinical and laboratory parameters respectively. A total of 242 patients enrolled in this study were suffering with COVID-19 pneumonia and admitted immediately to the Covid-19 facility/ICU of super speciality COVID-19 hospital (L3 facility), BRD Medical College, Gorakhpur. In all COVID-19 positive cases the average duration of symptoms before admission was 10 days. Among the total cases, 106 (44%) patients were died during the ICU hospitalization and 136 patients recovered from SARS-CoV-2 and discharged from the hospital. Table (1,2,3) represents the demographics, clinical characteristics, and severity of the patients in each group of the study subjects.

Among these 242 patients included in the study, 92 (38%) were critically ill and 150 (62%) non-critically ill based on the different clinical and diagnostic criteria. During the above mentioned duration of age range, the average age was 48.88±15.49 years. In terms of severity, the maximum number of patients were belongs to age group 33 to 47, which was 29.75 %, of non-critical and in critically ill cases it was13.2% and mean age of critically and non-critical ill patient were found to be 55.26+15.5 and 43.52+13.4respectively. Only 2.5% of critically ill patients belong to age group 78 and above, which was higher in comparison with non-critically ill cases in the same age group. The

average age was found to be higher in critically ill as compared with non-critically ill patient. The most common complaints in patient were; fever (50.4%), altered taste and smell (49.58%), headache (24.4%), shortness of breath (26.9%) as shown in table 3. Some of these patients had more than one underlying co-morbid disease, among which diabetes (52/242) was the most common (21.5%), cardiovascular diseases (26/242, 10.7%) and systemic hypertension (52/242, 21.5%) as shown in Table 3.

The laboratory findings include different haematological and biochemical parameters which help to make a comparison between critical and non-critical groups as shown in Table 4. It was found that platelet count, neutrophil/lymphocyte ratio (NLR) and PCV values were found much lower in both group of patient, whereas eosinophils count was found to be higher in non-critically ill patients. TLC was found to be higher in critical as compare to non-critically ill patients. The haemoglobin and RBC count was seen to be normal (within range) in this study.

Among different biochemical parameters such as D-dimer, serum ferritin, LDH, SGOT, SGPT and CRP; these were found to be higher in both groups as shown in Table 4. The values of biochemical parameters were much higher in critically ill as compare to non-critically ill patients. LDH was seen to be normal in the both group while ALP was seen to be higher in non-critically ill patient and serum creatinine was raised mildly in critically as compare to non-critically ill patient. These results were statically significant higher level of biochemical parameters

in COVID-19 patients as shown in table 4. In the present study, among critically ill participants the mean±SD was platelets (92213.48±61415.07), TLC(13846.13±3903.76), haemoglobin (13.0±1.8), PCV (36.8±8), neutrophils (66.6±30.1), NLR(36.5±30.4) and it was found and similarly in non-critically ill participants was 137328±86956.4, 9881.6±10868.73, 12.09±2.51, 29.82±9.7, 62.1±1.9, 24±35.5 respectively.

In present study patients with positive RT-PCR results, the mean±SD of the biochemical parameters of critically ill participant was; CRP 44.7±35.4 (95% CI 25.06,2.93), D. Dimmer 2.9±2.6 (95% CI 1.69,0.10), serum ferritin 1204.7±750.7 (95% CI 581.8,60.1), LDH 397.2±180.8 (95% CI 133.01,0.0163), SGOT 78.9±27.9 (95%CI21.11,1.28), SGPT 68.4±43.4 (95% CI 30.45,5.74), ALP 150.2±75.8(95% CI 64.4,18.26), serum creatinine 1.9±0.6(95% CI 0.55,0.04). Similarly in non-critically ill participant mean ± SD of CRP, D-dimer, serum ferritin, LDH, SGOT, SGPT, ALP and serum creatinine were; 30.7±25.9, 2±1.8, 883±673, 330.7±178.5, 67.7±26, 50.3±25.3, 173.3±128.5, and 1.6±0.74 respectively and this result show significant higher level of biochemical parameters in COVID-19 patients.

Among 242 SARS-CoV-2 patients, 124 (51.23%) had been discharged,106 (43.8%) patients died, and 12 (5.0%) patients were still under observation in hospital and not a single patient need to be re-admitted in the entire follow up time. The main physiological deficit which leads to death was refractory hypoxia, massive pulmonary thrombosis and multiple organ failure. The maximum length of stay by few cases in the

Table 1. Age wise distribution of COVID-19 study subjects

S.N.	Age (Years)	Critically ill	Non-critically ill	Grand total
1	18-32	4 (1.7%)	32(13.2%)	36
2	33-47	32 (13.2%)	72(29.75%)	104
3	48-62	24(9.9%)	32(13.2%)	56
4	63-77	26(10.7%)	14 (5.8%)	40
5	78 and above	6(2.5%)	0	6
	Grand Total	92(38%)	150 (62%)	242 (100%)

Table 2. Sex wise distribution of Covid-19 study subjects

Demographic parameters	Non-Critically ill (150)	Critically ill (92)	Total number (242)
Age in years (Mean value)	43	55	48
Sex	108	70	178
1. Male	(72%)	(76%)	(73.6%)
2. Female	42	22	64
	(28%)	(24%)	(26.44%)

Table 3. Comparisons of different risk factors and clinical features among critically ill and non-critically ill COVID-19 cases

Risk factors	Non-Critically ill (150)	Critically ill (92)	Total (242)
i) Systemic Hypertension	18(12%)	34(36.9%)	52(21.5%)
ii) Other Cardiovascular disease	9 (6%)	17(18.4%)	26(10.7%)
iii) Diabetes mellitus	14(9.3%)	38(41.30%)	52(21.5%)
Signs and symptoms	69 (46%)	53(57.60%)	122 (50.4%)
i) Fever			
ii) Fatigue	13 (8.6%)	23(25%)	36(14.87%)
iii) Dry cough	32 (21.3%)	21(22.8%)	53 (22.72%)
iv) Shortness of breath	36 (24%)	29 (31.52%)	65(26.9%)
v) Altered taste and smell	67 (44.6 %)	53 (57.6%)	120(49.58%)
vi) Vomiting	26 (17.33%)	15(16.30%)	41(16.9%)
vii) Headache	46(30.7%)	13(14.13%)	59(24.4%)
viii) Diarrhoea	24 (16%)	31(33.69%)	55 (22.7%)

Table 4. Comparisons of different haematological and biochemical parameters among critically ill and non-critically ill COVID-19 cases

Lab. parameters	Critically ill (92)	Non- critically ill(150)	p – value	t-value	95% CI Maximum	95% CI Minimum
HB	13.0±1.8	12.09±2.51	0.0342	2.143	1.7510	.0690
RBC	4.9±3.9	4.24±0.98	0.1645	1.399	1.5944	0.2744
TLC	13846.13±3903.76	9881.6±10868.73	0.0190	2.378	7264.94	663.85
PCV	36.8±8	29.82±9.7	0.0001	4.098	3.6	10.35
E	0.4±0.3	0.7±0.7	0.0068	2.752	0.5158	0.0842
N	66.6±30.1	62.1±1.9	0.1982	1.294	11.3863	2.3863
L	3.5±3.9	6.0±6.7	0.0232	2.301	4.6516	0.3484
NLR	36.5±30.4	24±35.5	0.0497	1.983	24.98	0.017
Platelet Counts	92213.48±61415.1	137328±86956.4	0.0026	3.077	74144.23	16084.8
SGOT	78.9±27.9	67.7±26	0.027	2.23	21.11	1.28
SGPT	68.4±43.4	50.3±25.3	0.0044	2.901	30.45	5.74
ALP	150.2±75.8	173.3±128.5	0.27	1.106	64.461	18.26
Serum Urea	44.1±17.7	42.8±16.7	0.685	0.406	7.63	5.03
Serum Creatinine	1.9±0.6	1.6±0.74	0.02	2.32	0.55	0.04
CRP	44.7±35.4	30.7±25.9	0.0136	2.504	25.06	2.93
D-Dimer	2.9±2.6	2±1.8	0.0264	2.248	1.69	0.10
Serum Ferritin	1204.7±750.7	883±673	0.0163	2.437	581.8	60.1
LDH	397.2±180.8	330.7±178.5	0.0501	1.980	133.01	0.0163

Table 5. Clinical outcome among critically ill and non-critically COVID-19 cases (n=242)

Clinical outcome	Critically ill	Non-critically ill	Grand Total
Death	50(54.3%)	56(37.3%)	106 (43.8%)
Survived	42 (45.7%)	94 (62.7%)	136(56.2%)
Grand total	**92 (38.01%)**	**150(61.98%)**	**242(100%)**

hospital was approximately 26 days but the average stay by other patients was 14 days and then hospitalization to discharge the patient. The average duration of patient's death was on 10[th]day after admission; overall 43.8% patients were died in our study subject.

4. DISCUSSION

The emergence of the SARS-CoV-2 infection, affected many countries have taken a heterogeneous and evolving approach for diagnosis of infection in patients to determine

using As per our study we analysed several clinical haematological and biochemical laboratory parameters. They plays an important role for the early diagnosis and management of COVID-19 positive cases and proves its pivotal role by providing the clinical status of patients and a number of useful prognostic markers.

A commercial supplied Real-time RT-PCR kit (multiplex Seegene AllPlex, South Korea) and manual/automation methods of RNA extraction was used to detect SARS-CoV-2 RNA in given specimen under the guidelines given by WHO 2020, which was similar with other study by Palmas et al. [22] but a study conducted by Centre for disease control and prevention (CDC) 2020, they used nasal mid-turbinate swab, saliva specimen and nasopharyngeal aspirate as specimen for the detection of similar virus [23].

In our study, *rdrp*, nucleocapsid protein (N) genes, envelope (E) and Rnase P (RNP) as internal control gene were targeted simultaneously, amplified and tested similar types of study conducted by Zou et al. [24]. Clinical samples firstly quantified and the results were expressed in terms of the cycle threshold value (Ct value), which is defined as the number of cycles required for the fluorescence signal to cross the threshold. Samples were considered positive if the Ct-value was below 40 as per kit protocol.

Out of all 242 confirmed cases of SARS-COV-2, the male female ratio was 3:1. The average age in male and female was 46.2 ± 15.5 and 45.5 ± 14.6 years respectively. The average age was found to be higher in critically ill as compared with non-critically ill patient. This was supported by a similar study conducted by Guan et al. [25] and another study conduct by Li et al. [26]. So, we can conclude that, the risk of infection of SARS-CoV-2 was seen more frequently in males with middle-aged group as compared to females in our study population.

The most common complaints in patients suffering from infection of SARS-COV-2 during first visit of hospital was fever (50.4%) followed by shortness of breath (49.58%), cough(22.72%), fatigue (17.87%) these parameter were seen to be higher in critically ill as compare to non-critically ill patient which was higher in a study conduct by the Huang et al. [27] in which fever (98%) followed by cough (76%) and myalgia or fatigue (44%).

In a study which was conducted by Yang et al [28] mortality was 62% among critically ill patients but in our study the mortality was slightly towards the lower side ie. 54.3% in critically ill patients. Most of the critically ill patients were older and had a greater number of co-morbid conditions associated with high levels of D-dimer, ferritin, CRP, and LDH than patients with non-critically illness, similar finding was shown in Goldstein et al. [29] where mortality was reported high approximately 75% in older cases belongs to age group 70 years and above as compared to the younger cases.

In our study the haematological parameters [TLC, lymphocytosis, neutrophilia, eosinophilia, decreased platelets count and PCV (polycythemia)] was found statistically significant (p < 0.05) these values were similar in study conducted by Usul et al. [30]. Author Huang et al. [31] and Hu et al. [32] suggested in his study conducted over 323 COVID-19 positive patients in Wuhan, China that the mean of neutrophil counts and TLC were seen to be higher in critically ill COVID-19 positive cases and 87.5% of critical patients having neutrophilia, which is similar with our study where TLC and neutrophil was higher in critically ill patients as compared to non-critically ill covid-19 positive patients. TLC and increased neutrophil were found independent predictors of an adverse clinical outcome [33].

This study also shows lymphocytes and eosinophils was low in critically ill patients as compare to non-critically ill positive patients whereas, some other studies suggested that a remarkable decrease in the total number of lymphocytes, indicates that the coronavirus affects many immune cells and also responsible for the inhibition of cellular immune functions [34].

This study shows, haemoglobin was found to be higher in critically ill (13 g/dL) patients as compare to non-critically (12.09 g/dL) and the difference was statistically non-significant. These values are found similar in study conducted by Terpos et al. [35] and Henry et al. [36], suggested that increase of inflammatory factors can lead to a reduced erythropoiesis and increased damage of red blood cells, resulting in anemia. On the other hand the COVID-19 positive adult patients associated with increased severity and high mortality, they had low level of platelet count and the mean±SD was 92213.48±61415.07 and 137328±86956.4 in critically ill and non-critically ill group respectively(95% CI 74144.23,16084.8) which

was similar in study done by Henry et al. [36]. In continuation, patients with low platelet level (less than 80000) and also have symptoms like pneumonia and respiratory distress syndrome are more subjected to admit in the respiratory care unit. Furthermore; platelet counts more than 145000-155000/cumm and above have good recovery rate and less complication with limited use of antibiotics and supportive treatment, required as compared with lower one. This data is in accordance with research by Yousif et al [37] that showed thrombocytopenia is common in patients with COVID-19, and it is associated with increased risk of in-hospital mortality.

It is possible that haematological findings are affected by certain other factors, such as the presence of co-morbidities, anaemia, diet, habits of smoking, and drinking alcohol. It should be mandatory to acknowledge these habits and history in bedside head ticket (BHT) when patients get admission in hospital [38].

In this study since around 75% of the positive patients group is comprised of males, this is likely to also have an effect on the results. Biochemical parameter like LDH, D- Dimer, CRP, ferritin, SGOT, SGPT and creatinine, all of these parameters seems to be significantly high in critically ill COVID-19 infection as compare to non-critically ill.

Our study highlighted that the level of D-dimer, ferritin, CRP, and LDH was higher in severe and critically ill patients than non-critically ill patients. This higher parameters suggested that the inflammatory response was more prominent in severe and critically ill as compared to non-critically ill patients and it also may be due to some other chronic & acute viral infection, secondary bacterial infections. The increased levels of different biochemical parameters like serum creatinine, SGOT and SGPT were positively correlated with disease severity in COVID-19 patients.

The value of LDH was statistically significant in our study, several other studies also concluded that LDH is significantly increased in patients experiencing severe course of the disease compared to those with non-critically ill, thereby demonstrating its role as the most potential biomarker in predicting COVID-19 severity. Study conducted by Zheng et al. [39] and Velavan et al [40] also observed in their study that LDH is an important biomarker for disease progression and severity. In these studies, the levels of LDH were lower in moderate severity groups of patients as

compared to the levels of LDH in our study and both the authors and this article concluded a substantial association between LDH levels and disease severity.

In our study CRP was another important predictor of severity of the COVID-19 disease.CRP was statically significant (p 0.014) in critically ill cases as compared to the non-critically ill. The mean value of CRP was 44.7 ±35.4 in critically ill and 30.7±25.9 in non-critically ill (95%CI 25.06, 2.93). The level of CRP was five to six times higher from its normal reference range and it was a significant marker for the severe manifestation of COVID-19 cases. Qin et al. [41] also found a significant association of increased CRP levels in their study and for severe prognosis of the disease, but the values reported in the severe group of patients were 2.5 times higher than the levels in CRP in our study, however, a ratio of 1.74:1 between the severe and non-severe groups was almost similar, whereas the marker levels in the mild course of the disease are equal to levels in severe disease mentioned in this study.

The level of serum ferritin was a significant biomarker for this disease, the significant increase of serum ferritin as a biomarker for the assessment of severity of disease as compared to CRP, LDH and D-dimer was clinically more important. A strong association of serum ferritin as a marker and several times high levels of serum ferritin in both surviving (non-critically ill and severe (critically ill) groups of patients was supported by a study conducted in Wuhan, China [41].

In this study, there were also some other abnormal biomarkers that had significant differences between the critically and non-critical ill groups, AST, ALT, LDH, ferritin, CRP and creatinine. These abnormalities suggested that SARS-CoV-2 infection may be associated with myocardial injury, hepatic injury, kidney and other vital organ damage. Based on analysis between critical and non-critical patients group, we can monitor progression of disease to some extent. One major limitation of this study is that, some predisposing factors have influence on certain biomarkers, which we have not evaluated.

5. CONCLUSION

In conclusion, several laboratory parameters could be associated with the severity and mortality of COVID-19 pandemic since

December 2019 but in the lack of the scientific knowledge about the SARS-CoV-2 origin, virulence and its spread is still not well established. The continuous research and measurement required to understand the progression of the infection, its pathophysiology and for the discovery of an effective antiviral drug therapy and a vaccine. Therefore, the search for haematological, biochemical or other suggestive laboratory parameters are extremely necessary in this scenario to evaluate and establish early clinical diagnosis of SARS-CoV-2 infection. These parameters included WBCs count, lymphocytes, platelet count, D-dimer (except congestive heart failure), LDH, CRP and S. ferritin could be helpful to monitor the severity of the disease and symptomatic treatment because of the lengthy procedure and large numbers of suspected individual samples are awaiting diagnosis through RT-PCR (gold standard). [40], [41]

Available data suggest that several hematological and biochemical parameters might be responsible for the change in the duration of SARS-CoV-2 infection and some of them can be considered significant predictors of unfavourable clinical outcomes and also help in reflecting changes in systemic inflammation of the renal, hepatic, cardiac, immune, hemostatic, bone marrow, and peripheral blood systems.

In continuation to this article, we need to concentrate more on studies which help to understand the good clinical laboratory and biosafety practices prevail and that the patient be the centre of attention. Advance training required for laboratory personnel's who are responsible for collecting, transporting and handling biological samples and carrying out the various laboratory tests for patients with COVID-19 is recommended. Development of advance skills in data interpretation and feasible techniques to diagnose infection in lesser time is also required.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

Written informed consent was obtained from the patient's attendant for publication of this case and any accompanying images.

ETHICAL APPROVAL

The study was approved by the Baba Raghav Das Medical College Ethical Committee (register IHEC/BRDMCGKP/04/09-2020).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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