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Original Research Article

Hematological Parameters and its Association with Disease Severity among COVID-19 Patient

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Abstract: ***Objective*:** To find out the relationship between hematological parameters and disease severity among COVID-19 patients. ***Methods:*** This cross-sectional analytical study was conducted in the COVID unit of Dhaka Medical College and Hospital (DMCH). The study was conducted from October 2020 to September 2021. Patients diagnosed with COVID-19 infection detected by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) admitting in the Corona unit of DMCH, Dhaka, Bangladesh. Purposive sampling technique was applied to collect the samples. 96 patients were included for the study. ***Result*:** In case of severity of patients, 17 (17.7%) patients had mild illness, 29 (30.2%) had moderate illness and 50 (52.1%) patients had severe illness due to COVID-19 infection. Furthermore, according to gender, the proportion of male patients (70.0%) were significantly more in severe group compared to mild/moderate group (47.8%). Moreover, the mean age of the patients in severe group (56.2± 14.3) were significantly higher than the mean age of the patients in mild/moderate group (48.7±12.5) as p=0.007. Patients in severe group had significantly higher neutrophil count than mild/moderate group (p<0.001). On the other hand, patients in severe group had significantly lower lymphocyte, eosinophil and platelet count than mild/moderate group (p<0.05). The neutrophil-to-lymphocyte ratio (NLR) was significantly higher in severe group compared to mild/moderate group (p<0.001).

Keywords: Hematological, parameters, disease, severity, covid-19 patients.

INTRODUCTION

Coronaviruses are enveloped non-segmented positive sense RNA viruses belonging to the family Coronaviridae and broadly distributed in humans and other mammals [1]. Although most human coronavirus infections are mild, the epidemics of the two beta-coronaviruses, severe acute respiratory syndrome coronavirus and Middle East respiratory syndrome coronavirus have caused more than 10,000 cumulative cases in the past two decades, with mortality rates of 10% for SARS-CoV and 37% for MERS-CoV [2, 3]. The virus can be transmitted by an infected person or an asymptomatic carrier and is a highly contagious disease. Respiratory droplets are the main route of transmission, but can also be transmitted by contact and digestive tract [4].

In December 2019, a novel coronavirus labelled 2019- nCoV, which is believed to have originated in Wuhan, the capital city of Hubei province, began spreading rapidly across China. The virus is transmitted mainly via respiratory droplets and/or contact, and human-to-human transmission and family clustering have been reported [5]. In Bangladesh, the first case was identified on 8th March 2020 & up to 11 October 2021, there have been 1,562,958 confirmed cases of COVID-19 with 27,699 deaths, reported to WHO [6].

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Reported illnesses have ranged from patients with little or no symptoms to patients being severely ill and dying [7]. The main clinical manifestations include fever, cough, fatigue, and dyspnea [8]. As compared to young and middle-aged patients with COVID-19, elder infected patients with chronic comorbidities have an increased risk of developing organ dysfunctions, including shock, acute respiratory distress syndrome (ARDS) acute cardiac injury, and acute kidney injury, resulting in a higher mortality rate [9].

Several studies have shown that hematological parameters are markers of disease severity and suggest that they mediate disease progression [10]. Patients with positive RT-PCR had significantly higher neutrophil count ($p = 0.0001$), compared to patients with negative RT-PCR. In addition, patients with positive RT-PCR had lower white blood cell (WBC) count ($p = 0.0001$) compared to others. Neutrophil (AUC = 0.858) had very good accuracy in predicting cases with positive RT-PCR for COVID-19 [11]. Patients with COVID-19 with poor outcome showed significant differences in results of routine hematological parameters compared with patients that recovered. Especially lymphocyte count can be helpful in the prediction of a patient's outcome [12]. Other study suggested that the lymphocyte counts, red blood cell counts and the immunoglobulin G antibodies of COVID-19 patients were impaired to varying degrees and this were more obvious in critically ill patients [13].

Coagulation parameters show abnormal results related to sepsis or DIC. Prothrombin time (PT) and D-dimer are useful indicators of prognosis and severity of disease in COVID-19 suggested that D-dimer levels can be used to estimate the severity of COVID-19 [14, 15]. Previous study reported that the potential risk factors of old age, high baseline D-dimer, and dynamic co-variates of fibrinogen, platelets, CRP, and LDH could help clinicians to identify and treat subjects with poor prognosis [16]. Early recognition of these abnormal coagulation results will be useful to predict the disease severity, support to guide the therapy, and improve the patients' clinical outcome [17]. Therefore, this study is conducted to familiarize clinicians with the primary hematological manifestations of COVID-19 infection and also determined its association with severity of disease. Although diagnosis of coronavirus disease (COVID-19) is challenging in the early stages due to non-obvious manifestations, hematological parameters provide clues to aid diagnosis.

METHODS

This cross-sectional analytical study was conducted in the COVID unit of Dhaka Medical College and Hospital (DMCH). The study was conducted from October 2020 to September 2021. Patients diagnosed with COVID-19 infection detected by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) admitting in the Corona unit of DMCH, Dhaka, Bangladesh. Purposive sampling technique was applied to collect the samples. 96 patients were included for the study. Patient was interviewed for their socio-economic information. Blood sample was collected and send to the lab for the investigations. Routine blood tests (white blood cell [WBC] count, lymphocyte count, mononuclear count, neutrophils count) was performed on the blood samples. Blood biochemistry parameters (alanine aminotransferase [ALT], serum ferritin, creatinine and C-reactive protein [CRP]) were measured. Coagulation parameter such D-dimer was determined. Data were collected in a predesigned data collection sheet by face-to-face interview and medical record review.

Selection Criteria

Inclusion criteria

- Hospitalized Patients diagnosed with COVID-19 infection confirmed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR)
- All adult patients (age >18 years)

Exclusion criteria

- Patients suffering from hematological malignancy
- Suspected and probable case of COVID-19 infection

Data Analysis

The statistical analysis was conducted using SPSS (statistical package for the social science) version 26 statistical software. The findings of the study had been presented by frequency, percentage in tables. Means and standard deviations for continuous variables and frequency distributions for categorical variables were used to describe the characteristics of the total sample. In this study, patients with mild or moderate symptoms were classified as mild/moderate group, and patients with severe symptoms were classified as severe group. Association of categorical variables was assessed by Chi square test or Fisher Exact test. Association of continuous variables was assessed by Student t test. In case of skewed data, Mann Whitney U test was done to find out association between continuous data.

RESULT

Table 1: Distribution of patients by severity (N=96)

Severity	Frequency (N)	Percentage (%)
Mild	17	17.7
Moderate	29	30.2
Severe	50	52.1

Table 1 shows that out of 96 patients, 17 (17.7%) patients had mild illness, 29 (30.2%) had moderate illness and 50 (52.1%) patients had severe illness due to COVID-19 infection. In this study, the

patients with mild or moderate symptoms are classified as mild/moderate group (n=46) and rest of are as severe group (n=50).

Table 2: Distribution of patients by socio-demographic characteristics (N=96)

Socio-demographic status	Total (N=96)	Mild/moderate (n=46)	Severe (n=50)	p value
Gender				
Male	57 (59.4%)	22 (47.8%)	35 (70.0%)	**0.027***
Female	39 (40.6%)	24 (52.2%)	15 (30.0%)	
Age (in years) (Mean ± SD)	52.6 ±13.9	48.7±12.5	56.2± 14.3	**0.007****
Educational status				
Illiterate	25 (26.0%)	13 (28.3%)	12 (24.0%)	0.755*
Primary to secondary	24 (25.0%)	10 (21.7%)	14 (28.0%)	
Higher secondary and above	47 (49.0%)	23 (50.0%)	24 (48.0%)	
Occupational status				
House maker	29 (30.2%)	15 (32.6%)	14 (28.0%)	0.393*
Service holder	30 (31.3%)	17 (37.0%)	13 (26.0%)	
Businessman	16 (16.7%)	7 (15.2%)	9 (18.0%)	
Others	21 (21.9%)	7 (15.2%)	14 (28.0%)	

Footnotes: SD=Standard deviation, *=Chi-square test, **=Independent sample t test, bold indicate significant p value

Table 2 shows that proportion of male patients (70.0%) were significantly more in severe group compared to mild/moderate group (47.8%) as p=0.027. Moreover, the mean age of the patients in severe group (56.2± 14.3) were significantly higher than the mean age of the patients in mild/moderate group (48.7±12.5) as p=0.007. No significant statistical difference was observed between the groups regarding educational and occupational status as p>0.05.

Table 3: Frequency of specific comorbidity according to disease severity (N=96)

Comorbidity	Total (N=96)	Mild/moderate (N=46)	Severe (N=50)	p value
Hypertension				
Present	40 (41.7%)	15 (32.6%)	25 (50.0%)	0.084*
Absent	56 (58.3%)	31 (67.4%)	25 (50.0%)	
Diabetes mellitus				
Present	38 (39.6%)	16 (34.8%)	22 (44.0%)	0.356*
Absent	58 (60.4%)	30 (65.2%)	28 (56.0%)	
Cardiovascular disease				
Present	15 (15.6%)	4 (8.7%)	11 (22.0%)	0.073*
Absent	81 (84.6%)	42 (91.3%)	39 (78.0%)	
COPD				
Present	10 (10.4%)	4 (8.7%)	6 (12.0%)	0.743**
Absent	86 (89.6%)	42 (91.3%)	44 (88.0%)	
Chronic kidney disease				
Present	10 (10.4%)	4 (8.7%)	6 (12.0%)	0.743**
Absent	86 (89.6%)	42 (91.3%)	44 (88.0%)	

Footnotes: COPD: Chronic obstructive pulmonary disease, *=Chi-square test, **=Fisher Exact test

Table 3 demonstrates that there was no significant statistical difference was observed between the groups regarding hypertension, diabetes mellitus, cardiovascular disease, COPD and chronic kidney disease as p>0.05.

Figure 1 indicates that among the 46 patients with mild/moderate disease severity, 24 (52.2%) patients had at least one comorbidity while among the 50 patients with severe disease, 36 (72.0%) patients had at least one comorbidity. Chi-square test showed that presence of comorbidity was significantly more in severe group as p=0.045.

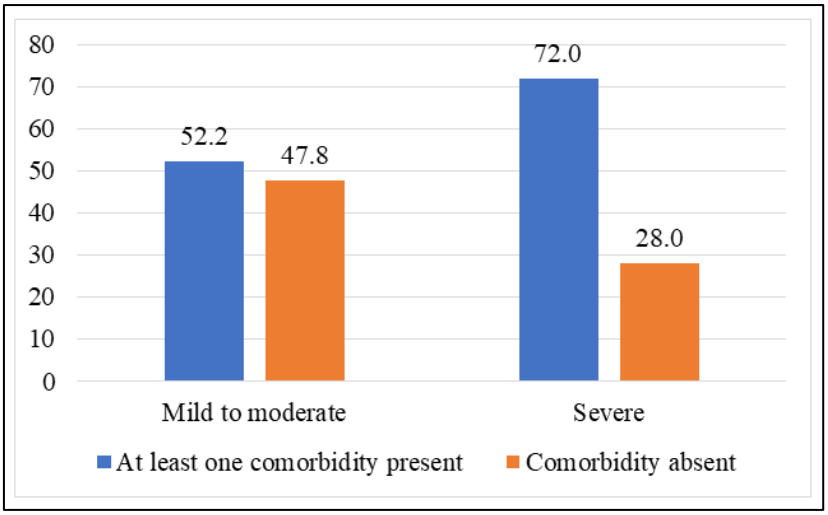

Figure 1: Frequency of comorbidity according to disease severity (n=96)

Table 4: Frequency of presenting symptoms of the patients (N=96)

Symptoms	Total (N=96)	Mild/moderate (N=46)	Severe (N=50)	p value
Fever	88 (91.7%)	40 (87.0%)	48 (96.0%)	0.147**
Cough	82 (85.4%)	38 (22.6%)	44 (88.0%)	0.455*
Fatigue	74 (77.1%)	31 (67.4%)	43 (86.0%)	**0.030***
Breathing difficulty	77 (80.2%)	27 (58.7%)	50 (100%)	**<0.001***
Myalgia	64 (66.7%)	30 (65.2%)	34 (68.0%)	0.773*
Nausea	62 (64.6%)	26 (56.5%)	36 (72.0%)	0.113*
Anorexia	61 (63.5%)	25 (54.3%)	36 (72.0%)	0.073*
Headache	41 (42.7%)	12 (26.1%)	29 (58.0%)	**0.002***
Anosmia	28 (29.2%)	10 (21.7%)	18 (36.0%)	0.125*
Diarrhoea	19 (19.8%)	9 (19.6%)	10 (20.0%)	0.957*
Arthralgia	9 (9.4%)	4 (8.7%)	5 (10.0%)	0.999**

Footnotes: *=Chi-square test, **=Fisher Exact test, bold indicate significant p value

Table 4 shows that patients in severe group had significantly more fatigue, breathing difficulty and headache as p<0.05. However, no significant statistical difference was observed between the groups regarding fever, cough, myalgia, nausea, anorexia, anosmia, diarrhea and arthralgia as p>0.05.

Table 5: Hematological parameters of the patients (N=96)

Hematological findings	Total (N=96) Median [IQR]	Mild/moderate (N=46) Median [IQR]	Severe (N=50) Median [IQR]	p value
Leukocytes (/mm3)	10025.0 [7105.0, 12670.0]	8565.0 [7117.0, 12747.0]	10250.0 [7040.0, 12650.0]	0.411*
Neutrophil (/mm3)	8316.0 [4696.2, 10733.2]	6200.5 [3995.0, 9808.4]	8952.1 [5598.0, 11520.0]	**0.025***
Lymphocyte (/mm3)	1246.5 [773.5, 2095.0]	1678.6 [1262.5, 2544.9]	886.5 [592.9, 1250.7]	**<0.001***
Monocyte (/mm3)	258.0 [185.7, 380.1]	260.2 [196.6, 400.0]	256.0 [159.6, 352.0]	0.545*
Eosinophil (/mm3)	114.8 [50.0, 235.5]	137.7 [51.1, 270.3]	109.4 [13.1, 150.7]	**0.033***
Platelet (/mm3)	252500 [164000, 310000]	277000 [233000, 318500]	196000 [139250, 304000]	**0.004***
Hemoglobin (mg/ dl)	11.9 [10.3, 13.1]	12.0 [10.3, 13.1]	11.9 [10.1, 13.0]	0.719*
Erythrocytes (M/µL)	4.4 [3.9, 4.7]	4.5 [4.1, 4.8]	4.3 [3.6, 4.6]	0.123*
Hematocrit (%)	36.7 [31.5, 39.7]	37.2 [31.6, 40.1]	36.5 [27.7, 39.5]	0.399*
NLR	5.7 [3.1,11.5]	3.9 [2.3, 5.5]	10.1 [5.7, 14.2]	**<0.001***

Footnotes: IQR=Inter-quartile range, NLR= neutrophil-to-lymphocyte ratio *=Mann Whitney U test, bold indicate significant p value

Table 5 shows that patients in severe group had significantly higher neutrophil count than mild/moderate group (p<0.001). On the other hand, patients in severe group had significantly lower lymphocyte, eosinophil and platelet count than mild/moderate group (p<0.05). The neutrophil-to-lymphocyte ratio (NLR) was significantly higher in severe group compared to mild/moderate group (p<0.001). However, no significant statistical difference was observed between the groups regarding total leukocytes, monocytes, hemoglobin, erythrocytes and hematocrit values as p>0.05.

Table 6: Biochemical and coagulation parameters of the patients (N=96)

Laboratory findings	Total (N=96) Median [IQR]	Mild/moderate (N=46) Median [IQR]	Severe (N=50) Median [IQR]	p value
LDH (n=31) (U/L)	504.0 [420.0, 640.0]	443.0 [222.0, 664.0]	580.0 [438.0, 640.0]	0.148*
Ferritin (n=63) (ng/ml)	343.0 [170.0, 889.1]	170.0 [55.0, 315.0]	729.4 [320.0, 1050.0]	**<0.001***
ALT (n=55) (U/L)	46.0 [32.0, 74.0]	38.0 [32.0, 77.0]	46.0 [36.0, 67.7]	0.933*
Creatinine(n=87) (mg/dl)	1.1 [0.8,1.8]	1.0 [0.8,1.2]	1.3 [0.9, 1.9]	0.094*
CRP (n=73) (mg/L)	20.4 [7.0, 56.0]	8.0 [1.0, 28.0]	48.0 [12.0, 120.0]	**<0.001***
D-dimer (µg/ml)	0.5 [0.2, 1.2]	0.2 [0.2, 0.4]	0.9 [0.6, 1.5]	**<0.001***

Footnotes: IQR=Inter-quartile range, LDH= Lactate dehydrogenase, ALT= Alanine aminotransferase, CRP= C-reactive protein, *=Mann Whitney U test, bold indicate significant p value

Table VI shows that patients in severe group had significantly higher ferritin level, C-reactive protein (CRP) level and D-dimer level than mild/moderate group (p<0.001). However, no significant statistical difference was observed between the groups regarding Lactate dehydrogenase (LDH), Alanine aminotransferase (ALT) and creatinine values as p>0.05.

Table VII: Correlation between laboratory findings and severity of disease (N=96)

Variable	r_s	p value
D-dimer (µg/ml)	0.632	**<0.001***
Lymphocyte (/mm^3)	-0.535	**<0.001***
NLR	0.513	**<0.001***
Ferritin (ng/ml)	0.516	**<0.001***
CRP (n=73) (mg/L)	0.488	**<0.001***
Neutrophil (/mm^3)	0.230	**0.024***
Platelet (/mm^3)	-0.292	**0.004***
Eosinophil (/mm^3)	-0.218	**0.033***

Footnotes: r_s=Spearman's rank correlation coefficient, NLR= neutrophil-to-lymphocyte ratio, CRP= C-reactive protein, *=Spearman correlation coefficient test, bold indicate significant p value

Table VII shows that significant moderate positive correlation was present between severity of disease and D-dimer, ferritin, neutrophil-to-lymphocyte ratio (NLR), C-reactive protein (CRP) level (p<0.001). Significant moderate negative correlation was present between severity of disease and lymphocyte count (p<0.001). Moreover, significant weak correlation was present between severity of disease and neutrophil eosinophil and platelet count. Proportion of male patients (70.0%) were significantly more in severe group compared to mild/moderate group (47.8 %). Similar observation was reported.

DISCUSSION

COVID-19 is a systemic infection with a significant impact on the hematopoietic system and hemostasis. The present study was a cross-sectional study conducted in the COVID unit of Dhaka Medical College and Hospital (DMCH) to find out the relationship between hematological parameters and disease severity among COVID-19 patients. A total of 96 patients diagnosed with COVID-19 infection were included in the study. Patients suffering from hematological malignancy were purposively excluded from the study.

Out of the 96 patients, majority had severe illness while near about one third had moderate illness. As this was a hospital-based study, the proportion of severe patients were higher compared to others. In this study, the patients with mild or moderate symptoms are classified as mild/moderate group (n=46) and rest of are as severe group (n=50).

Majority of the patients of this study was male patients. The study conducted in Dhaka Medical College and Hospital also found male predominance [18]. Proportion of male patients (70.0%) were significantly more in severe group compared to mild/moderate group (47.8 %). Similar observation was reported another study [19].

The mean age of the patients in severe group (56.2± 14.3) were significantly higher than the mean age of the patients in mild/moderate group (48.7±12.5). Most severe ill patients were older than patients with mild to moderate illness [9, 20].

Patients in severe group had significantly higher neutrophil **c**ount than mild/moderate group (p=0.025) which was similar to other studies [12, 13, 16, 20]. The present study also found that the peripheral blood lymphocyte count of COVID-19 patients decreased in severe group than others which matched the other studies [13, 16]. Neutrophilia is an expression of the cytokine storm and hyperinflammatory state which have an important pathogenetic role in COVID-19 and related infections such as SARS [21, 22].

Platelet count was significantly lower in patients in severe group than mild/moderate group ($p<0.05$). Other study also had similar findings [12]. Although patients with a severe disease may be at a risk of developing thrombocytopenia, the mechanism of this condition remains obscure. It has been postulated that thrombocytopenia may result from three predominant mechanisms, including decreased platelet production, as well as increased destruction and increased consumption of platelets [23].

Patients in severe group had significantly higher C-reactive protein (CRP) than mild/moderate group ($p<0.001$). This finding was supported by other studies [13, 19]. Patients in severe group had significantly higher D-dimer than mild/moderate group ($p<0.001$). Similar observation was reported in other studies [13,19]. The best cut-off point of D-dimer was 0.51 µg/L with a sensitivity of 80.0% and specificity of 81.0%. Another study revealed that the cut off value of D-dimer to predict the severity of pneumonia was 0.750 [14]. Other study stated that disease severity was associated with D-dimer > 0.96 ug/mL. The lower value of D-dimer in the present study might be due the exclusion of critical patients from the study [20].

CONCLUSION

In conclusion, COVID-19 disease has prominent manifestations from the hemopoietic system and is often associated with a major blood hypercoagulability. Careful evaluation of hematological parameters and other relevant laboratory indices at baseline can assist clinicians for prediction of severity of COVID-19 patients.

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