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Original Article

Correlation between Haematological Features and Severity of RT-PCR Positive COVID-19 Patients

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Abstract

Background: *Haematological tests have an important role in early diagnosis of the COVID-19 patient's disease condition, considering the information they provide to physicians regarding the inflammatory process. This information includes leukocyte count and characteristics such as neutrophil or lymphocyte-dominance, inflammation (CRP) Serum ferritin and coagulation profile (D-dimer). Aim of this study was to investigate the association of hematological parameters with severity of the disease and identify the association of magnitude of any cytopenia and pancytopenia with the severity of COVID-19 patients.*

Materials and methods: *This prospective observational study was conducted at dedicated COVID unit of Chattogram Maa O Shishu General Hospital from March 2021 to August 2021. A total of 100 RT-PCR$^{(+ve)}$ COVID cases were included in this study. Automated hematology analyzer was used to determine complete blood count (Total WBC count, absolute and relative count of each WBC type, RBC, and platelet count).*

Results: *Majority patient were female (61%). Among all patient 87% patients were above 35 years of age. Patient with mild disease were comparatively younger than patient with severe disease. Major presenting complaints were cough (88%) shortness of breath (60%) fever (59%) sore throat (30%) and fatigue (26%). Raised pulse and respiratory rate were seen according to disease severity. Total WBC (p=0.0038) Neutrophil (p=0.0001) serum ferritin (p=0.0205) CRP (p=0.0004) and D-dimer (p=0.0004) were significantly increased in severe group and the patient with critical condition. No statistically significant difference was seen in the median value of platelet count (p=0.4364).*

Conclusion: *Leukocytosis, Neutrophilia, elevated serum ferritin, CRP and D-dimer are significantly increased in patients with severe and critical disease condition of COVID-19 patient. Lymphopenia also featured in these patients, which is associated with the severity of disease.*

Key words: COVID-19; CRP; D-dimer; Haematological feature; Lymphocyte; Neutrophil; S. ferritin.

INTRODUCTION

COVID-19 is caused by novel Severe Acute Respiratory Syndrome Corona Virus (SARS-CoV-2) which first appeared in December 2019 in Wuhan, China and quickly transformed into a global pandemic through symptomatic and asymptomatic person-to-person transmission mainly through respiratory droplets.[1-4] On March 11, 2020 CODIV-19 was declared as a pandemic state by WHO.[5] As of 31 May 2022, there have been more than 526.5 millions of confirmed cases were reported, including 6.29 millions deaths.[6]

Although, primarily it was documented as a respiratory tract infection, emerging researches indicted that COVID-19 causes an illness which has a wide variety of clinical features, ranging from mild to moderate upper respiratory tract infection to severe systemic disease which involves respiratory as well as other body systems including cardiovascular, gastrointestinal, neurological, immunological and hematopoietic system.[7-9] Bangladesh stands as eight the most populated countries in the world, the first case was reported by IEDCR on 8th March, 2020.[10,11]

The association of hematological abnormalities in severe COVID-19 pneumonia is multifactorial. Hematological abnormalities in COVID-19 are related with disease progression, severity and mortality. Lymphopenia, thrombocytopenia, abnormal coagulation profile and sepsis leading to Disseminated Intravascular Coagulation (DIC) is very well documented in patients of COVID-19.[12] Thus, abnormal hematological values in patient with COVID-19 may aid in earlier risk stratification and prognostication of these patients, ultimately leading to earlier interventions and ideally more favorable outcomes.

Blood tests have an important 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, inflammation (CRP), collateral organ damage (Acute renal failure, acute liver failure) and the severity of the disease. Furthermore, biomarkers provide information regarding the nature of pneumonia, meaning that physicians can determine whether a disease is bacterial or due to other etiologies by analyzing blood test results.[13] Complete Blood Counts (CBC) are easily performed and inexpensive. Included in the CBC are values such as white blood count, neutrophil, lymphocyte and Platelet Count (PLT), mean platelet volume and certain ratios of this values.[2]

Early identification and timely treatment of COVID-19 patients at higher risk of developing critical disease are pivotal to prevent unfavorable clinical outcomes. Coagulopathies like disseminated intravascular coagulation, Sepsis-Induced Coagulopathy (SIC) local microthrombi, Venous Thrombo-embolism (VTE) arterial thrombotic complications, and thrombo-inflammation have been associated with COVID-19.[14] Hematological manifestations of this particular virus should be tracked closely as this epidemic evolves.

These hematological and inflammatory biomarkers like CBC, d-dimer, C-reactive Protein, Ferritin and coagulation profile can play a vital role in early prediction of disease severity and can provide a better guide for prompt management of patients, thus, can help in deceasing the disease morbidity and mortality. Aim of this study was to investigate the association of hematological parameters with severity of the disease and identify the association of magnitude of any cytopenia and pancytopenia with the severity of COVID-19 patients.

MATERIALS AND METHODS

This prospective observational study was conducted at dedicated COVID unit of Chattagram Maa-O-Shishu General Hospital from March 2021 to August 2021. A total of 100 cases were included in this study. Purposive sampling technique was used to select the patient who tested positive for COVID-19 by RT-PCR and who were admitted at the dedicated unit. Both genders patients who were 15-90 years of age were included. Patient who had been previously diagnosed with chronic disease such as kidney failure, heart and liver disease as well as those who underwent immunosuppressive therapy and pregnant women were excluded due to the fact that these conditions would affect hematological parameters. Socio-demographic data, clinical sign and symptoms and disease severity status of admitted patients were collected by reviewing medical records. Three milliliters of venous blood was collected in an EDTA vacutainer tube from each patient by experienced laboratory professionals working at the center. Automated hematology analyzer was used to determine complete blood count (total WBC count, absolute and relative count of each WBC type, RBC and platelet count).

According to WHO clinical management of COVID-19 interim guidance of May 27, 2020 and National Guidelines on Clinical Management of COVID-19 of 06 May 2021 by Directorate General of Health Services, Bangladesh patients were categorized into the following four groups to assess disease severity. [15-16]

Mild Case (Influenza like illness) **-** Clinical symptoms are mild, and there is no evidence of pneumonia. Symptoms may be: Fever, Cough, Loss of taste or smell, Myalgia, Fatigue, Anorexia, Headache, Diarrhoea, Vomiting, Rhinorrhoea, Sore throat, Husky voice, Red eye, Abdominal pain, Skin lesion (Chilblain, nodule) etc.

Moderate Case (Pneumonia) **-** Adolescent or adult with clinical signs of pneumonia (Fever, cough, dyspnoea, fast breathing) but no sign of severe pneumonia ($SpO_2 \geq 90\%$ on room air).

Severe Case (Severe Pneumonia) **-** Adolescent or adult with clinical sign of pneumonia (Fever, cough, dyspnea, fast breathing) plus one of the following: respiratory rate >30 breath/min, severe respiratory distress or $SPo_2<90\%$ on room air.

Critical Case (Cases requiring ICU care) **-** Severe COVID-19 cases meeting any of the following criteria like- Respiratory failure and requiring mechanical ventilation, Sepsis, Septic shock, ARDS or Any organ failure that require ICU care.

All statistical operations were analyzed by STATA SE 13 Version. Statistical analysis using descriptive statistic was done. All non-parametric distributed measurement variables were presented using the median (Interquartile range). Continuous variables were analyzed by using non parametric, Kruskal Wallis test. Categorical variables were presented in frequency distribution and analyzed by chi-square test. All tests statistically significant results were considered when p value < 0.05.

This study was approved by Ethical Review Committee (ERC) of Chattagram Maa O Shishu General Hospital. Written informed consent was obtained from each participant and confidentiality of the data was maintained throughout the study.

RESULTS

Out of 100 patients 61 were female and 39 were male. Among all patient 87% patients were above 35 years of age. Patient with mild disease were comparatively younger than patient with severe disease. In our study there were 50, 29 and 20 number of mild, moderate and severe cases respectively, there was only one critical case was reported. The covid-19 disease was significantly more prevalent in female as compare to male (p=0.028). Age wise gender distributions of patient are shown in Table I.

Table I Age and Gender distribution of the study population

Age distribution	Male n (%)	Female n (%)	Total n (%)	p
16-25	-	4 (4%)	4 (4%)	
26-35	2 (2%)	7 (7%)	9 (9%)	
36-45	4 (4%)	15 (15%)	19 (19%)	
46-55	11 (11%)	4 (4%)	15 (15%)	
56-65	10 (10%)	18 (18%)	28 (28%)	0.028
66-75	9 (9%)	10 (10%)	19 (19%)	
76-85	2 (2%)	3 (3%)	5 (5%)	
86-100	1 (1%)	-	1 (1%)	
Total	39 (39%)	61 (61%)	100 (100%)	

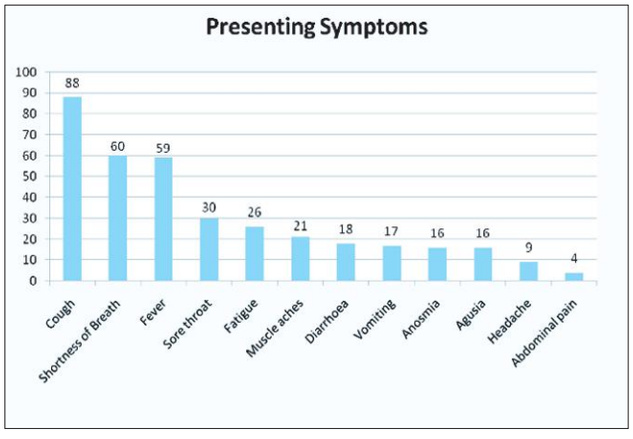

Figure 1 Presenting clinical symptoms of the cases

The clinical presentation of the cases varied from cough, shortness of breath, and fever to non-specific symptom like headache or abdominal pain (Figure 1). Major presenting complaints were cough (88%), shortness of breath (60%), fever (59%), sore throat (30%), fatigue (26%) muscle aches (21%) and diarrhea (18%). Other minor presenting complaints including, anosmia (16%), agusia (16%), headache (9%) and abdominal pain (4%). The median (IQR) age of the patients of the mild, moderate and severe were 50(27), 55(28) and 65(5) years respectively and the age of the critical patient was 75 years (Table II). Over-all clinic-hematological findings of all study patients are shown in Table III.

Table II Age (years) distribution of the cases according to disease Severity (Category)

Disease Category	Number N (%)	Female/ male	Median (IQR)	Min	Max	p
Mild	50 (50%)	32/18	50(27)	17	83	0.0123
Moderate	29 (29%)	18/11	55(28)	30	90	
Severe	20 (20%)	11/9	65(5)	50	75	
Critical	1 (1%)	0/1	75	-	-	
Total	100 (100%)	61/39	60(23)	16	90	

Table III Overall Clinico-hematological findings of study population (n=100)

Clinico-haematolocal	Unit	Median (IQR)	Min	Max
Temperature	^{O}f	100(2.9)	98	104
SpO_2	%	94.5(7)	76	99
Pulse	b/min	94(23.5)	70	130
Respiratory rate	b/min	28(9)	20	44
BP (Systolic)	mm of Hg	125(22.5)	85	71
BP (Diastolic)	mm of Hg	70(10)	52	90
Haemoglobin	g/dl	11.2(2.55)	7	15.9
Total Count	$x10^3/\mu l$	8.5(5.2)	3	20
Neutrophil	%	80(17)	44	96
Lymphocyte	%	18(16.5)	3	45
Platelet count	$x10^3/\mu l$	270(135.5)	123	500
Ferritin	ng/ml	297(769.25)	16	2275
CRP	mg/dl	32.8(83.95)	0.3	271.2
D-Dimer	mg/ml	0.71(1.56)	0.21	9.95

The median (IQR) respiratory rates were higher in patients with severe disease 34(6) per minute and in the critical patient it was 36 b/min (p=0.0001). And the median (IQR) pulse rates were higher in patients with severe disease 104(23) per minute and in the critical patient it was 120 b/min (p=0.0001. Median (IQR) of O_2 saturation was 97(3)%, 92(3)%, 85(6)% in mild, moderate and severe case group, O_2 saturation of critical patient was 76% (p=0.0001) Among 100 cases, 59 patient have raised body temperature. There was no significant difference of body temperature and systolic and diastolic pressure among the groups (p=0.0750, p=0.3375 and p=0.7841 respectively). The hematological and coagulation parameters in mild, moderate, severe & critical disease are shown in table V & VI. Median value of WBC (p=0.0038), Neutrophil (p=0.0001), serum ferritin (p=0.0205), CRP (p=0.0004) and D-dimer (p=0.0004) were significantly increased in severe group and the patient with critical condition. The median value of Lymphocyte (p=0.0001) and Haemoglobin (p=0.0013) were significantly decreased in severe group and the patient with critical condition. No statistically significant difference was seen in the median value of platelet count (p=0.4364). There was a statistically significant moderate correlation of CRP (Pearson correlation =0.427, p=0.000) and D-dimer (Pearson correlation =0.330. p=0.001) with disease category (Table VII).

Table IV Clinical findings (Sign) in different disease category groups

Clinical Sign	Mild (N=50) Median(IQR) min-max	Moderate (N=29) Median(IQR) min-max	Severe (n=20) Median(IQR) min-max	Critical (N=1) Median(IQR) min-max	p
Temperature (°f)	100(2.4) 98-104	98.6(2.4) 98.4-103	102(3.7) 98.4-104	98 -	0.0750
SpO_2 (%)	97(3) 95-99	92(3) 90-94	85(6) 76-89	76 -	0.0001
Pulse (b/min)	88(18) 70-106	100(36) 70-128	104(23) 76-130	120 -	0.0001
Respiratory rate (b/nim)	24(6) 20-36	28(5) 20-44	34(6) 22-40	36 -	0.0001
BP (Systolic) mm of Hg	130(10) 85-171	120(23) 90-150	117.5(35) 100-160	150 -	0.3375
BP (Diastolic) mm of Hg	70(10) 52-90	73(17) 60-90	75(15) 60-90	80 -	0.7841

Table V Hematological (CBC) findings in different disease category groups

Hematological finding	Mild (N=50) Median(IQR) min-max	Moderate (N=29) Median(IQR) min-max	Severe (N=20) Median(IQR) min-max	Critical (n=1) Median(IQR) min-max	p
Haemoglobin g/dl	11.65(2.09) 8-15	11.2(3) 7.4-13.29	10.45(1.4) 7-15.19	10.5 -	0.0013
Total Count of WBC $x10^3/\mu l$	7.8(4.4) 4-18.4	8.5(3.2) 3-20	11.2(7) 6.8-20	19 -	0.0038
Neutrophil %	70(20) 44-90	80(13) 57-94	81(9.5) 69-96	84 -	0.0001
Lymphocyte%	26.5(15) 7-45	15(11) 4-37	15.5(9) 3-27	12 -	0.0001
Platelet count $x10^3/\mu l$	274.5(110) 150-470	270(200) 123-400	225(191) 130-500	250 -	0.4364

Table VI Inflammatory and Coagulation factors findings in different disease category groups

Factors	Mild (N=50) Median(IQR) min-max	Moderate (N=29) Median(IQR) min-max	Severe (N=20) Median(IQR) min-max	Critical (N=1) Median(IQR) min-max	p
Serum Ferritin ng/ml	162.6(600) 16-2000	250(702.82) 96.39-2275	636.54(1328) 88.1-2104	119.2 -	0.0205
CRP mg/dl	19(38.7) 0.5-121	54(108.5) 0.3-258	72.35(118.2) 18.5-271.2	151 -	0.0004
D-dimer mg/ml	0.5(0.33) 0.21-4	1.1(3.59) 0.34-8	1.75(1.94) 0.3-9.95	5 -	0.0004

Table VII Correlation analysis of Inflammatory and Coagulation factors with disease category

Factor	Pearson Correlation value with disease category	Pearson Correlation status	p
Serum Ferritin	0.248	Weak	0.013
CRP	0.427	Moderate	0.000
D-dimer	0.330	Moderate	0.001

DISCUSSION

Finding of the study illustrates hematological, inflammatory and coagulation manifestation and their correlation with the severity of the disease in COVID-19 patient. Our study reported that, females were predominantly affected (p=0.028) from the disease than male. Instead majority of the study reported male were more affected from Covid-19.[2,7,11] Among the total 100 cases 50 were mild, 29 were moderate, 20 were severe and only one patient was critical in condition. Proportion of critical case was comparatively less frequent in our study than others.[3,7]

As for the symptom associated with COVID-19, cough (88%), shortness of breath (60%) and fever (59%) were commonly seen, symptoms were similar with the study of Usal E et al, Manna A et al, Wang D[2,10,17]. But compare with cough, fever was less frequent in our study. Diarrhoea and vomiting were also widely observed in this study, which are also common in the study of Manna A et al Assiri A et al, Guan WJ et al.[10,18,19].

A significant increase in the WBCs level (p=0.00380) in the critical case and severe disease group were observed. It has been previously reported that WBCs count increased with severity of the COVID-19 disease.[20] Our study confirmed that Leukocytosis, neutrophilia (p=0.0001) and increased neutrophil to lymphocyte ratio, have a significant association with the disease severity, which might be due to inflammatory response. Neutrophil to lymphocyte ratio was highest in patients with critical disease. Liao D et al. also found elevated neutrophil to lymphocyte ratio as a useful predictor for severity and mortality of SARS-CoV-2 infection.[21]

Our study did not show significant association of platelet count (p=0.4346) with the severity of disease while Liao D et al. found significantly lower platelet count in patients with critical and severe disease while Fan BE et al., found mild thrombocytopenia in 20% of his study cases and leukopenia (WBC ≤ 4 x 109/L) in almost 19% of the total admitted patients.[21,22] Lymphopenia (p=0.0001) was also featured in these patients which was associate with the severity of disease. Though Hemoglobin of the COVID-19 patients showed no association with the severity of disease in the study of Taj S et al, but we found significant association (p=0.0013) of decreasing of Hemoglobin (g/dl) in the severity of the disease condition of our patients.[7]

Values of serum ferritin (p=0.0205) and CRP (p=0.0004) were significantly increased in severe and critical patients as compared to mild and moderate patient. In a retrospective cohort study from Wuhan, China, Terpo E et al. reported that increased ferritin were risk factors for Acute Respiratory distress syndrome, ICU support and mortality. Higher CRP has also been related to adverse aspects of COVID-19 disease, such as ARDS development, higher troponin-T levels and myocardial injury, and death. Association of increasing serum ferritin and CRP according to disease severity were also reported in the studies of Taj S et al, Mannan A et al.[23,7,10].

In our study, coagulation profile, D-dimer levels was tested for abnormal clotting. We found significantly different of D-dimer (p=0.0004) across the severity of disease in our study. Study of Taj S et al, Mannan A et al, Bhuiyan MNZ & Bansal A et al also showed significant association of D-Dimers with the severity of the COVID -19 disease.[7,10,11,24] Patients with increased D-Dimer levels had worst clinical outcome.[24]

CONCLUSION

The conclusive finding of the study is that Leukocytosis, Neutrophilia, elevated serum ferritin, CRP and D-dimer are significantly increased in patients with severe and critical disease condition of Covid-19 patient. Lymphopenia also featured in these patients, which is associated with the severity of disease. CBC, inflammatory and coagulation profile are significantly associated to the COVID-19 disease. So these markers may be an useful prognosticator for early prediction of disease severity.

DISCLOSURE

All the authors declared no competing interest.

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