VOLUME - 11, ISSUE - 07, JULY - 2022 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Pathology

HEMATOLOGICAL PARAMETERS IN COVID-19: A RETROSPECTIVE STUDY OF 50 PATIENTS IN A TERTIARY CARE CENTRE IN EASTERN INDIA.

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ABSTRACT **Introduction:** The recent pandemic of coronavirus disease 2019 (COVID-19) has major public health and economic impact. The hematological features reported in COVID-19 patients are leucocytosis, neutrophilia, lymphopenia and thrombocytopenia. **Objectives:** We aimed to determine the hematological parameters: total leukocyte count (TLC), absolute neutrophil count (ANC), absolute lymphocyte count (ALC) and platelet count (PC), in COVID-19 patients and compare them between discharged (after recovery) and expired patients. We also looked for any association between these parameters and number of days of hospitalisation of the discharged (after recovery) patients. **Materials And Methods:** We conducted a retrospective observational study on 50 COVID-19 patients. Their TLC, ANC, ALC and PC were recorded. They were divided into two broad categories: - Group A: Discharged from hospital after recovery; Group B: expired at hospital. Group A was divided into Group A1: Discharged from hospital after recovery, number of days of hospitalisation <=10; Group A2: Discharged from hospital after recovery, number of days of hospitalisation >10. **Results:** Patients in group B had significantly higher TLC and ANC than group A. Patients in group A2 had significantly higher TLC and ANC than group A1. Patients in group A2 had significantly lower ALC than group A1. **Conclusion:** High TLC, high ANC and low ALC predict worse outcome in patients (hospitalisation >10 days and/or death).

KEYWORDS : COVID-19, leukocyte, lymphocyte, neutrophil, platelet

INTRODUCTION

The recent pandemic of coronavirus disease 2019 (COVID-19) has major public health and economic impact. COVID-19 is caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).[1] The clinical spectrum of COVID-19 ranges from asymptomatic carrier state to severe, bilateral, and diffuse pneumonia, leading to acute respiratory distress syndrome (ARDS), respiratory failure and multiple organ dysfunction. [2,3] The common clinical features of COVID-19 include cough, fever, dyspnea, fatigue, myalgia, headache, anosmia, ageusia and diarrhea. [4,5] SARS-CoV-2 spreads among people through direct contact routes and by droplet, airborne and, fomite transmission. It is important to identify early manifestations of COVID-19 and patients at risk for disease progression in order to alleviate the major stress on healthcare systems.

During the incubation period and during the early phase of the disease, peripheral blood leukocyte and lymphocyte counts are normal or slightly reduced. Following viremia, SARS-CoV-2 affects the tissues expressing high levels of ACE2 like the lungs, heart and gastrointestinal tract. There is a surge in the clinical manifestations of the disease, approximately 7 to 14 days from the onset of the initial symptoms because there is significant increase of inflammatory mediators and cytokines, including interleukin (IL)-6, IL-8, IL-10, granulocyte-colony stimulating factor (G-CSF), monocyte chemoattractant protein 1 (MCP1), macrophage inflam matory protein (MIP) 1, and tumor necrosis factor (TNF)- . This may be characterized as a cytokine storm which affects hematopoietic system. [6,7]

The hematological features that have been reported in COVID-19 patients are anemia, leucocytosis, neutrophilia, lymphopenia, and thrombocytopenia. [6,8,9,10] Neutrophils are involved in early anti-viral defence and during severe pneumonia, neutrophils become cytotoxic through degranulation and lysis. [11] There are studies that suggest that neutrophil recruitment may exacerbate COVID-19 immuno pathology. [12] There are several factors that may contribute to COVID-19 associated lymphopenia. Lymph ocytes have been shown to express the ACE2 receptor on their surface so SARS-CoV-2 may directly infect those cells and ultimately lead to their lysis. [13] The cytokine storm may also promote lymphocyte apoptosis and be associated with atrophy of lymphoid organs. [14-17]

In this study we aimed to determine the hematological parameters viz. total leukocyte count (TLC), absolute neutrophile count (ANC), absolute lymphocyte count (ALC) and platelet count (PC), in COVID-19 patients and compare these parameters between discharged (after recovery) and expired patients. We also looked for any association between these parameters and number of days of hospitalisation of the discharged (after recovery) patients.

MATERIAL AND METHODS

We conducted a retrospective observational study that included 50 COVID-19 patients admitted at our institution, from July to September 2020. This study was approved by the ethics committee of our institute. Data pertaining to the patients were retrieved from institutional archives.

Real-time polymerase chain reaction (RT PCR) confirmed SARS-CoV-2 infected patients were included in this study. Patients who had hematological malignancies and immunodeficient states were excluded from the study. Clinical data pertaining to the cases such as age, sex, signs and symptoms as well as relevant history were obtained from medical records. Hematological parameters viz. TLC, ANC, ALC and PC of the patients were recorded.

The RT PCR confirmed SARS-CoV-2 infected patients were divided into two broad categories based on outcome: - Group A: Discharged from hospital after recovery; Group B: expired at hospital. Group A was further divided into Group A1:

Discharged from hospital after recovery, with number of days of hospitalisation <=10; Group A2: Discharged from hospital after recovery, with number of days of hospitalisation >10. The cut off for high TLC, high ANC, low ALC and low PC were 11,000/mm^3, 8000/mm^3, 1000/mm^3 and 1,00,000/mm^3 respectively.

The values of TLC, ANC, ALC and PC of the of RT PCR confirmed SARS-CoV-2 infected patients were expressed as mean and range. These variables were compared between group A and group B, and group A1 and group A2 using unpaired t test- two tailed. Categorical variables were expressed as percentages, and compared between the groups using Fischer's exact test or Chi square test. All statistical analyses were done using GraphPad Prism 6™. p Value of <0.05 was considered to be statistically significant.

RESULTS

Our study included 50 patients. The mean age of the patients was 61.9 years with a range of 27–89 years. 37 patients (74%) were male and 13 patients (26%) were females. Group A and group B included 40 (80%) and 10 (20%) patients, respectively. The mean age of patients in group A was 59.6 years (range: 27-89 years) and that in group B was 70.7 years (range: 62-85 years) (p = 0.01). Group A1 and group A2 included 26 (65%) and 14 (35%) patients, respectively. The mean age of patients in group A1 was 60.6 years (range: 27-89 years) and that in group B was 57.9 years (range: 32-79 years) (p = 0.5). Among males, 29 (78.3%) patients were discharged while 8 (21.7%) patients died. Among females, 11 (84.6%) patients were discharged while 2 (15.4%) patients died.

The mean TLC of group A was 8,088/mm^3 (range: 2,000-18,200/mm^3) and that of group B was 18,144/mm^3 (range: 8800-39000/mm^3) (p < 0.001). 90% (9 out of10) of patients in group B had TLC > 11,000/mm^3 while in group A, only 14.2% (5 out of 40) had TLC >11,000/mm^3 (p <0.0001). The mean ANC of group A was 6,656/mm^3 (range: 1,240- 17,108/mm^3) and that of group B was 16,235/mm^3 (range: 3,649- 37,440/mm^3) (p < 0.0001). 90% (9 out of10) of patients in group B had ANC > 8,000/mm^3 while in group A, 37.5% (15 out of 40) had ANC> 8,000/mm^3 (p =0.004). Lymphocytopenia (<=1,000/mm^3) was seen in 56% (28/50) of all the patients. The mean ALC of group A was 969/mm^3 (range: 305- 2,100/mm^3) and that of group B was 1,241/mm^3 (range: 328- 2,400/mm^3) (p= 0.1). 50% (5 out of 10) of patients in group B had ALC<= 1,000/mm^3 while in group A, 57.5% (23 out of 40) had ALC<= 1,000/mm^3 (p =0.7). The mean PC of group A was 2,10,000/mm^3 (range: 90,000-4,60,000/mm^3) and that of group B was 2,20,000/mm^3 (range: 30,000- 5,00,000/mm^3) (p=0.5). 10% (1 out of 10) of patients in group B had PC<= 1,00,000/mm^3 while in group A, 5% (2 out of 40) had PC of<=1,00,000/mm^3 (p =0.4). (Table 1 and 2)

The mean TLC of group A1 was 6,907/mm^3 (range: 2,000-11,400/mm^3) and that of group A2 was 10,207/mm^3 (range: 4,200- 18,200/mm^3) (p < 0.002). 35.7% (5 out of 14) of patients in group A2 had TLC > 11,000/mm^3 while in group A1, only 3.8% (1 out of 26) had TLC >11,000/mm^3 (p 0.01). The mean ANC of group A1 was 5,659/mm^3 (range: 1,240- 10,032/mm^3) and that of group A2 was 8,509/mm^3 (range: 3,696- 17,108/mm^3) (p = 0.001). 23% (6 out of 26) of patients in group A1 had ANC > 8,000/mm^3 while in group A2, 64.3% (9 out of 14) had ANC> 8,000/mm^3 (p =0.01). The mean ALC of group A1 was 1059/mm^3 (range: 450- 1,792/mm^3) and that of group A2 was 822/mm^3 (range: 308- 2,100/mm^3) (p= 0.1). 42.3% (11 out of 26) of patients in group A1 had ALC<= 1,000/mm^3 while in group A2, 78.5% (11 out of 14) had ALC<= 1,000/mm^3 (p =0.04). The mean PC of group A1 was 1,90,000/mm^3 (range: 90,000-4,60,000/mm^3) and that of group A2 was 2,59,000/mm^3 (range: 1,50,000- 3,70,000/mm^3) (p=0.5). 7.7% (2 out of 26) of patients in group A1 had PC<= 1,00,000/mm^3 while in group A2, none (0 out of 14) had PC of<=1,00,000/mm^3 (p=0.5).

(Table 1 and 2)

DISCUSSION

The mean age of patients included in this study was 61.9 years. The mean age of the patients who expired was significantly higher than that of the patients who were discharged. Other studies have also found poorer outcome of COVID-19 among elderly population. [8] Several studies have reported an independent association of male sex with poorer outcome in COVID-19 patients but in our study the outcome of the illness was not associated with the sex of the patient.[18]

The aim of our study was to look for any association between the hematological findings and patients who were discharged from hospital after recovery (group A), patients who expired (group B), patients who were discharged from hospital after recovery, with number of days of hospitalisation <=10 (group A1) and patients who were discharged from hospital after recovery, with number of days of hospitalisation >10 (group A2). The hematological tests are common laboratory investigations that are performed on COVID 19 patients. The results of the hematological tests can aid in predicting the outcome of COVID 19 and the patients can be managed accordingly.

In our study, patients in group B (expired) had significantly high TLC and ANC as compared to that in group A. Patients in group A2 (discharged and hospitalisation > 10 days) had significantly high TLC and ANC as compared to that in group A1 (discharged and hospitalisation <= 10 days). Several studies showed similar findings. [8,9,19] Zhao et al in their study on COVID 19 patients showed that COVID-19 patients with increased TLC were more likely to develop systemic inflammatory response syndrome (SIRS). Patients with increased TLC exhibited increased concentrations of procalcitonin, CRP and IL-6 in the serum when compared with the patients with non-increased TLC. COVID-19 patients with increased TLC had significantly higher level of systemic inflammation response, which is related to the development of critical illness, high admission to an ICU and a high mortality rate. They also found the blood levels of leukocyte count and neutrophil count, the serum concentrations of CRP and IL-6 were significantly increased in the patients with increased TLC who have underlying chronic diseases, compared with the patients with increased TLC who have no underlying chronic diseases. [20]

We saw in our study that COVID-19 patients with increased TLC had a higher ANC. Zhao et al had similar results. The COVID-19 patients with leucocytosis had a higher level of systemic inflammatory response and IL-6 in the serum as comparison with the patients with non increased leukocyte count. IL-6 can promote differentiation of Th17 cells from naive CD4 T cells, and Th17 cells can induce an inflammatory response through the production of IL-17A and IL-17F. These cytokines cause neutrophils migration, recruitment and activation. The activated neutrophils are involved in phagocytosis, release of granular contents and production of cytokines as protective immune response against the virus. However, excessive increased neutrophils can lead to cytokine storm and tissue damage, causing severe pneumonia and death. Therefore, neutrophilia in COVID-19 patients with increased leukocyte count is related to the development of critical illness, high admission to an ICU and a high mortality rate.[20]

In our study, 56% of all the COVID 19 patients showed lymphocytopenia. Patients in group A2 (discharged and hospitalisation > 10 days) had significantly lower ALC as compared to that in group A1 (discharged and hospitalisation <= 10 days). Several studies have shown similar findings. Guan et al reported lymphocytopenia in 83.2% of COVID 19

patients on admission. 92.6% of patients who were admitted to an ICU/ on mechanical ventilation/ died presented with lymphocytopenia and 82.5% of patients who were discharged had lymphocytopenia. Severe cases presented lymphocytopenia more frequently (96.1%) vs non-severe cases (80.4%). [3] Huang et al showed that patients who needed ICU had significantly lower lymphocyte than those who did not need ICU. [21]

In our study only 3 patients had low PC. There was no significant difference in PC between group A and group B and between group A1 and group A2. Wang et al reported no significant difference in PC between ICU cases vs non-ICU cases. [22] Wu et al did not find any significant difference in PC between dead patients and alive patients with ARDS. [23]

CONCLUSION

We observed that COVID-19 can lead to significant alteration in the hematological parameters. High TLC, high ANC and low ALC predict worse outcome in patients (hospitalisation >10 days and/or death) and there is no association between PC and outcome. This study can help prognosticate different Covid-19 affected patients on the basis of easily and cheaply determined hematological parameters, and is of specific relevance to resource-constrained environments like underdeveloped countries.

Table 1

	Comparison between group A and B			Comparison between group A1 and A2		
	Group A (Discharged)	Group B (Death)	P value	Group A1 (Hospitalisation <= 10 days)	Group A2 (Hospitalisation >10 days)	P value
Mean TLC	8088/mm3	18144/mm3	<0.001	6907/mm3	10207/mm3	0.002
Range of TLC	2000-18200/mm3	8800-39000/mm3		2000-11400/mm3	4200-18200/mm3	
Mean ANC	6656/mm3	16235/mm3	<0.0001	5659/mm3	8509/mm3	0.001
Range of ANC	1240-17108/mm3	3649-37440/mm3		1240-10032/mm3	3696-17108/mm3	
Mean ALC	969/mm3	1241/mm3	0.1	1059/mm3	822/mm3	0.1
Range of ALC	305-2100/mm3	328-2400/mm3		450-1792/mm3	308-2100/mm3	
Mean PC	2,10,000/mm3	2,20,000/mm3	0.4	1,90,000/mm3	2,59,000/mm3	0.5
Range of PC	90,000-4,60,000/mm3	30,000-5,00,000/mm3		90,000-4,60,000/mm3	1,50,000-3,70,000/mm3	

Comparison of mean and range of TLC, ANC, ALC and PC between group A and group B and between group A1 and group A2.

Table 2

	Comparison between group A and B			Comparison between group A1 and A2		
	Group A (Discharged)	Group B (Dead)	p value	Group A1 (Hospitalisation <=10 days)	Group A2 (Hospitalisation >10 days)	p value
TLC			<0.0001			0.01
TLC <=11,000	35 (85.8%)	1 (10%)		25 (96.2%)	9 (64.3%)	
TLC >11,000	5 (14.2%)	9 (90%)		1 (3.8%)	5 (35.7%)	
ANC			0.004			0.01
ANC <= 8,000	25 (62.5%)	1 (10%)		20 (77%)	5 (35.7%)	
ANC > 8,000	15 (37.5%)	9 (90%)		6 (23%)	9 (64.3%)	
ALC			0.7			0.04
ALC <= 1,000	23 (57.5%)	5 (50%)		11 (42.3%)	11 (78.5%)	
ALC > 1,000	17 (42.5%)	5 (50%)		15 (57.7%)	3 (21.5%)	
PC			0.4			0.5
PC <= 1,00,000	2 (5%)	1 (10%)		2 (7.7%)	0	
PC > 1,00,000	38 (95%)	9 (90%)		24 (92.3%)	14 (100%)	

Comparison of TLC, ANC, ALC and PC between group A and group B and between group A1 and group A2.

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