

Original article

Study of haematological parameters in Covid-19 positive patients at tertiary care hospital

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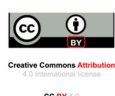
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Abstract:

Introduction: Apart from clinical symptoms and pulmonary computed tomography (CT) findings in, confirmed COVID-19 patients' Blood tests have an important role in early diagnosis of the disease and they provide valuable information to physicians regarding the inflammatory status in body.

Materials and methods: A retrospective cross-sectional study was conducted from January 2020 to March 2020 in the Pathology Department of Rural Medical College, Loni. Total of 120 patients from different groups, both genders and between 18 and 75-year age were studied.

Results: TLC, Neutrophil, NLR, PLR, D-Dimer values were found to have statistical significant ($p < 0.05$) positive correlation with Covid -19 severity. Blood investigations like Lymphocyte and Monocyte count have statistical significant ($p < 0.05$) negative correlation with Covid -19 severity. No significant correlation was observed between haematological tests like Hb, HCT, PLT, LMR and tests of coagulation like PT & APTT with Covid -19 severity.

Conclusion: We concluded that TLC, NLR and D-dimer tests are important to predict about the severity of disease.

Keywords: Covid – 19 , Hematological parameters , D-Dimer

Introduction:

Coronavirus disease (COVID-19) is an infectious disease caused by the SARS CoV-2 virus which was first described in the December 2019 in Wuhan, China. The COVID-19 is a highly contagious disease and it has spread around the globe within a short time, and the world health organization (WHO) has declared it a pandemic on March 12, 2020.¹ Most people infected with the virus are asymptomatic, some suffer from mild to moderate to severe respiratory illness. Those, who become seriously ill and require medical attention are especially elderly people and those with underlying medical comorbidities like diabetes, hypertension, obesity etc. Anyone can get sick with COVID-19 and become seriously ill or die at any age.^{2,3} Covid-19 causes an illness which has a wide variety of clinical features

involving 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.^{4,5}

Apart from clinical symptoms and pulmonary computed tomography (CT) findings in, confirmed COVID-19 patients' Blood tests have an important role in early diagnosis of the disease and they provide valuable information to physicians regarding the inflammatory status in body.⁶ To find out the prognosis and prediction of hyperinflammatory state, a combination of laboratory tests have been evaluated. These tests either in single or in combination can be easily performed and includes various tests such as Complete blood count, serological markers and coagulation profile are easily

performed and inexpensive. COVID-19 leads to variation in the hematological parameters, including white blood cells, neutrophils, lymphocytes, monocytes, hemoglobin, hematocrit, platelets and coagulation profile like PT, APTT and D-dimer. The combination of the various test includes platelet-to-lymphocyte ratio (PLR), Lymphocyte to monocytes ratio (LMR) and neutrophils to lymphocyte ratio (NLR).⁷ Serum Ferritin levels were used as an indicator for the severity of disease along with above ratio.⁸

Objective:

The objective of our study was to investigate the changes in hematological parameters and to find out any correlation of these with disease severity.

Materials and methods:

A retrospective cross-sectional study was conducted from January 2020 to March 2020 in the Pathology Department of Rural Medical College, Loni. Total of 120 patients from different groups, both genders and between 18 and 75-year age were studied. Patients were categorized into in three groups : Group A included 40 patients in whom ventilation was required (Severe patients), Group B comprised of 40 patients who were symptomatic but ventilation was not required and Group C was of 40 patients who were asymptomatic.

All those individuals who were tested positive for the RT-PCR(COVID-19) according to the WHO and CDC guidelines for the detection and diagnosis of COVID-19 were included in this study. Individuals below 18 years of age and those with missing data were excluded in our study.

About 2 ml of venous blood was collected on the second day of admission to the hospital using the EDTA vacutainer tube. The blood samples collected from each individual were analyzed for complete blood count (CBC) examination using Automatic Hematology Analyzer Sysmex XN-3100. For Coagulation profile collected 2 ml blood in citrate vacutainer tube and was performed on ACL TOP 300 while serum Ferritin was performed on VITROS 5600 and 4 ml blood in plain bulb.

Method of data analysis

Statistical Product and Service Solutions(SPSS) version 21 for Windows (Armonk,NY:IBM corp software was used to analyse the data. Statistical analysis was done by using tools of descriptive

statistics such as Mean, and SD for representing quantitative data. Probability $p < 0.05$, considered as significant as alpha error set at 5% with confidence interval of 95% set in the study. Power of the study was set at 80% with beta error set at 20% One-way ANOVA 'F' test was applied to compare haematological parameters in three groups of COVID-19 subjects. Post hoc data analysis which follows One way ANOVA test was done by using Tukey's multiple comparison test was also used. Post hoc test analyses multiple pair -wise individual comparisons.

Results:

A total of 120 COVID-19 positive individuals were examined in this study. These patients were previously screened through RT-PCR for the SARS-CoV-2 infection and were found positive for viral infection. Among the total individuals ($n = 120$), 84 (70%) were males while 36 (30%) were females. Males were more affected than females. The severe and symptomatic patients were mostly older individuals compared with asymptomatic patients.

Hematological parameters like TLC, Hemoglobin, Hematocrit, Platelet, Neutrophils, Lymphocytes, Monocytes, NLR, PLR, LMR, PT, ApTT, D-dimer and Ferritin were examined across comparative groups.

TLC was highly significantly increased (p value < 0.001) in group A ($15.67 \times 10^6/\mu\text{L}$) compared to Group B ($9.74 \times 10^6/\mu\text{L}$) and Group C ($6.58 \times 10^6/\mu\text{L}$). However, significant difference (p value = 0.01) of TLC was observed between the Group A and Group B but not as much as in Group B and Group C ($p = 0.029$). TLC results showed positive correlation with severity of the disease. (Table 1).

A small difference in the hemoglobin (p value = 0.772), hematocrit (p value = 0.772) and platelet level (p value = 0.745) was observed which indicates no significant association with the severity of the disease (Table 1).

Serum ferritin value was significantly increased in Group A(533.8) than Group B (476.64) and Group C (134.4) (p value = < 0.001) (Table 1)

The Neutrophils were highly significantly increased (p value < 0.001) in group A (86%) compared to Group B (82.5 %) and Group C (67.9%). There is no significance difference between Group A and Group B (p value = 0.0514). (Table 2)

The lymphocyte count was decreased in group A (7.6%) compared to Group B (11.9 %) and Group C (23.25 %) patients (p value = <0.001), thus indicating no significance difference between Group A and Group B patients. (p value = 0.321).(Table-2)

The monocyte count was decreased in group A (4.3%) compared to Group B (11.9 %) and Group C (23.25 %) patients (p value = <0.001) reflecting no significance difference between Group A vs Group B patients (p value = 0.321) and Group B vs Group C patients. (p value = 0.016). (Table-2)

A significant increase in NLR values was observed in various disease groups. The highest value of NLR was recorded in Group A patients (21.09) followed by Group B (8.58) and Group C (4.77). A significant

association (p value = <0.001) was observed in various disease groups as shown in (Table 3).

PLR was increased in the group A patients (318.6) as compared to Group B patients (234.6) and Group C patients (191.4). (p value = 0.024). (Table 3.)

A small difference in the LMR was observed which indicates no significant association with the severity of the disease (p value = 0.171). (Table 3).

Pt (p value=0.409) and aptt values were (p value=0.508) observed which indicates no significant association with the severity of the disease (Table 4).

D-dimer value was comparatively increased in Group A (5657.7) than Group B (305.5) and Group C (172.4). (p value = 0.028) (Table 4)

Table:1 Comparison of haematological parameters in three groups of COVID-19 subjects.

	TLC Mean (SD)	HB Mean (SD)	HCT Mean (SD)	PLT Mean (SD)	S.Ferritin Mean (SD)
Group A (BIPAP)	15696 (991)	13.22 (2.14)	40.57 (6.04)	204.8 (86.42)	533.8 (339.32)
Group B (Symptomatic)	9774.5 (3451)	13.63 (1.37)	40.75 (3.55)	216.7 (110.3)	476.64 (303.86)
Group C (Asymptomatic)	6585.2 (1842)	13.53 (2.03)	41.69 (5.78)	225.8 (53.37)	134.4 (214.74)
p value[^] (overall comparison – A vs B vs C)	p<0.001**	p = 0.772	p = 0.772	p =0.745	P<0.001**
Group A vs B#	p=0.01*	p=0.771	p=0.994	p=0.901	P=0.809
Group A vs C #	p<0.001**	p=0.857	p=0.781	p=0.725	P<0.001**
Group B vs C#	p=0.229	p=0.986	p=0.84	p=0.941	P=0.001*

Table:2 Comparison of haematological parameters in three groups of COVID-19 subjects.

	Neutrophil Mean (SD)	Lymphocyte Mean (SD)	Monocyte Mean (SD)
Group A (BIPAP)	86.0 (7.2)	7.6 (6.11)	4.3 (2.51)
Group B (Symptomatic)	82.5 (10.11)	11.9 (8.61)	5.5 (2.39)
Group C (Asymptomatic)	67.9 (13.79)	23.25 (12.28)	7.8 (2.7)
p value[^] (overall comparison – A vs B vs C)	p<0.001**	p<0.001**	p<0.001**
Group A vs B#	p=0.514	p=0.321	p=0.302
Group A vs C #	p<0.001**	p<0.001**	p<0.001**
Group B vs C#	p<0.001**	p=0.001*	p=0.016*

Table:3 Comparison of haematological parameters in three groups of COVID-19 subjects.

	NLR Mean (SD)	LMR Mean (SD)	PLR Mean (SD)
Group A (BIPAP)	21.09 (14.96)	2.11 (2.43)	318.6 (182.2)
Group B (Symptomatic)	8.58 (5.81)	2.22 (1.31)	234.6 (149)
Group C (Asymptomatic)	4.77 (4.95)	3.15 (1.78)	191.4 (86.5)

p value[^] (overall comparison – A vs B vs C)	p<0.001**	p =0.171	p =0.024*
Group A vs B#	p<0.001**	p=0.982	p=0.167
Group A vs C #	p<0.001**	p=0.201	p=0.02*
Group B vs C#	p=0.435	p=0.273	p=0.615

Table 4: Comparison of haematological (coagulation) parameters in three groups of COVID-19 subjects

	DDIMER Mean (SD)	PT Mean (SD)	APTT Mean (SD)
Group A (BIPAP)	5657.7 (937.1)	13.97 (1.89)	45.19 (16.32)
Group B (Symptomatic)	305.5 (294.2)	13.23 (3.45)	40.54 (11.07)
Group C (Asymptomatic)	172.4 (88.5)	13.01 (1.16)	40.45 (15.89)
p value[^] (overall comparison – A vs B vs C)	p=0.028*	p=0.409	p =0.508
Group A vs B#	P=0.057	p=0.584	P=0.577
Group A vs C #	P=0.047*	P=0.409	P=0.564
Group B vs C#	P=0.997	P=0.954	P=1.000

p>0.05 – no significant difference *p<0.05 – significant difference **p< 0.001 – highly significant

DISCUSSION

The world has faced an outbreak of a novel Corona virus. Widespread distribution of this virus has led to a major concern, globally.⁹ This is the third contagious Corona virus leading to an epidemic in the 21st century after MERS and SARS.¹⁰ Several inflammatory and hematological parameters are thought to associate with the severity of COVID-19.¹¹ The aim of our study is to investigate the changes in hematological parameters and to find out any correlation of these with disease severity of COVID-19. In our study we analyzed several blood parameters and their association with the severity of the COVID-19 disease. In this study, we classified the patients into different categories based on severity. The patients were categorized into three groups : Group A included 40 patients in whom ventilation was required, Group B comprised of 40 patients who were symptomatic but ventilation was not required and Group C was of 40 patients who were asymptomatic.

The study reported that men were more affected than women from the disease. A study conducted by Jin JM et al., reported that according to the clinical classification of severity, men had more severe disease than the women¹². SARS-CoV-2 and SARS-CoV attack cells via the same receptor, ACE2.¹² It has been shown that circulating ACE2 levels are higher in men than in women and in patients with diabetes or cardiovascular diseases.¹³ Abdul Waris et. al.¹⁴ and Sadia Taj et. al¹⁵ also reported men being affected more than women. A significant increase in the WBCs level was observed in the severe and symptomatic individuals compared to asymptomatic patients, which might be due to inflammatory response, having a significant association with the disease severity. Brandon Michael Henry et al. also found Patients with severe and fatal disease with significantly increased white blood cell (WBC) count.¹⁶

We observed increased neutrophils and decrease in the lymphocytes, monocytes in severe and symptomatic patients compared to asymptomatic patients. Based on our observation, the lymphocytes count depletion is directly associated with the COVID-19 disease severity and the survival rate of the patients could be linked with the ability of T lymphocytes which are essential for the destruction

of infected viral particles.¹⁷ The observation in this study supports previous investigations by Aditya Anurag et. al. related to differential white blood cell count in COVID-19.¹⁸ Also Yang et.al.¹⁹ and Abdul Waris et. al.¹⁴ found similar results. The decreased lymphocyte count and increased granulocytes in the individuals which could be attributed to increased inflammation and suppression of the immune system caused by SARS-CoV-2 infection. The elevation in granulocytes and decrease in lymphocytes can be, therefore, easily used for severity and mortality analysis of COVID-19 and as routine blood tests like CBC are easily and readily available.²⁰

In present study NLR ratio was increased in severe and symptomatic patients compared to asymptomatic patients, similar findings also found in Aditya Anurag et. Al.¹⁸, Abdul Waris et. al.¹⁴, Sadia Taj et. al¹⁵ and . Liu J et. al.²¹ In pandemic difficulty is the shortage of medical resources, especially critical care resources so, early identification critical illness and risk stratification management will help alleviate insufficient medical resources and might reduce mortality. The NLR ratio is the most promising predictive factor for critical illness incidence of COVID-19 pneumonia. The early application of NLR will be beneficial to patient classification management and relief of medical resource shortage.²¹

In our study LMR ratio did not show significant difference between 3 groups whereas Yang et.al.¹⁹ and Abdul Waris et. al.¹⁴ found LMR ratio was significantly decreased with severity of disease. Decreased LMR ratio is as an indicator of poor prognosis so increased chances of mortality among patients suffering from COVID-19.

This study did not find changes of platelet count with the severity of disease. Similar findings were found in Sadia Taj et. al¹⁵ study while Lippi G et. Al.²², yang et.al.¹⁹ and Abdul Waris et. al.¹⁴ found significantly thrombocytopenia is associated with significantly ill patients of coronavirus disease 2019 (COVID-19). The platelet count was decreased in the severe and critical patients which could be linked to thrombin generation, immunological destruction of platelets, impaired megakaryopoiesis, and inappropriate platelet consumption.²³ Platelet could be a suitable biomarker for recognition of coagulopathy and its severity. In our study we found

PLR ratio increased in Severe patients compared to symptomatic and asymptomatic patients, this findings support by previous study of Daniel et. al.²⁴, Abdul Waris et. Al.¹⁴ and Yang et.al.¹⁹ PLR ratio determining the severity and mortality of COVID-19 disease. Inflammation plays a considerable role in the pathophysiology of COVID-19, with cytokine storm as a hallmark condition in severe disease with poorer prognosis. Elevated PLR value suggests an overactive inflammatory response and subsequently, worse prognosis.²⁴

In this study it was observed that hemoglobin and hematocrit of the COVID-19 patients showed no significant difference with the severity of disease, similar observation also found in Abdul Waris et. al.¹⁴ and Sadia Taj et. al study.¹⁵ Patients presenting with infection may eventually develop into sepsis. Sepsis is well established as one of the most common causes of DIC; when monocytes and endothelial cells are activated to the point of cytokine release following injury, expression of tissue factor and secretion of von Willebrand factor which causes circulation of free thrombin and this process is uncontrolled by natural anticoagulants, can activate platelets and stimulate fibrinolysis. So levels of fibrin-related markers (D-dimer and FDP) elevated in all sever patients, which suggested a common coagulation activation and secondary hyperfibrinolysis condition in these patients.²³ For these reason coagulation profile, including prothrombin time, activated partial thromboplastin time and D-Dimer levels is the screening test for abnormal clotting.

A significant difference in D-dimer across severity of disease was noted in our study. Previous studies by Jeffrey et al.²⁵, Mikami et al.²⁶ and Sadia Taj et. al¹⁵ showed similar findings like D-Dimer

increase with the severity of the COVID -19 disease. Elevation of D-dimer suggests coagulopathy, particularly disseminated intravascular coagulation (DIC), which may contribute to mortality in COVID-19. Patients with increased D-Dimer levels had worst clinical outcome.²³ PT/APTT values showed changes in different groups, however these changes were not statistically significant with severity of disease whereas Toshiaki Iba et al.²⁷, Tang et al.²³, Mikami et al.²⁶ and Sadia Taj et. al¹⁵ found PT/APTT increased with severity of disease.²⁷ In this study we found serum Ferritin level significantly increased in severe patients compared to asymptomatic patients and ethical dilemma we should consider.²⁸ Similar findings were observed by investigators -Sadia Taj et. al¹⁵, Hussein et al.⁸ and Abbaspour et al.²⁹ Ferritin is an acute phase protein which can be discharged from destroyed hepatocytes. Covid-19 patients with abnormal ferritin levels have increased risk for liver injury and severe illness⁸. Ferritin is also a key mediator of immune dysregulation, especially under extreme hyperferritinemia, via direct immunosuppressive and pro-inflammatory effects, contributing to the cytokine storm²⁸.

CONCLUSION:

TLC, Neutrophil, NLR, PLR, D-Dimer values were found to have statistical significant ($p < 0.05$) positive correlation with Covid -19 severity. Blood investigations like Lymphocyte and Monocyte count have statistical significant ($p < 0.05$) negative correlation with Covid -19 severity. No significant correlation was observed between haematological tests like Hb, HCT, PLT, LMR and tests of coagulation like PT & APTT with Covid -19 severity. So we concluded that TLC, NLR and D-dimer tests are important to predict about the severity of disease.

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