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Original Research Article

Variations in complete blood counts and cellular morphological alterations in blood smear of COVID-19 patients from a tertiary care center

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ABSTRACT

Background: Corona viruses are a large family of viruses of which Corona virus disease 19 (COVID 19) infection evokes a hyperinflammatory response and severe acute respiratory distress syndrome in human beings. Complete blood count evaluation and peripheral smear examination is an essential tool for early diagnosis and management of COVID-19 positive patients.
Aims: This study was aimed to evaluate the variations in complete blood counts (CBC) and changes in the cellular morphology in the blood smear in patients affected with COVID-19.
Settings and Design: This was an observational study, undertaken in a tertiary care center.
Materials and Methods: The study included baseline blood samples from 96 proven COVID-19 positive cases. The samples were subjected for automated complete blood count analysis and peripheral smear examination.
Statistical Analysis: The quantitative data were expressed by mean and Standard deviation. Qualitative categorical variables were expressed as numbers and percentages.
Results: Out of the total 96 cases, the most common quantitative finding was neutrophilic leukocytosis with relative lymphopenia. Five cases of absolute lymphopenia was observed. Majority of the cases had normal hemoglobin values and normal platelet counts. The characteristic morphological changes were seen in neutrophils and lymphocytes. The neutrophils showed presence of toxic granules, cytoplasmic vacuolations and hypolobation.The morphological alteration in lymphocytes included reactive lymphocytes with plasmacytoid and monocytoid morphology, large granular lymphocytes and Turk cells. Infrequent finding of leukoerythroblastic reaction was also observed in our study.
Conclusion: To summarize, hematological investigation of COVID 19 cases may show several abnormal findings. Quantitative findings like Neutrophilia and lymphopenia were characteristic, whereas, the most common morphological alteration observed was atypical lymphocytes. Quantitative and qualitative alterations of hematological parameters are one of the investigations which would aid timely diagnosis of the disease. Toxic granules and cytoplasmic vacuolations in neutrophils may indicate ongoing sepsis in COVID 19 patients which in turn aids in efficient management of the patients to reduce the mortality rate.

1. Introduction

Coronaviruses (CoV) are a large family of viruses which can cause broad spectrum diseases ranging from mild flu to Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS coV).[1] Towards the end of the year 2019, a new disease called COVID 19, related to corona virus species emerged from the city of Wuhan located in China.[2] It was first observed in a group of patients who developed fever, cough and

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shortness of breath.[1] The causative virus, severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2) is capable of human- to- human transmission. This disease had further spread rapidly to other localities of the country (China) then worldwide[3] giving rise to a pandemic in a few months causing about two million deaths.[2] In addition to the archetypal respiratory symptoms, COVID 19 affects other organs as well, as shown by several other studies.[3]

The primary routes of SARSCoV-2 transmission is either through respiratory droplets or direct contact. Approximately about 80% of SARS-CoV-2 infections are mild, having flu-like symptoms with few cases being asymptomatic. About 15% of cases evolve into severe disease characterised by pneumonia and dyspnea. A meagre percentage of SARS-CoV-2-infected individuals require intensive care support as they experience acute respiratory distress syndrome (ARDS), septic shock eventually leading to multi-organ failure.[4]

Few of the studies have shown that the pathogenesis is brought about by penetration of virus into the host cell by interacting with the angiotensin-converting enzyme 2 (ACE2), a monocarboxypeptidase present on the cell surface of the epithelial cells of the respiratory tract and capillary endothelial cells.[5]

Imaging modalities and laboratory tests have shown certain abnormalities in COVID 19 patients. Lymphopenia and neutrophilia were the first reported laboratory findings in complete blood count analysis. A growing body of evidence suggests that the changes in the blood parameters are dynamic and it varies in patients according to the stage of disease.[6]

Assessment of blood cell morphology in peripheral blood film is a potent tool to evaluate patients' blood samples with abnormalities in their blood counts. Unexplained cytopenia, suspected hematologic diseases, patients with suspected organ failure, infections and sepsis are the other few conditions which behooves blood smear examination.[2] The major morphological alterations found in COVID 19 patients include atypical, reactive, and large granular lymphocytes (LGL), smudge cells, myeloid left shift, giant platelets, anisocytosis and poikilocytosis such as schistocytes has been described in literature.[7–11] Alteration in lymphocyte count and the morphology are usually associated with other viral infections. But coronavirus appears to have a trilineage involvement of hematopoietic cells.[12]

In this study we aim to evaluate the alterations in the morphological details of the formed elements in peripheral blood film and the quantitative analysis of complete blood count in patients diagnosed with COVID 19 infection in our tertiary care centre.

2. Materials and Methods

It was an observational study which was carried out in a tertiary care center. The study was conducted from August 2021 to October 2021. The study population included patients who got admitted in the COVID 19 ICU and ward after being diagnosed as COVID 19 positive via Rapid antigen test or a real-time reverse transcription–polymerase chain reaction (RTPCR). A total of 96 cases were included in the study.

2.1. Inclusion criteria

All the blood samples of patients tested positive for COVID 19 (by either of the method) that was sent for complete blood counts and peripheral smear examination to the department of Hematopathology.

2.2. Exclusion criteria

The samples which couldn't be analysed i.e, hemolysed samples or clotted samples.

2.3. Study tools

All medical records of the COVID 19 patients managed in ICU and ward.

2.4. Data collection

Clinical data of the patients were collected from the ward, medical records section and electronic hospital health records. For all the subjects included in the study, only the baseline values of complete blood count and peripheral smear reports were analysed.

2.5. Processing of blood samples

The blood samples (of subjects included in the study) from the COVID ICU and COVID ward was received in the hematopathology laboratory. The received blood samples were run in Beckman coulter-Coulter LH 780 Hematology Analyzer. Automatic counts of all samples were processed, analysed and documented. The values and changes in CBC parameters and its derived parameters were evaluated using the laboratory biological reference interval (Table 1).

2.6. Blood smear preparation

Venous samples of COVID 19 patients were collected in EDTA vacutainers following which peripheral blood smears were made. A drop of blood was placed on a clean glass slide at one centimeter distance from one end. The drop of blood was spread quickly with a spreader at an angle of 30 degrees. The slides were air dried and were stained with Leishman stain for two minutes. After two minutes, buffer water of about twice the volume of stain used was added and left for about 8 minutes after which the slides were washed

Table 1: Biological reference interval

S. No	Parameter	Normal reference range		Inference
1.	Hemoglobin (Hb)	12 – 15 g/dl in females 13 – 17 g/dl in males		<12 g/dl – Anemia <13 g/dl – Anemia
2.	Total WBC count	4 – 10 x10^3 /μl		< 4 x10^3 /μl – leucopenia >10 x10^3 /μl – leukocytosis
3.	Platelets	150-450x10^3 /μl		<150 x10^3 /μl – thrombocytopenia >450 x10^3 /μl – thrombocytosis
4.	Mean corpuscular Volume(MCV)	83-101 fL		<80 fL – microcytic picture >101 – macrocytic picture
	Differential Leukocyte count			
	Cells	DC	AC	
5.	a) Neutrophils	40%-80%	2000-7000/cu.mm	Absolute count of <2000/cu.mm – neutropenia Absolute count of >7000/cu.mm – neutrophilia
	b) Lymphocytes	20%-40%	1000-3000/cu.mm	Absolute count of <1000/cu.mm – lymphopenia Absolute count of >3000/cu.mm - lymphocytosis
	c) Monocytes	2%-10%	200-1000/cu.mm	Absolute count of >1000/cu.mm - monocytosis
	d) Eosinophils	0-6%	20-600/cu.mm	Absolute count of >600/cu.mm - eosinophilia
	e) Basophils	0-1%	20-100/cu.mm	Absolute count of >100/cu.mm - basophilia

DC – Differential count, AC – Absolute count

under slow flowing tap water. The stained slides were placed upright on a rack to dry.

2.7. Peripheral smear analysis

The slides were scanned at scanner view. One hundred leukocyte counts were performed at 100X magnification on each peripheral blood smear by the reporting pathologists. The differential counts were documented in percentage. The red blood cell morphology was studied at 100x magnification and the morphological data was documented for each case included in the study.

Ethical committee approval has been obtained from institutional human ethics committee.

2.8. Statistical data analysis

Data was entered into MS Excel 2007 version. Following this, the data was analyzed using SPSS version 20. Quantitative data were presented as mean and Standard deviation. Qualitative categorical variables were expressed as numbers and percentages.

3. Results

The total number of cases studied were 96. The mean age was 49.4 with a standard deviation of 16.8. Males constituted about 67.7% and females were 32.3% with a male to female ratio of 2.09.

3.1. Quantitative changes

The hemoglobin was normal in majority of the cases which was seen in 64 (66.7%) cases. Three out of these 64 cases (4.7%) showed macrocytic blood picture with an MCV value of above 101.

The total WBC counts were increased (leukocytosis) in majority of the cases which was observed in 75(78.12%) cases. In the total 96 cases studied, absolute lymphopenia was seen in 5 (5.2%)cases, relative lymphopenia was seen in 82 (85.5%).

Majority of the patients, 74 (77.1%) had platelets within normal limits (Table 2).

3.2. Morphological changes

Hemoglobin was low in 32 (33.3%) cases, which was categorized as anemia and further classified according to the morphology seen in peripheral smear in conjuction with the MCV values on coulter reading into, microcytic anemia and normocytic anemia (Table 3).

Out of the 75 cases with leukocytosis, Neutrophilic leukocytosis was seen in 71 (94.7%) cases. Shift to left of neutrophil series along with presence of toxic granules (Figure 1) in the cytoplasm of neutrophils were seen in 70 (72.9%) of the cases. Lymphocytes with reactive changes (Figure 2) were seen in 20.83% of cases as explained in the table. One case had a leukoerythroblastic blood picture in our study (Table 4).

Table 2: Characteristics of blood parameters in COVID-19 patients

Variables	Number of cases [total (n)=96]	Percentage of cases
Hemoglobin		
Within normal limits	64	66.7%
Low (anemia)	32	33.3%
Total WBC counts		
Within normal limits	20	20.84%
Increased (leukocytosis)	75	78.12%
Decreased (leucopenia)	1	1.04%
Differential WBC counts		
Normal distribution	8	8.3%
Neutrophilia [includes absolute and relative neutrophilia]	83	86.4%
Absolute Lymphopenia	5	5.3%
Platelets		
Within normal limits	74	77.1%
Increased in count (thrombocytosis)	7	7.3%
Decreased in count (Thrombocytopenia)	15	15.6%

Table 3: Morphological type of Anemias observed in COVID-19 patients

Type of anemia	Number of cases with anemia [total (n)=32]	Percentage of cases
Normocytic anemia	22	68.7%
Microcytic hypochromic anemia	10	31.3%

Table 4: Morphological abnormalities of formed elements in peripheral blood smear of COVID 19 patients

Leukocyte	Morphological change	Number of cases Total (n)=96	Percentage of cases
Neutrophils	Toxic granules	60	62.5%
	Toxic granules and Cytoplasmic vacuolation	10	10.41%
	Hypolobated neutrophils	3	3.12%
Lymphocytes	Reactive lymphocytes	Total=20	Total % = 20.83%
	a) Plasmacytoid	11	11.45%
	b) Monocytoid	7	7.3%
	c) Turk cell	2	2.08%
	d) Large granular lymphocytes	1	1.04%
RBC and WBC	Leukoerythroblastic picture	1	1.04%
Platelets	Giant platelets	4	4.16%

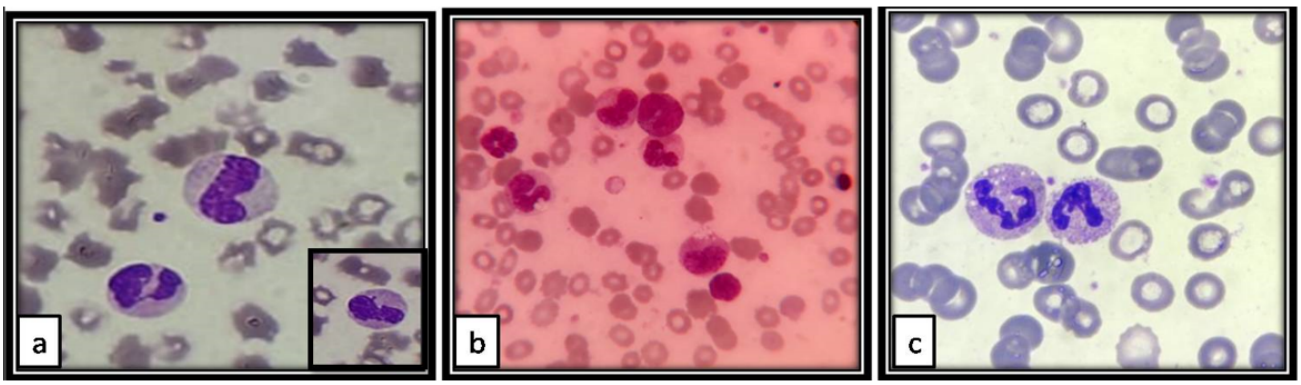

Fig. 1: Morphology of neutrophils in blood smear of COVID 19 patients:(**a**) Hypolobated neutrophils; (**b**) Shift to left of neutrophils showing metamyelocyte, band form and segmented forms (**c**) Segmented neutrophils with cytoplasmic vacuolations and toxic granules. [X1000, Leishman stain]

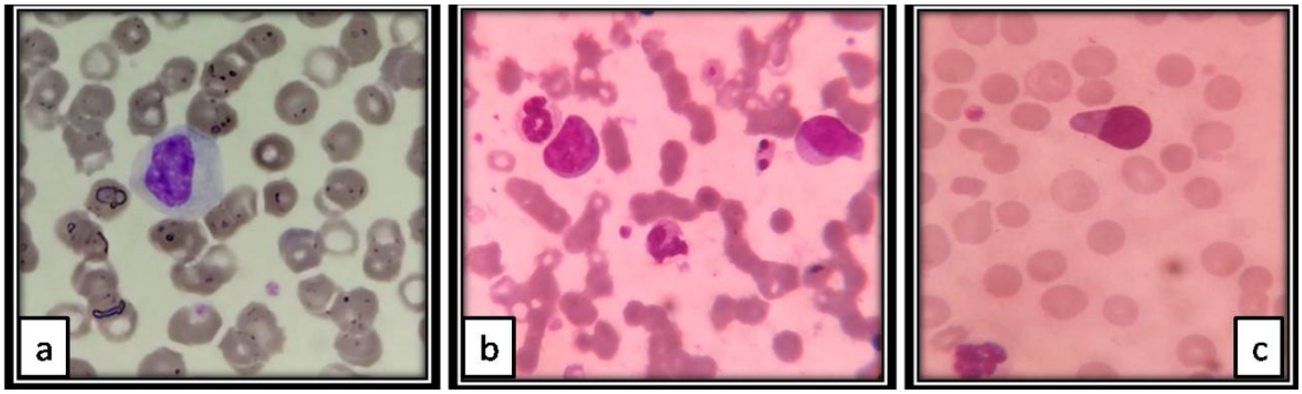

Fig. 2: Morphology of lymphocytes in blood smear of COVID 19 patients: (**a**): Monocytoid morphology of reactive lymphocyte; (**b**): Reactive lymphocytes and neutrophils; (**c**): Plasmacytoid morphology of reactive lymphocyte. [X1000, Leishman stain]

4. Discussion

COVID-19 has rapidly spread and evolved into a pandemic after it has been identified in the year 2019 at Wuhan China.[8] The spike protein present on the surface of the virus viral plays a key role in host attachment and penetration. SARS-CoV-2 infection remarkably affects the respiratory system. But it has been found that it may also involve the hematopoietic system and has a role in homeostasis.[13] Previous studies elucidate that TLC is normal or slightly low in the incubation period of disease ranging from 1 to 14 days. However, after 7 to 14 days of illness, the course of illness is governed by inflammatory mediators and cytokines leading to a cytokine storm.[14] The hematopoietic system actions a thoughtful character in the notable hyperinflammation, mainly in severely ill patients.[15]

The alterations in the laboratory parameters such as hematologic abnormalities have been reported in COVID-19 patients. The association of these alterations with early diagnosis, disease prognosis and severity has been discussed time and again in the literature. Complete blood count analysis is a basic, easy and cost effective haematological test that is customarily being performed which plays an imperative role in early diagnosis of the disease.[13]

Fibrinogen, prothrombin time (PT), activated partial thromboplastin time (APTT) and D dimer tests are the other additional tests performed for the diagnosis of COVID 19.[16] Prevailing haematological deviance seen in COVID-19 are: anaemia, leucocytosis or leucopenia, neutrophilia, low eosinophil count or eosinophilia, thrombocytopenia, and rarely thrombocytosis.[17]

In the present study, 33.3% of the cases showed anemia, whereas hemoglobin within normal limits were observed in 66.7% of the cases. Peripheral smear examination of these patients showed normocytic and normochromic blood picture. In a study done by Nath D et al,[18] similar finding was observed.

Elevated neutrophil count is seen in severe COVID-19 patients[19] which implies association of the myeloid series in COVID 19, which is unapparent in other viral infections.[12] In our study, the most characteristic WBC finding was neutrophilic leukocytosis in majority of the cases and leucopenia was seen in only one case.

Neutrophils with toxic granulation was seen in 72.9% of the cases which was similar to the study done by Singh A et al[20] and Kannan G et al.[21] Shift to left of myeloid series was seen in our study which was similar to a study done by Zini G et al,[22] Kaur G et al[5] and khandelwal A et al.[23] In all these studies, the smears of patients with neutrophilia showed shift to left of neutrophil series with presence of band forms, metamyelocytes, and myelocytes. Cytoplasmic vacuolations were appreciated in cases which had critical clinical outcome. Kaur G et al[5] has described similar findings in their study.

Reduction in the nuclear lobularity were also seen in a few (3.12%) cases in our study and that was described by Nazarullah et al[8] and Zini et al[22] in their respective studies. Previous literatures have shown that these findings may be attributed to the fact that there is direct infection of the hematopoietic stem cells and the progenitor cells. Apart from the action of inflammatory cytokines, in the setting of infection, there can be a possible change in the bone marrow microenvironment.[22,24]

Neutrophilia is a feature that correlates with the hyper-inflammatory state and cytokine storm, which is pathognomonic of COVID-19. With the advancement of the disease stage, there has been a quantitative increase in the circulating neutrophils. Gradual increase in neutrophil count is seen with the advancement of COVID 19. Neutrophilia can thus be considered as a marker of poor outcome following severe respiratory disease.[25]

In contrast to most of the literature on COVID 19, our study showed minimal number of cases (5.3%) with absolute lymphopenia, which is comparable to a study done by Nazarulla A et al,[8] where absolute lymphopenia was

seen only in two cases. A subset of total cases showed presence of reactive lymphocytes. Predominant type of reactive lymphocyte in this study showed plasmacytoid appearance seen in 11.45% of the cases. Monocytoid appearance of reactive lymphocytes were appreciated in 7.3% of the cases. Fan BE et al[17] has done a study in which he describes about lymphopenia with reactive lymphocytes. In his study few of the cases showed monocytoid and or lympho-plasmacytoid appearance of reactive lymphocytes. The B-lymphocytes become activated to form lympho-plasmacytoid type of reactive lymphocytes with a distinct morphology and also forms immunoglobulin-secreting plasma cells during a viral infection. The mechanism behind peripheral blood lymphopenia remains unclear. It could possibly be justified due to mobilization of the cells to the infected site, virus induced destruction of T cells,[26] direct viral toxicity and cytokine-induced lymphopenia.[27] Large granular lymphocytes were seen in just one case in our study comparable to a study done by Singh et al.[20]

Leukoerythroblastic reaction, an exceptional finding have also been described in this study in 1% of the cases. Mitra et al[7] and Pezeshki A et al,[2] have described similar features in their respective studies. Platelet count was normal in majority of the cases in our study which constituted about 77.1% similar to Kaur G et al[5] study.

Thrombocytopenia was observed in 15.6% cases similar to studies done by Sundari A et al[28] and Dudve S S et al.[29] However, the percentage of cases with thrombocytopenia was variable. Lippi et al[30] in his study has shown correlation of low platelet count with increased severity of the disease and association with augmented mortality. Three mechanisms of a cascade can be assumed to explain thrombocytopenia in SARSCoV- 2 infections: 1) Infection of the marrow by virus thereby inhibiting platelet synthesis; 2) Destructive mechanism of the immune system on platelets; 3) Further consumption of platelets due to formation of platelet aggregate and microthrombi in the lungs.[25]

Platelet synthesis will be reduced due to the role played by the viruses in their interaction with the megakaryocytes in the bone marrow.[31] There are specific receptors upon which SARS-CoV-2 acts and inhibits the bone marrow hematopoiesis there by depressing the formation of platelets resulting in peripheral thrombocytopenia.[32]

The morphological abnormality observed in platelets were giant platelets seen in 4.16% in our study, similar to studies done by Pezeshki A et al,[2] Mitra A et al,[7] Sadigh S et al,[11] Marchi G et al.[33]

5. Limitation of the Study

The study was performed for a short duration and with relatively small sample size. Only the baseline values were analysed in the study and no conjuction with biochemical tests were made. A study with longer duration with more number of study subjects and samples taken during different disease stages (according to severity) would represent a better clinico-pathological correlation.

6. Conclusion

Analysis of the parameters of complete blood counts and peripheral blood smear examination are the basic tests done for diagnosis of any clinical condition. In COVID 19 patients, one of the investigative findings include alterations in CBC values and the morphology of cellular elements in the peripheral blood smear. Toxic granules and cytoplasmic vacuolations in neutrophils may indicate ongoing sepsis in COVID 19 patients. This would predict prognosis earlier and can aid the clinicians in timely management. Follow up evaluation of CBC and blood smear of COVID 19 patients during different disease stages would help in early detection of complications, thus reducing the mortality rate. However, evaluation of other parameters along with CBC and peripheral smear would be of paramount importance for diagnosis of the disease.

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None.

8. Conflict of Interest

None.

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