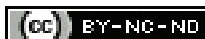


Morphological Changes in Red Blood Cells, White Blood Cells, and Platelets in COVID-19 Infected Patients: A Cross-sectional Study

KOVURI UMADEVI¹, MOHD IMRAN ALI², LAVANYA MOTRAPU³, NAGARJUNACHARY RAJARIKAM⁴

ABSTRACT

Introduction: Coronavirus Disease-2019 (COVID-19) is a highly infectious virus that mutates rapidly. This has led to the emergence of newer variants that can spread more easily and may be more resistant to current treatments and vaccines. It is possible that new variants could emerge in the future that could be even more dangerous than the current ones.

Aim: To identify and describe characteristic changes in peripheral smears of COVID-19 patients.

Materials and Methods: A cross-sectional study was conducted at Government Medical Hospital Nizamabad, Telangana, India from March 20, 2021, to June 10, 2021. A total of 600 COVID-19 patients who were tested positive via Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) were included, and blood tests were performed on the day of admission before any prior intervention. Samples were analysed using a Complete Blood Count (CBC) analyser, and peripheral smears were examined to identify the morphological changes of Red Blood Cells (RBCs),

White Blood Cells (WBCs), and Platelets in COVID-19-affected patients. These changes help assess the disease process, its severity, and aid in its proper management.

Results: Out of the 600 cases, 384 (64%) were males, and 216 (36%) were females. The study showed the following RBC changes: microcytic hypochromic RBCs 333 (55.5%), macrocytic RBCs 66 (11%), dimorphic picture 33 (5.5%); WBCs showed neutrophilia 535 (89.17%), lymphopenia 535 (89.17%), pseudo Pelger-Huët cells 468 (78%), smudge cells 360 (60%), reactive lymphocytes 360 (60%), large granular lymphocytes 72 (12%), and large giant platelets 444 (74%) in peripheral smears of COVID-19 patients.

Conclusion: As COVID-19 patients showed specific morphological changes in RBCs, WBCs, and Platelets, CBC and peripheral smear analysis can be interpreted in conjunction with other clinical and confirmatory tests for prognostication and management of COVID-19 patients.

Keywords: Atypical lymphocytes, Large and giant platelets, Pseudo Pelger-Huët cells smudge cells, Reactive lymphocytes

INTRODUCTION

COVID-19 is a viral infection caused by the novel coronavirus Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2). The disease was discovered in Wuhan, China, in December 2019 and has since spread worldwide, resulting in a pandemic.

COVID-19 symptoms can range from mild to severe, including fever, cough, fatigue, muscle aches, sore throat, loss of smell or taste, and difficulty in breathing [1]. While the majority of COVID-19 patients have mild to moderate symptoms, some may develop severe illness, such as pneumonia, Acute Respiratory Distress Syndrome (ARDS), and multiorgan failure, which can be fatal [2].

Peripheral smears, also known as peripheral blood smears or blood films, are laboratory tests that involve looking at a thin layer of blood through a microscope. Peripheral smears play a major role in supportive diagnoses in many conditions, such as the diagnosis of blood-related disorders like anaemia and leukaemia, evaluation of infection and inflammation, monitoring treatment effectiveness, and screening for blood-borne diseases like malaria and other parasitic infections [3,4].

COVID-19 has been linked to changes in blood cell counts and morphology, including changes in WBC count, RBC count, and platelet count, according to some research. The significance and clinical relevance of these changes, however, are still being investigated, and more research is required to determine the precise relationship between COVID-19 and peripheral smears [5,6]. COVID-19 patients are at an increased risk of developing blood clotting disorders such as Deep Vein Thrombosis (DVT) and Pulmonary

Embolism (PE) [7]. Peripheral smears can detect abnormalities in blood cell morphology, such as giant platelets, large platelets, and platelet clumps, which can help predict clotting disorders [7].

Furthermore, COVID-19 can cause respiratory complications, such as ARDS, which can result in low blood oxygen levels. Changes in RBC morphology or haemoglobin levels detected by peripheral smears may indicate anaemia or other blood-related complications that may contribute to decreased oxygen delivery [8]. ARDS is a lung condition that is mainly linked to the recruitment, activation, and retention of neutrophils in the pulmonary vasculature. Neutrophils recruited in the lung are more resistant to apoptosis. All these inflammatory changes can be detected in peripheral smears through morphological alterations of cells [8]. CBC is a simple and useful test that can provide important information about a person's overall health and help healthcare providers diagnose and manage a wide range of conditions. Peripheral smears can be easily performed with a rapid turnaround time and are also available at grassroots levels, cost-effective, and easily accessible to the public.

Present study aims to identify morphological changes in RBCs, WBCs, and platelets that play a significant role in the early diagnosis of COVID-19. These changes can serve as a helpful screening test followed by a definitive test in the future, as morphological changes associate better with disease progression.

MATERIALS AND METHODS

A cross-sectional study was conducted on 600 patients at the Department of Pathology, Government Medical College and General

Hospital, Nizamabad, Telangana, India, from March 20, 2021, to June 10, 2021. Ethical committee clearance was obtained for the study with IEC no: ECR/144/Inst/TG/2019. The study obtained informed consent from the participants allowing the collection of specimens for research and subsequent publication.

Inclusion criteria: COVID-19 patients with positive RT-PCR admitted to Government General Hospital were included in the study.

Exclusion criteria: RT-PCR positive OPD patients and patients under the age of 10 years were excluded from the study. Children have been less severely affected by COVID-19 due to a robust and adaptable immune system and lower Angiotensin-Converting Enzyme (ACE) receptor expression in the respiratory tract compared to adults. Additionally, children have some degree of pre-existing immunity or cross-immunity that protects them from coronaviruses [9].

Data collection: A 3 mL blood sample was collected from COVID-19 patients on the day of admission before any intervention. Using the Sysmex xn-1000 analyser, haematological parameters such as haemoglobin (reference range males: 13.7-17.5 gm/dL, females: 11.2-15.7 gm/dL), RBC count (reference range males: $4.6-6.1 \times 10^6$ /microliter (μ L), females: $3.9-5.2 \times 10^6$ /mcl), WBC count (males: $3.7-10.3 \times 10^3/\mu$ L, females: $3.7-10.3 \times 10^3/\mu$ L), platelet count (males, females: $155-369 \times 10^3/\mu$ L), absolute neutrophil count ($1.6-6.1 \times 10^3/\mu$ L), absolute lymphocyte count ($1.2-3.9 \times 10^3/\mu$ L), absolute monocyte count ($0.3-0.9 \times 10^3/\mu$ L), absolute eosinophil count ($0.0-0.5 \times 10^3/\mu$ L), mean cell volume (79-98 femtolitres), mean cell haemoglobin (26-32 picograms), and mean cell haemoglobin concentration (30.7-35.5 gm/dL) were recorded [10].

STATISTICAL ANALYSIS

Peripheral smears were prepared, stained with Leishman stain, examined, recorded in a Microsoft Excel sheet, and analysed. Results were explained in tables and percentages.

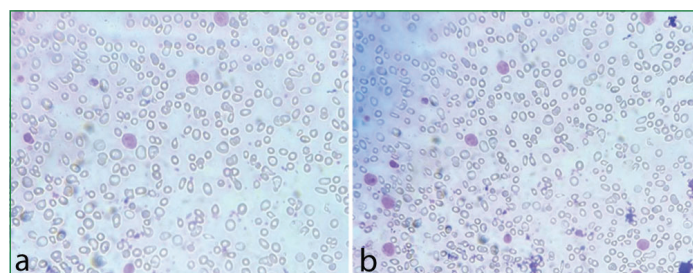
RESULTS

The data of 600 patients registered at Government General Hospital, Nizamabad, were included in the study. Out of the 600 cases, 384 (64%) were males, and 216 (36%) were females. 7 (1.2%) were in the age group of 10 to 20 years, 57 (9.6%) were in the age group of 21 to 30 years, 87 (14.4%) were in the age group of 31 to 40 years, 168 (28%) were in the age group of 41 to 50 years, 180 (30%) were in the age group of 51 to 60 years, and 101 (16.8%) were in the age group above 61 years. The mean age was in the range of 50 ± 12 years.

[Table/Fig-1] shows different RBC morphology, [Table/Fig-2a,b] Haematoxylin and Eosin (H&E) staining of RBC morphology.

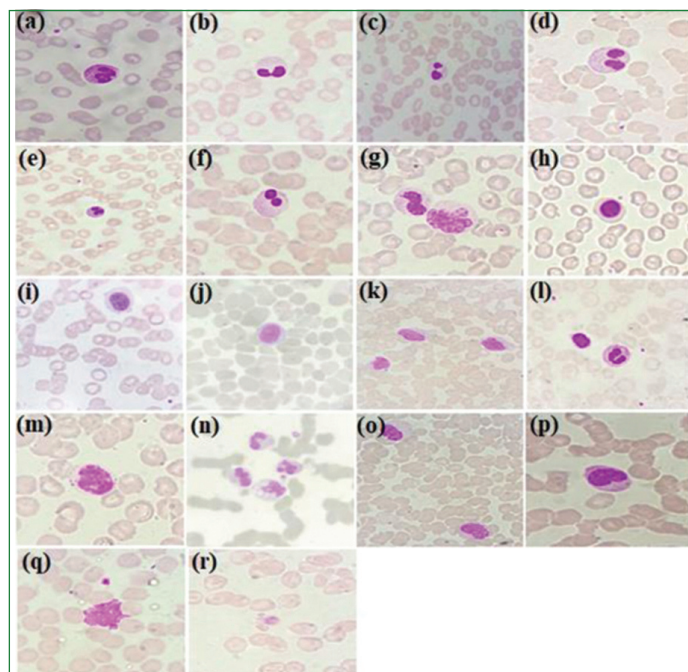
Parameters	n (%)
RBC Morphology	
Normocytic, Normochromic RBC	222 (37%)
Microcytic hypochromic RBC	333 (55.5%)
Macrocytic RBC	66 (11%)
Dimorphic RBC	33 (5.5%)

[Table/Fig-1]: Morphological changes in Red Blood Cells (RBC) on peripheral blood smear of COVID-19 patients.



[Table/Fig-2]: a) Showing microcytic RBC, macrocytic RBC with few normocytic RBC; b) Showing predominantly microcytic RBC, with few macrocytes. (Images from left to right)

Different morphological patterns seen in White Blood Cells (WBC) on peripheral smear are shown in [Table/Fig-3,4]. A total of 180 out of 600 (30%) patients showed reactive monocytes and multiple vacuolations in the cytoplasm.

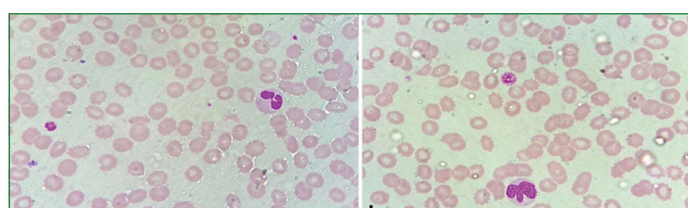


[Table/Fig-3]: Changes in neutrophil morphology in COVID-19 patients: (a) Showing neutrophil with C-shaped, foetus like nuclei; (b,c) Neutrophil showing bilobed nucleus looking like pincenez and agranular cytoplasm (Acquired pseudo Pelger-Huët anomaly (APHA)); (d) Neutrophil; (e,f) Degenerating neutrophil; (g) Dysplastic neutrophils; (h) Showing Nucleated Red Blood Cell (NRBC) Changes in lymphocyte morphology; (i-l) Showing large and atypical lymphocytes; (m) Showing large lymphocyte with granules. Changes in monocyte morphology; (n) Showing reactive monocyte showing irregular outline of cytoplasm and intranuclear and intracytoplasmic vacuolations; (o,p) Showing reactive monocytes with abundant cytoplasm and kidney shaped nucleus; (q) Showing smudge cell (r) Showing large platelet.

WBC morphology	n (%)
Neutrophilic leukocytosis	196 (32.7)
Leucopenia	49 (8.17)
Neutrophils	
Neutrophilia	535 (89.17)
Dysplastic neutrophils	372 (62)
Pseudo Pelger-Huët anomaly	468 (78)
Apoptotic cells	228 (38)
Smudge cells	360 (60)
Lymphocyte	
Lymphopenia	535 (89.17)
Reactive change	360 (60)
Large granular lymphocytes	72 (12)
Monocytes	
Reactive and vacuolated monocytes	180 (30)

[Table/Fig-4]: Morphological changes in White Blood Cells (WBC) on peripheral smear.

Large and giant platelets were seen in 444 (74%) patients and platelet clumps in 216 (36%) of COVID-19 affected patients, as shown in [Table/Fig-5a,b].



[Table/Fig-5]: a) Showing large platelet; b) Showing giant platelet. (Images from left to right)

DISCUSSION

RBC morphology changes are one of the many haematological abnormalities that COVID-19 has been linked to. Inflammation, hypoxia, and the direct effects of the virus on the bone marrow may all contribute to these changes [11]. Patients with COVID-19 may exhibit changes in RBC morphology, such as anisocytosis (variation in RBC size), poikilocytosis (variation in RBC shape), and hypochromia (pale RBCs with reduced haemoglobin content and central pallor more than 1/3rd of RBC). These alterations could be related to anaemia, which is a common complication of COVID-19 [11,12]. A higher risk of blood clotting conditions like DVT and PE has also been linked to COVID-19. Blood clots can appear as clusters of RBCs on peripheral smears, which are used to detect blood clot formation [13]. COVID-19 can affect the immune system, leading to changes in the numbers and types of immune cells in the bloodstream [13]. Neutrophils, which are a type of White Blood Cell (WBC) that plays an important role in the body's immune response to infections, may show changes in counts and morphology in COVID-19 patients. Neutrophils in COVID-19 patients' peripheral smears may exhibit changes such as an increase in immature forms (band neutrophils) and a decrease in mature forms (segmented neutrophils). This is referred to as a left shift and may indicate a more serious or prolonged infection. Pseudo PHA, along with apoptotic neutrophils, were seen in the majority of COVID-19 affected patients [14].

Lymphocytes, plays a key role in fighting viral infections, may show changes such as a decrease in the number of T lymphocytes. This decrease in T lymphocytes may be associated with the severity of the disease. In some cases, peripheral smears may also show an increase in atypical lymphocytes, which are larger than normal lymphocytes and have an irregular shape [15]. However, it is important to note that changes in lymphocytes on peripheral smears are not specific to COVID-19 and can be seen in other types of viral infections or inflammatory conditions. Therefore, peripheral smears should be interpreted alongside other clinical and laboratory findings.

COVID-19 can also affect monocytes, a type of white blood cell (WBC) that plays an important role in the body's immune response. Monocytes can differentiate into macrophages, which are immune cells that engulf and digest pathogens, as well as dead or damaged cells. In peripheral smears of COVID-19 patients, monocytes may show changes such as an increase in the number of circulating monocytes, which may indicate an ongoing immune response to the infection [16]. Some COVID-19 patients may have monocyte-derived macrophages that show changes in morphology, such as increased vacuolisation or cytoplasmic basophilia [17].

Platelet morphology in COVID-19 patients can show a range of abnormalities, including decreased platelet counts, large platelets, giant platelets, and platelet clumping. Some studies have reported an increased incidence of platelet aggregates, which are clusters of platelets that can lead to thrombosis (blood clots) and potentially serious complications [17].

Different studies have shown different morphology of WBC as can be seen in [Table/Fig-6] [18-21]. A study by Berber I et al., stated that pseudo-Pelger-Huet, pseudo Pelger-Huët/mature lymphocyte ratio, vacuolated monocytes, atypical lymphocytes, degenerating neutrophils, and band forms were higher in COVID-19 affected patients [20]. Present study also showed similar findings. According to Jain S et al., a study on 80 COVID-19 positive cases on peripheral smear findings, there were specific changes in the morphology of WBCs, especially neutrophils, lymphocytes, and monocytes [19]. The neutrophil series showed myeloid left shift, Dohle bodies, pseudo Pelger-Huët neutrophils, and monolobated neutrophils, smudged neutrophils, ring-shaped neutrophils, apoptotic neutrophils, and hypersegmented neutrophils. Present study showed similar findings, but there were no specific hypersegmented neutrophils noted except

in two cases where the patient had B12 deficiency. In their study, most of the lymphocytes were reactive lymphocytes, plasmacytoid lymphocytes, large granular lymphocytes, and atypical lymphocytes, which were same as present study. Monocyte vacuolisation and vacuoles in lymphocytes were seen in their study, whereas present study showed only reactive monocytes with multiple vacuoles and no vacuoles were observed in lymphocytes. Toxic changes in neutrophils and large and giant platelets were observed in both studies. Present study also showed similar findings to Pezeshki A et al., and Singh A et al., [18,21] studies.

Present study	Pezeshki A et al., 2021, Isfahan hospital Iran [18]	Jain S et al., 2022 [19] New Delhi, ABVIMS and RML hospital	Berber I et al., 2021, Inonu University [20]	Singh A et al., 2020 Ludhiana, India [21]
Neutrophils				
C shaped foetus like nuclei	-	-	-	C shaped foetus like nuclei
Pelger-Huët anomaly	-	Pelger-Huët anomaly	-	-
Pseudo Pelger-Huët anomaly	-	Pseudo Pelger-Huët anomaly	Pseudo Pelger-Huët anomaly	-
Apoptotic neutrophils	-	Apoptotic neutrophils	-	Apoptotic neutrophils
Dysplastic neutrophils	-	-	-	-
Myeloid shift left	-	Myeloid shift left	-	-
Lymphocytes				
Large granular lymphocyte	Large granular lymphocyte	Large granular lymphocyte	-	Large granular lymphocyte
Plasmacytoid lymphocytes	-	Plasmacytoid lymphocytes	Plasmacytoid lymphocytes	Plasmacytoid lymphocytes
Atypical lymphocytes	Atypical lymphocytes	Atypical lymphocytes	Atypical lymphocytes	Atypical lymphocytes
Monocytes				
Reactive monocytes with multiple vacuoles in cytoplasm	-	Reactive monocytes with multiple vacuoles in cytoplasm	Reactive monocytes with multiple vacuoles in cytoplasm	Reactive monocytes with multiple vacuoles in cytoplasm
Platelets				
Large and giant platelets	Giant platelets	-	-	Large and giant platelets
Smudge Cells	Smudge cells	-	Smudge cells	-
NRBC	NRBC	-	-	-
Other findings- Toxic granulations in neutrophils, fragmented RBC.	Schistocytes	- Ring shaped neutrophils - Dohle bodies - Hypersegmented neutrophils - Toxic changes in neutrophils		- Toxic granulations and vacuolations - Ring nuclei
[Table/Fig-6]: Comparison of findings on smears with most relevant and recent research [18-21]. NRBC: Nucleated red blood cell				

In the study conducted by Ahnach M et al., on peripheral smear findings in COVID-19, peculiar changes were found, such as eosinophils containing multiple vacuoles, giant platelets of different sizes, large lymphocytes, and neutrophils with hyposegmented and hypogranular cytoplasm [22]. However, in this study, no morphological changes were observed in eosinophils.

Neutrophilia (an increase in the number of neutrophils, a type of white blood cell) and lymphopenia (a decrease in the number of lymphocytes, another type of white blood cell) are among the most

commonly reported changes in COVID-19 patients. These changes in white blood cells can potentially indicate a severe inflammatory response to the infection [14]. Smudge cells, also known as basket cells, are lymphocytes that appear to have ruptured on peripheral blood smears. They are typically seen in various infections, including viral infections, and may indicate an abnormal immune response [23,24]. Reactive and atypical lymphocytes are lymphocytes that have undergone changes in response to an infection, which can be seen on peripheral blood smears. Large and giant platelets are also reported in some COVID-19 patients [25]. In this study, neutrophilia, lymphopenia, Pelger-Huët cells, smudge cells, reactive and atypical lymphocytes, and large and giant platelets were the major morphological changes observed in peripheral smears of COVID-19 patients.

Limitation(s)

Follow-up and post-COVID smears were not done. Severity of the disease was not assessed. As it was a single-centre study with a small sample size, large-scale studies are needed to identify peculiar findings and assess disease severity based on morphological findings.

CONCLUSION(S)

CBC can provide valuable information about a patient's immune response and help monitor disease progression in COVID-19 patients. Neutrophilia and lymphopenia have been reported in COVID-19 patients and may indicate the severity of the disease. Other morphological changes, such as Pelger-Huët cells, pseudo Pelger-Huët cells, smudge cells, reactive and atypical lymphocytes, and large and giant platelets, may also be indicative of COVID-19 infection and can potentially be used for early diagnosis.

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