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Haematological and morphological alterations in peripheral blood cells of patients with COVID -19 infections

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Abstract

Introduction: *COVID-19 infection is a highly communicable disease caused due to Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is responsible for a widespread pandemic with millions of confirmed cases and numerous deaths, which are reported in almost all countries. Peripheral blood smears shows changes in the morphology and count of white blood cells (WBCs), as well as abnormalities of platelets. The most common hematological abnormalities seen in these patients include decreased lymphocytes, increased neutrophils, low eosinophils, as well as changes in the platelets.*

Aims: *The objective of this study was to evaluate changes in blood smears of COVID-19 patients which can help in the diagnosis and to a certain extent in the patient's prognosis.*

Materials and Methods: *This study was performed on 52 patients diagnosed with COVID- 19 infection in district hospital Udhampur between April 2021 to June 2021. Blood samples were collected from the patients and examined for the estimation of hematological parameters and blood cell morphology.*

Results: *A total of 52 patients who were diagnosed with COVID-19 infection were enrolled in the study.WBC count was normal in 59.6% of the patients and leukocytosis was observed in 38.4%cases. In addition, differential WBC count indicated that 30.7% of cases had neutrophilia. Lymphopenia was found in 21.1% and 61.5 % had a low eosinophil count. The most notable morphologic changes were neutrophils with clumped chromatin, multiple abnormal nuclear shapes, pseudo-Pelger-Huet deformity, and smudged neutrophils'-shaped, fetus-like nuclei were noted with aberrant nuclear projections referred to as COVID nuclei. Atypical lymphocytes with deeply basophilic cytoplasm were seen in 19.8% of the cases. Activated monocytes were observed that demonstrated marked anisocytosis with significant cytoplasmic vacuolization and few granules.*

Conclusion: *The understanding of the hematological manifestations of SARS-CoV-2 is still evolving. This research identifies and describes a summary of morphological changes in peripheral blood cells of patients suffering fromCOVID-19. Knowledge of such morphologic changes in peripheral blood of affected leucocytes, if substantiated with larger studies, may help physicians diagnose in the absence of a negative RT-PCR or antibody results.*

Introduction

COVID-19 infection is a highly communicable disease caused due to Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It originated in the Chinese town of Wuhan and has attained pandemic status in just a few months. Recognized for the first time in December 2019, it was declared global pandemic by the World Health Organization (WHO) on 11 March 2020 as it has rapidly spread worldwide. So far it has affected about 300 countries globally, leading to significant morbidity and mortality. The novel corona virus is responsible for a widespread pandemic with millions of confirmed cases and numerous deaths, which are reported in almost all countries[1]. It is primarily transferred through droplets and contact surfaces. The Covid-19 virus is predominantly respiratory infection and is responsible for a series of clinical manifestations, some of which show the subclinical course, while others are related to multisystem manifestations.

It istypically diagnosed by a nasopharyngeal swab with the help of reverse transcriptase polymerase chain reaction (RT-PCR). Real time RT-PCR (reverse transcriptase polymerase chain reaction) has been considered as the gold standard diagnostic test in diagnosing Covid-19 infection, but research is still underway to determine the prognostic effectiveness of different tests that diagnose Covid-19 and can be useful for predicting patient's outcome . The clinical severity of this illness has been correlated with several diagnostic modalities such as radiography, lung function test, SPO2 etc.

Numerous studies on the role of D-dimer and fibrinogen levels along with Complete blood count (CBC) in early diagnosis of COVID infection have been done. However, very few studies have been done to study the peripheral blood findings, in Covid-19 patients.

There have been claims of virus infecting hemoglobin levels, peripheral blood lymphocytes and monocytes count[2]. The literature is full of quantitative hematological parameter's correlating with clinical findings where studies prove CBC as an indicator of Covid-19 progression. Peripheral blood smears show changes in the morphology and count of white blood cells (WBCs), as well as abnormalities of platelets. The most common hematological abnormalities seen in these patients include decreased lymphocytes, increased neutrophils, low eosinophils, as well as changes in the platelets. The patients may also have mild thrombocytopenia, while the significant decrease in platelet count is relatively rare[1,3]. The morphological changes in the cells like atypical and reactive lymphocyte, inclusion bodies, vacuolization are also observed. The monocytes with vacuoles are also increased. Pyknosis and karyorrhexis are known to be phases of cell death and appear with roundness and opacity of nuclei of infected cells in response to the cytopathic effect of viral infection. Their numbers can increase in infections and bleeding. Two or single lobed neutrophils called pseudo-Pelger-Huet abnormality having defects of lobulation or maturation are also observed.

The objective of this study was to evaluate changes in blood smears of COVID-19patients which can help in the diagnosis and to a certain extent in the patient's prognosis.

Material and Methods

This study was performed on 52 patients diagnosed with COVID- 19 infection in district hospital Udhampur between April 2021 to June 2021. Blood samples were collected from the patients and examined for the estimation of hematological parameters and blood cell morphology. We have examined 52 Romanowsky stained smears of Covid-19 cases to study blood cell morphology to emphasize the significance of peripheral blood smear examination in Covid-19.Peripheral blood smears of the patients were prepared from blood sample drawn in an EDTA tube and stained with Leishman's stain.

Blood films were examined at low magnification ($\times$ 10 objective), and WBC differential count was carried out manually after reading 100 cells at a

power of 40X. In the cases with abnormal WBC counts, up to 200 cells were counted.

According to World Health Organization (WHO) recommendations, anemia is diagnosed if the Hb levels are less than 12.0 g/dL among women (normal: 12 - 15 g/L) and less than 13.0 g/L among men (normal: 13 - 17 g/L).

Leukocytosis is defined as the total WBC count greater than 11×10^9/L, and leucopenia is defined as the total WBC count less than 3×10^9/L.

Neutropenia is defined as the total WBC count less than 1.5×10^9/L, and neutrophilia is defined as the absolute neutrophil count greater than 7.5×1^9/L.

Lymphopenia is defined as the absolute lymphocyte count below 1.0×10^9/L, and lymphocytosis is defined as the absolute lymphocyte count above 3.5×1^9/L.

Monocytosis is defined as the absolute monocyte count above 1.0×1^9/l, and monocytopenia is defined as the absolute monocyte count below 0.2×1^9/L.

The normal range of eosinophils is $0.02 - 0.5 \times 11^9$/L , and eosinophilia means count of $> 0.5 \times 1^9$/L.

Thrombocytopenia is defined as the number of platelets less than the lower limit of normal ($< 150 \times 10^9$/L), and thrombocytosis refers to the number of platelets greater than the upper limit of normal ($> 450 \times 10^9$/L)[4-9].

Lymphocytes split into two groups; mature lymphocytes and atypical or reactive lymphocytes, which have an eccentric nucleus, dark basophilic cytoplasm and pale sunflower region adjacent to the nucleus.

Neutrophils were divided into three subgroups; (I) the nuclei composed of 3–4 segments connected with thin chromatin as segmented neutrophils, (II) single-lobe or bilobed neutrophils as a pseudo-Pelger-Huet anomaly (III) cell size ranging from 10 to 16 microns with horseshoe-shaped undivided nuclei resembling the C, U, S letters as bands (club, stab).

The cells with a nucleus having features like loose chromatin network, curved, resembling kidney and located in the middle or edge of the cell, with gray-blue colored cytoplasm, and thin granules were evaluated monocytes.

Monocytes containing vacuoles in their cytoplasm were reported as monocytes with vacuoles.

Cells with bilobed nuclei and large pink granules in their cytoplasm were reported as eosinophils.

Pyknosis refers to the shrinkage of the cell nucleus. Karyolysis involves melting of nucleus chromatin with enzymes released by lysosomes of dead cells. Karyorrhexis is the rupture of the nuclear membrane, chromatin division into small basophilic granules, and spreading into the cytoplasm.

Results

A total of 52 patients who were diagnosed with COVID-19 infection with the mean age of 49.2 years were enrolled in the study. Males comprised 73.1% of the sample population, and the majority having normal Hb concentrations (Table/Figure 1). WBC count was normal in 59.6% of the patients and leukocytosis was observed in 38.4%cases. 86.5 % had a normal platelet count and thrombocytopenia was reported in 11.5%. Changes observed in RBC morphology were primarily nonspecific that could not be correlated to the viral etiology. Normocytic normochromic blood picture was observed in most of the cases i.e. 67.3%patients. Certain smears showed occasional polychromatophils, target cells and nRBCs.

Table/Figure 1. Characteristics of Patients and associated Blood Parameters

| Variables(n=52)	No.	%
Gender		
Male	38	73.1
Female	14	26.9
Hemoglobin		
Normal	28	53.8
Low	24	46.1
MCV		
Normocytic	37	71.1
Microcytic	9	17.3
Macrocytic	6	11.5
WBC		
Normal	31	59.6
Leucocytosis	20	38.4
Lymphopenia	1	1.9
Platelets		
Normal	45	86.5
Thrombocytosis	1	1.9
Thrombocytopenia	6	11.5
Peripheral Blood Picture (PBF)		
Normocytic normochromic	35	67.3
Microcytic hypochromic	6	11.5
Dimorphic	11	21.1

In addition, differential WBC count indicated that 65.3% of the patients had normal neutrophils while 30.7% of cases reported neutrophilia. Lymphopenia was found in 21.1% and 61.5 % had a low eosinophil count (Table 2/Figure 2).

Table /Figure 2. Differential Cell Count of Patients

Variables (n=52)	No.	%
Neutrophils		
Normal	34	65.3
Neutrophilia	16	30.7
Neutropenia	2	3.8
Lymphocytes		
Normal	38	73.1
Lymphocytosis	3	5.7
Lymphopenia	11	21.1
Monocytes		
Normal	46	88.4
Monocytopenia	2	3.8
Monocytosis	4	7.6
Eosinophils		
Normal	18	34.6
Eosinophilia	2	3.8
Low Eosinophil	32	61.5

The most notable morphologic changes were neutrophils with clumped chromatin, multiple abnormal nuclear shapes, pseudo-Pelger-Huet deformity, and smudged neutrophils. Neutrophils showed heavily clumped chromatin with toxic granules and cytoplasmic vacuoles. C-shaped, fetus-like nuclei were noted with aberrant nuclear projections referred to as COVID nuclei (Table/Figure 3).In addition, neutrophilic left shift with bands, metamyelocytes, and myelocytes was observed in the smears of patients with neutrophilia.
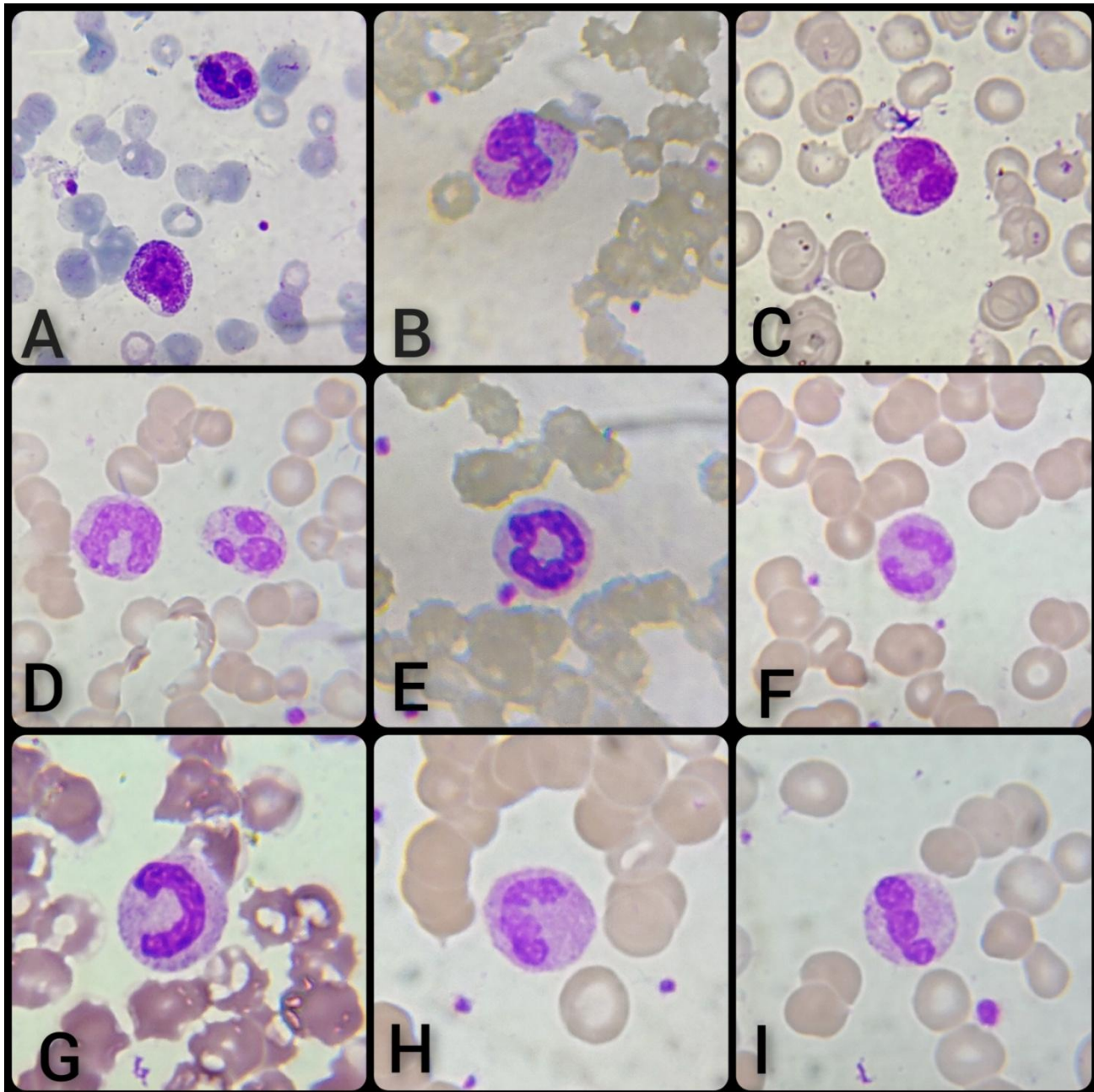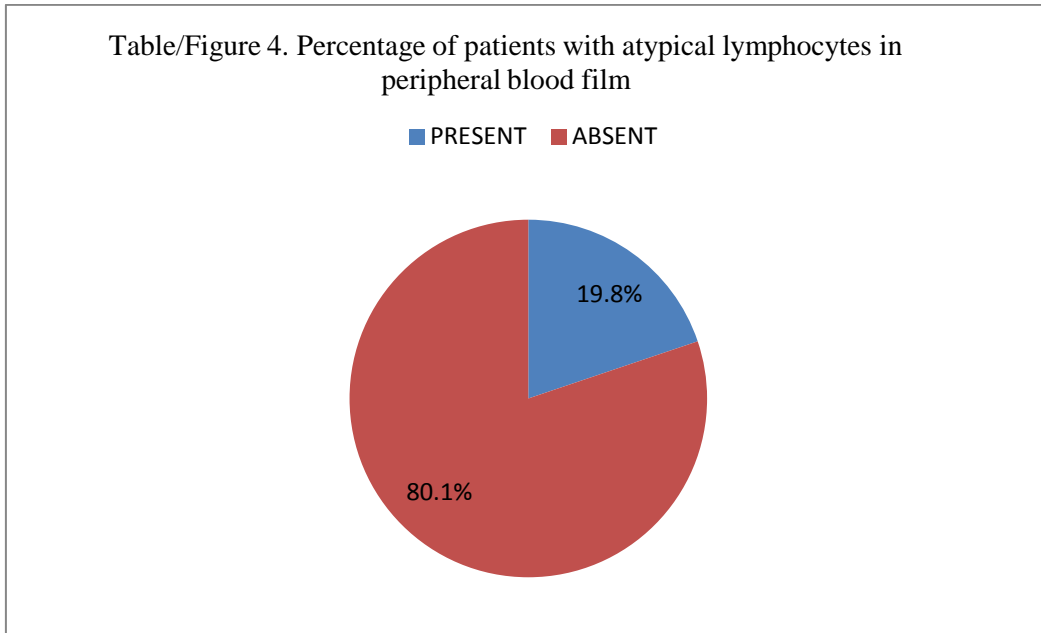
Table/Figure 3: Left shifted neutrophils with clumped chromatin and toxic granulations (A), with pseudo-Pelger-Huet deformity (B and C), with other abnormal shapes (D, F, and G) including ringneutrophils (E), and a fetus shaped nuclear deformity (H,I). (Original magnification ×1000 oil immersion {A through I}.)
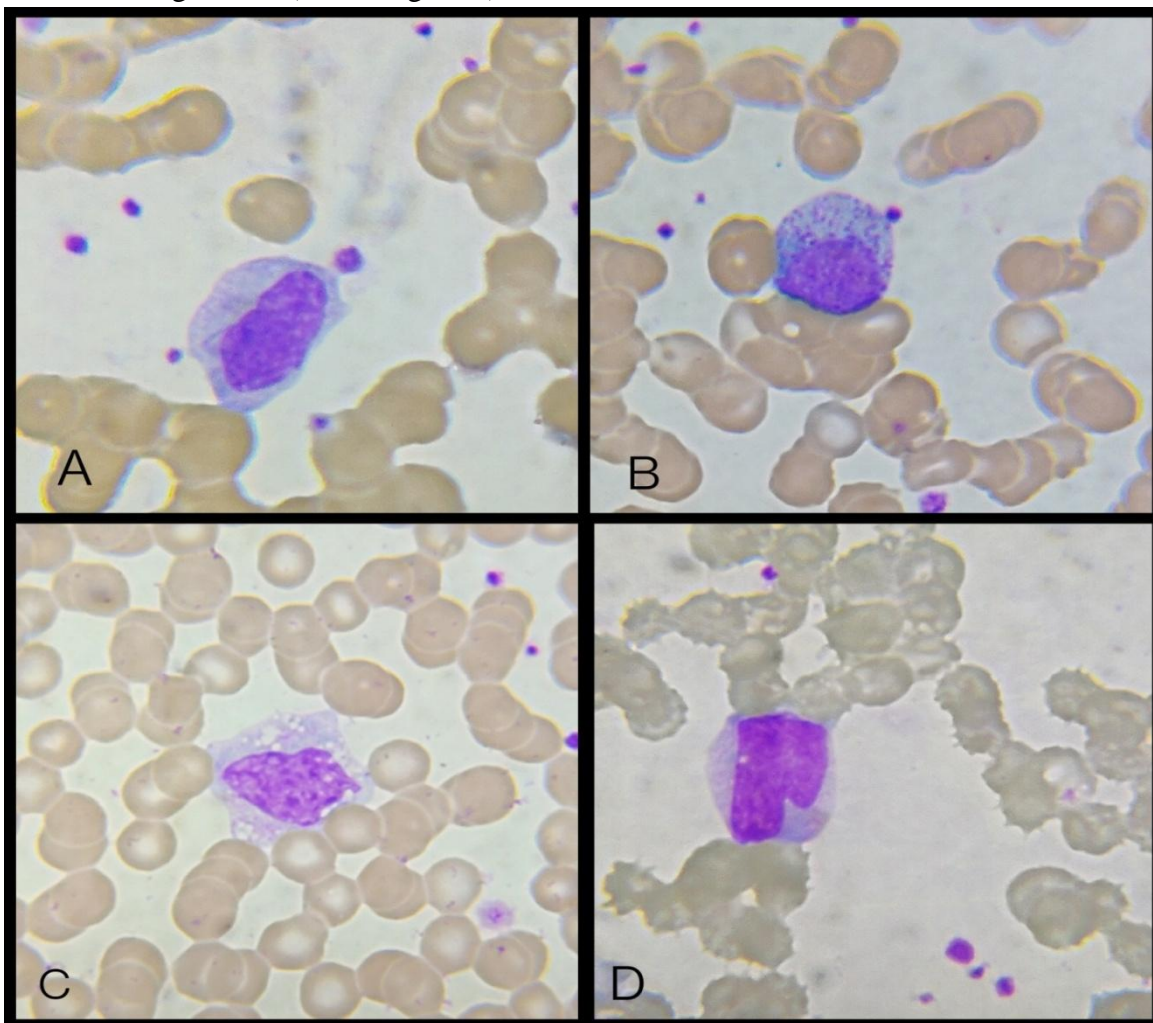
Lymphocytes showed abundant blue cytoplasm and/or lymphoplasmacytoid morphology. Most of the lymphocytes were seen as large granular lymphocytes with round to indented nuclei, condensed chromatin, prominent nucleoli in some, along with abundant pale blue cytoplasm with distinct variably sized azurophilic granules (Table/Figure 5).

The peripheral blood smear of the patients with COVID-19 infection revealed atypical lymphocytes with deeply basophilic cytoplasm in 19.8% of the cases (Table/Figure 4).

Table/Figure 4. Percentage of patients with atypical lymphocytes in peripheral blood film

■ PRESENT ■ ABSENT

19.8%

80.1%

Activated monocytes were observed that demonstrated marked anisocytosis with significant cytoplasmic vacuolisation and few granules (Table/Figure 5).

Table/Figure 5: Lymphocytes with abundant pale to dark blue cytoplasm (A) and with lymphoplasmacytoid features (B). Activated monocytes with abnormal shapes and cytoplasmic vacuolization(C,D). (Original magnification ×1000 oil immersion)

Thus, variety of morphological changes in all the cell lines examined in the peripheral blood smear noted and whether all these changes are attributable to the virus that infects them or are secondary to pathogenesis of Covid-19, has to be assessed through larger studies.

Discussion

There are many haematological abnormalities in patients diagnosed with COVID-19 infection, which are found in the measurement of blood parameters and blood film examinations. In this study, anemia was reported in 46.1% of the cases. Some studies have suggested that anemia in such patients can be due autoimmune origin although this has not yet been proven[1,10]. Leucocytosis was observed in 38.4% of the patients while neutrophilia was reported in 30.7% cases. Neutrophil count has been reported to increase within a few days after infection[3]. Most of the patients (61.5%) in our study had a low number of eosinophils.

Our study found a number of morphological changes in peripheral blood cells in COVID-19 patients. Neutrophils showed heavily clumped chromatin with toxic granules, cytoplasmic vacuoles abnormal nuclear shapes such as fetus-shaped nuclei, pi-shaped nuclei, C and donut-shaped nuclei, and cells with aberrant nuclear projections. The pseudo-Pelger-Huet deformity was also identified.

Several abnormal shaped lymphocytes, such as atypical lymphocytes with deeply stained basophilic cytoplasm, lymphocytes with spreading cytoplasm, and lymphocytes with plasma cytoid features were observed in our study. Detecting plasmacytoid lymphocytes in the peripheral blood film can support the diagnosis of COVID-19 infection[11]. Activated monocytes with abnormal shapes and cytoplasmic vacuolization were also seen.

The cells we examined in peripheral blood smear were morphologically similar to the those reported in the literature. Few authors have described in detail the morphologic anomalies of leukocytes of affected patients[12,13-17]. Zini et al. described similar changes in their study in neutrophils, lymphocytes, and platelets[13]. Singh et al. described similar characteristics in neutrophils, lymphocytes, and monocytes in a case report. The author also suggested that activated monocytes can indicate an improvement in the patient's clinical condition[14]. Zhang et al. described that monocytes express ACE-2 receptors and are directly affected by COVID-19 resulting in monocytosis and the presence of large, atypical, vacuolated monocytes[12].

In the present study, 86.5% of the patients had a normal platelet count, and the rate of thrombocytopenia was estimated to be 11.5%. The cause for thrombocytopenia is multifactorial, and it can be caused by the activation and platelet aggregation due to hypercoagulability and microthrombi formation, which has been reported in some cases[1,11,18].

Conclusion

The understanding of the haematological manifestations of SARS-CoV-2 is still evolving. To reach a definite conclusion as to the specificity and reliability of these viral cytopathic effects in peripheral blood smear, more COVID 19 patients should be evaluated in larger studies. This research identifies and describes a summary of morphological changes in peripheral blood cells of patients suffering fromCOVID-19. Knowledge of such morphologic changes in peripheral blood of affected leucocytes, if substantiated with larger studies, may help physicians diagnose in the absence of RT-PCR or antibody results. Neutrophilia and lymphopenia with the presence of atypical lymphocytes have been widely reported in these patients. Low eosinophil count is reported very frequently. Inspite of the changes in the platelets, they were either normal or reduced in majority of the cases.

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