

Original Research Article

Changes in morphology of white blood cells on peripheral smear in COVID-19 infection

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ABSTRACT

Background: COVID-19 is an infectious disease caused by a newly discovered coronavirus, and has spread around the world in a deadly pandemic. The first case of COVID-19 was reported from Wuhan, China in December 2019. This is also called as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) because of its homology with SARS virus. The most common hematological manifestation of coronavirus is lymphopenia which is due to depletion of lymphocytes by coronavirus infection. Other manifestations are neutrophilia and mild thrombocytopenia. Literature is full of quantitative hematological parameters but the researches on morphology of white blood cells is still ongoing. We at our institute done study on 60 confirmed positive cases of COVID-19, and analyzed those peripheral smears in terms of morphology of white blood cells.

Methods: The study was done using peripheral smear staining with methylene blue stain and was screened for various changes in white blood cells in peripheral smear.

Results: Changes in the white blood cells were examined in the peripheral smear and findings were made in the tabular form.

Conclusions: To conclude that all these changes are due to the virus infecting them or are secondary to pathogenesis of COVID disease, needs to be evaluated by larger studies.

Keywords: COVID-19, Neutrophilia, Blebbing, Lymphopenia, Toxic granules, Vacuolation

INTRODUCTION

COVID-19 is a highly contagious disease caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). It originated from the Chinese city of Wuhan and has reached the pandemic status in just a few months.¹ It has now affected around 300 countries worldwide, causing significant morbidity and mortality. The gold standard test is real-time reverse transcription polymerase chain reaction (RT-PCR) in diagnosis of COVID-19 infection. There are many hematological tests done for COVID-19 infection like complete blood count, fibrinogen,

prothrombin time (PT), activated partial thromboplastin time (APTT), D dimer etc., however there is less content in literature for the study of morphology of white blood cells in the peripheral smear of COVID-19 patients. We at our institute done study of 60 cases of peripheral smear of COVID-19. Neutrophilia, lymphocytopenia, mild thrombocytopenia are the common hematological findings present in hematological finding of COVID-19. 60 peripheral smear of COVID 19 positive patients were screened by us, and there was large range of morphological changes were seen in the white blood cells. Objective of our study is to affirm that this virus infects the white blood

cells in the blood that is neutrophils, lymphocytes, eosinophils and monocytes.

METHODS

This was the observational study conducted at Sharda hospital, Greater Noida. The duration of study was 6 months (20 November–20 May). All patients who were positive for COVID-19 on reverse transcriptase were selected for the study. The goal was to randomly identify and analyze data from at least 60 hospitalized patients. Patient blood was collected in ethylenediamine tetraacetic acid (EDTA) vials. The sample was first run on 6 part automated analyzer. Peripheral smear was prepared for the complete blood count (CBC) report showing leucocytosis. It was made by using the Leishmann stain. The smears were air dried and then methylene blue was put for 10 to 15 minutes. Then the slide was washed with distilled water and air dried and was viewed under the microscope.

RESULTS

60 patients were enrolled in the study. Their demographic details are given in Table 1.

Table 1: Demographic details.

Parameters	Range
Number of patients	60
Age range	<1 year to 85 years
Number of patients by parameter	
Pediatric	12 out of 60 (20%)
Gender	
Male	41 (68.3%)
Female	19 (31.6%)

Table 2: Percentage of various changes in WBC in peripheral smear in COVID-19 infection.

Changes in white blood cells	Percentage involved (out of 60)
Neutrophils	
Toxic granules	76.6 (46 cases)
Hypersegmented neutrophils	50 (30 cases) ⁵
Pseudopelger huet anomaly	43.3 (26 cases)
Dyspoietic forms	30 (18 cases) ³⁻⁴
C shaped or fetal shaped nuclei	28.3 (17 cases) ⁵
Ring forms	26.6 (16 cases) ⁵
Nuclear projections	16.6 (10 cases)
Hypogranular cytoplasm	6.66 (4 cases) ⁵
Hypolabated neutrophil	3.3 (2 cases) ⁵
Lymphocytes	
Blebbing	40 (24 cases)
Monocytes	
Cytoplasmic vacuolation	20 (12 cases)
Eosinophils	
Trilobed eosinophil	5 (3 cases)

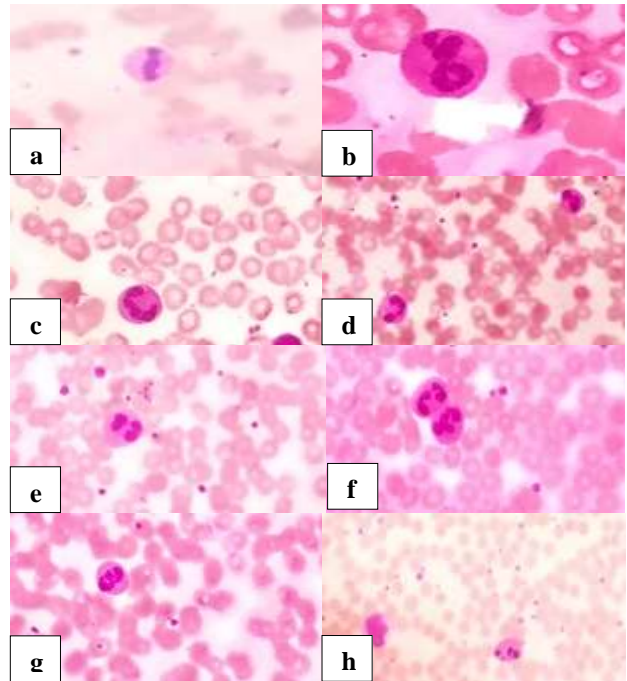


Figure 1: (a) Hyposegmented neutrophils, (b) ring shaped neutrophils, (c) C shaped neutrophil, (d) arrow head–hypogranular cytoplasm, arrow–hyposegmented neutrophil, (e) pseudopelger huet anomaly, (f) aberrant nuclear projections, (g) double nuclear rings, and (h) apoptotic nuclei of neutrophils.

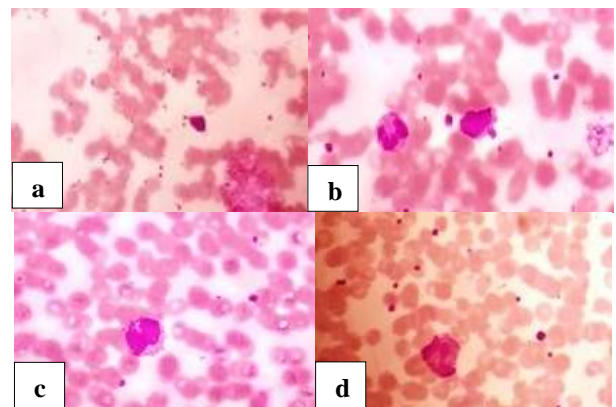


Figure 2: (a) and (b) Cytoplasmic blebbing, (c) monocyte vacuolation, and (d) trilobed eosinophil.

The age of patients vary widely ranging from less than one year to 85 years. Pediatric patients were also involved. Majority were males that is 68.3% and rest were females.

Results were analyzed by viewing the slides under the microscope and evaluating the morphology of various white blood cells separately. The most common changes in CBC were neutrophilia followed by lymphopenia, eosinopenia and mild thrombocytopenia.¹⁻²

Out of 60 cases 48 (80%) showed neutrophilic leucocytosis. Morphological changes are described in the Table 2.

46 cases shows toxic granules. Pseudopelger huet anomaly seen in 26 cases. Dyspoietic forms were seen in 18 cases.

Platelet count was normal in maximum patients with few patients showing thrombocytopenia.

The red blood cells were predominantly normocytic normochromic.

DISCUSSION

Understanding the hematological manifestations of COVID-19 is still in developing stage. Studies are very less in evaluating the morphological findings of white blood cells in COVID-19 infection.

In our study, majority of cases were males as concluded by Bwire that generally, females are more resistant to infections than men, and this is possibly mediated by several factors including sex hormones and high expression of coronavirus receptors, angiotensin converting enzyme (ACE 2) in men but also life style, such as higher levels of smoking and drinking among men as compared to women. Additionally, women have more responsible attitude toward the COVID-19 pandemic than men.⁹

Neutrophilia was the most consistent finding among the CBC parameters in our study. This finding was also present in the study done by Abhishek et al and Savitri et al.^{5,6} Increased neutrophil lymphocyte ratio is associated with worse prognosis in COVID-19 infection. This is the finding which is confirmed by many studies like Mehr et al, and Elnaz et al.^{7,8} Among the neutrophil series the variations were; dyspoietic neutrophils, ring shaped neutrophils, fetal or C shaped nuclei of neutrophils, hypogranular cytoplasm, hyposegmented neutrophils, toxic granules, aberrant nuclear projections. These findings were in accordance with the findings evaluated by Abhishek et al.⁶ Additional findings which we noted were pseudopelger huet anomaly, hypersegmented neutrophil. All these changes were also described by similar studies.¹¹⁻¹²

Most predominant findings among lymphocytes was lymphopenia. Several hypotheses exist for the cause of lymphopenia, including direct viral toxicity due to ACE-2 receptor expression and cytokine-induced lymphopenia.¹⁰ Lymphocytes were showing blebbing in our study.

Monocytes show vacuolation in our study which was the observation also studied by Abhishek et al.⁵ Zhang et al described that monocytes express ACE-2 receptors and are directly affected by COVID-19 leading to monocytosis and the presence of large, atypical, vacuolated monocytes in circulation.¹³

A finding unique to our study was the presence of eosinophil show three lobes which was the observation present only in our study.

Limitations

It needs more studies to evaluate whether all these morphological changes are due to virus infecting the white blood cells or they are secondary to pathogenesis of COVID disease.

CONCLUSION

In conclusion, our study identifies and describes a summary of morphologic changes in the peripheral blood cells of COVID-19 patients. Although this is a pilot study with a small sample, it is a step towards understanding the hematological manifestations of COVID-19. Knowledge of these constellations of morphologic changes in peripheral blood, if substantiated with larger studies, may help physicians diagnose COVID-19 in the absence of a negative RT-PCR/antigen test.

In addition, some authors have already suggested an association between morphological changes in CBC and disease progression/outcome. If validated by larger studies, serial CBC and blood smear review in hospitalized patients may become an essential tool to help clinicians assign patients to a higher risk category based on morphologic findings and take management decisions accordingly.

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REFERENCES

1. Tiwari N, Krishnan NR, Singh S, Madan J, Nath D, Tripathi S, et al. COVID 19 and pancytopenia: A single case experience of a pediatrics Institute. *Saudi J Pathol Microbiol.* 2020;5(5):308-10.
2. Thomas L. Does COVID-19 infect peripheral blood cells? Available at: <https://www.newsmedical.net/news/20200519/Does-COVID-19-infect-peripheral-blood-cells.aspx>. Accessed on 15 February 2021.
3. Mitra A, Dwyre DM, Schivo M, Thompson GR, Cohen SH, Ku N, Graff JP. Leukoerythroblastic reaction in a patient with COVID-19 infection. *Am J Hematol.* 2020;95(8):999-1000.
4. Zini G, Bellesi S, Ramundo F, d'Onofrio G. Morphological anomalies of circulating blood cells in COVID-19. *Am J Hematol.* 2020;95:870-2.
5. Singh A, Sood N, Narang V, Goyal A. Morphology of COVID-19-affected cells in peripheral blood film. *BMJ Case Rep.* 2020;13:e236117.

6. Singh S, Madan J, Nath D, Tiwari N. Peripheral Blood Smear Morphology- A Red Flag in COVID-19. *Int J Trop Dis Health.* 2020;41(8):54-8.
7. Imran MM, Ahmad U, Usman U, Ali M, Shaukat A, Gul N. Neutrophil/lymphocyte ratio-A marker of COVID-19 pneumonia severity. *Int J Clin Path.* 2021;75(4).
8. Vafadar Moradi E, Teimouri A, Rezaee R, Morovatdar N, Foroughian M, Layegh P, et al. Increased age, neutrophil-to-lymphocyte ratio (NLR) and white blood cells count are associated with higher COVID-19 mortality. *Am J Emerg Med.* 2021;40:11-4.
9. Bwire GM. Coronavirus: Why Men are More Vulnerable to Covid-19 Than Women? *SN Compr Clin Med.* 2020:1-3.
10. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, Kastritis E, Sergentanis TN, Politou M, et al. Hematological findings and complications of COVID-19. *Am J Hematol.* 2020;95(7):834-47.
11. Nazarullah A, Liang C, Villarreal A, Higgins RA, Mais DD. Peripheral blood examination findings in SARS-CoV-2 infection. *Am J Clin Pathol.* 2020;154:319-29.
12. Pozdnyakova O, Connell NT, Battinelli EM, Connors JM, Fell G, Kim AS: Clinical significance of CBC and WBC morphology in the diagnosis and clinical course of COVID-19 infection. *Am J Clin Pathol.* 2021;155:364-75.
13. Zhang D, Guo R, Lei L. COVID19 infection induces readily detectable morphological and inflammation related phenotypic changes in peripheral blood monocytes. *J Leukoc Biol.* 2021;109:13-22.

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