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 | 12 out of 60 (20%)         |
| Pediatric                   | 12 out of 60 (20%)         |
| Gender                      | 41 (68.3%)                 |
| 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)                  |
| Pseudopelger huet anomaly   | 43.3 (26 cases)                |
| Dyspoietic forms            | 30 (18 cases)                  |
| C shaped or fetal shaped nuclei | 28.3 (17 cases)                  |
| Ring forms                  | 26.6 (16 cases)                |
| Nuclear projections         | 16.6 (10 cases)                |
| Hypogranular cytoplasm      | 6.66 (4 cases)                 |
| Hypolabated neutrophil      | 3.3 (2 cases)                  |
| Lymphocytes                 |                                |
| Blebbing                    | 40 (24 cases)                  |
| Monocytes                   |                                |
| Cytoplasmic vacuolation     | 20 (12 cases)                  |
| Eosinophils                 |                                |
| Trilobed eosinophil         | 5 (3 cases)                    |

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. Dyspoeitic 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 Elnax et al.7,8 Among the neutrophil series the variations were; dyspoeitic 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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