



Original Article

Evaluation of Absolute Neutrophil Count in COVID-19 Patients in a Tertiary Care Hospital

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ABSTRACT

Background and objectives: Coronavirus disease 2019 (COVID-19) is a communicable disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The clinical manifestations of COVID-19 vary from asymptomatic to acute respiratory distress syndrome. In severely-ill patients, neutrophil count is significantly increased. This study aimed to evaluate absolute neutrophil count (ANC) in patients with COVID-19 and healthy individuals.

Methods: This retrospective, cross-sectional study was carried out on patients admitted to the outpatient department of OBGY Rural Hospital (Maharashtra, India) from April 2021 to June 2021. A peripheral venous blood sample (3 ml) was taken from 143 patients with COVID-19 and 143 healthy controls. The blood samples were analyzed using a fully automated blood cell counter for the estimation of complete blood count. A peripheral blood smear was prepared and stained with Leishman stain for evaluation of morphological changes.

Results: The ANC of COVID-19 patients was significantly higher than that of healthy control subjects ($p=0.00001$). Toxic granules (48.9%) were the most common morphological neutrophil abnormality, followed by hypolobation, hypogranulation, Pseudo-Pelger-Huet anomaly (20.9%), and shift to left with the presence of immature myeloid series cells.

Conclusion: High ANC along with morphological changes in neutrophils could be used as a simple and inexpensive surrogate marker of COVID-19 and its severity.

Keywords: [COVID-19 Testing](#), [Neutrophil](#), [Morphological and Microscopic Findings](#).

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which affects the lower respiratory tract. The clinical manifestations of the disease range from mild symptoms to severe illness, causing morbidity and mortality. Originating in Wuhan, China, COVID-19 rapidly spread across the world and turned into a pandemic, resulting in considerable human and economic loss (1, 2). Advanced age, male gender, and pre-existing comorbidities have been linked with increased disease severity (3-5). In severe patients, neutrophil count in blood is increased significantly. In COVID-19, hyper-inflammatory and immune thrombotic responses cause lung damage, thrombosis, fibrosis, and severe pneumonia resulting in respiratory failure (6, 7). It has been reported that inflammation biomarkers including D-dimer, interleukin-6 (IL-6), ferritin, and C-reactive protein along with neutrophil-lymphocyte ratio (NLR) are elevated in the early stages of SARS-CoV-2 infection (8-12). This study aimed to evaluate absolute neutrophil count (ANC) and morphological changes of neutrophils including segmentation, pseudo-Pelger-Huët (PHA), hypoblobation, pyknotic nuclei, abnormal granularity, and cytoplasmic vacuolation in peripheral blood smears of COVID-19 patients.

MATERIALS AND METHODS

This retrospective, cross-sectional study was carried out on patients admitted to the outpatient department of OBGY Rural Hospital (Maharashtra, India) from April 2021

to June 2021. The minimum sample size was estimated at 144 according to the following formula and considering a prevalence rate of 40% (8), 95% confidence interval, and 10% error:

$$N = (S.D)^2 \times Z\alpha^2 (\text{Diff})^2.$$

Inclusion criteria were having a positive reverse transcription polymerase chain reaction (RT-PCR) test for COVID-19 and willingness to participate in the study. Those aged less than 18 years and with pre-existing renal or vascular diseases, seizure disorders, severe anemia, and liver disease were excluded from the study. Overall, 143 patients with COVID-19 and 143 healthy subjects (with a negative RT-PCR for COVID-19) were enrolled in the study. Written informed consent was taken from all subjects. Ethics approval was also obtained from the Institutional Ethics Committee (Approval code: no.IEC/MIMER/2022/784).

A peripheral venous blood sample (3 ml) was taken from all subjects under aseptic precautions. The blood samples were collected in EDTA vacutainers and then transferred to the laboratory. The samples were analyzed using a fully automated blood cell counter for the estimation of complete blood count. A peripheral blood smear was prepared and stained with Leishman stain.

Data were analyzed using the SPSS software (version 26) at a statistical significance of 0.05.

RESULTS

The age-wise distribution of COVID-19 patients is presented in [table 1](#). Most patients were in the age range of 61-75 years and 31-45 years.

Table 1- Age-wise distribution of COVID-19 patients

Age range (years)	Number of COVID-19 cases	Percent
16-30	24	16.7
31-45	40	28
46-60	30	21
61-75	44	30.7
76-90	05	3.49
Total	143	100

Table 2- Comparison of study variables between COVID-19 patients and healthy controls

Parameters	Patients with COVID-19 (n=143)	Healthy controls (n=143)	P-value
Mean age (years)	50.04	52.89	>0.05
Gender	Males (n=98)	Males (n=79)	>0.05
	Females (n=45)	Females (n=64)	
ANC/ μ l	10561.85 \pm 6865.41	3946.93 \pm 1502.69	0.00001

As shown in table 2, the ANC of COVID-19 patients was significantly higher than that of healthy control subjects ($p=0.00001$).

Table 3- shows the frequency of morphological abnormalities in neutrophils of COVID-19 patients. The most common neutrophil abnormality was toxic granules (Figure 1).

Morphology abnormalities	Number	Percent
Toxic granules	70	48.9
Pseudo-Pelger-Huet (Hypolobation)	30	20.9
Shift to left	55	38.4

Table 3 shows the frequency of morphological abnormalities in neutrophils of COVID-19 patients. The most common neutrophil abnormality was toxic granules (Figure 1).

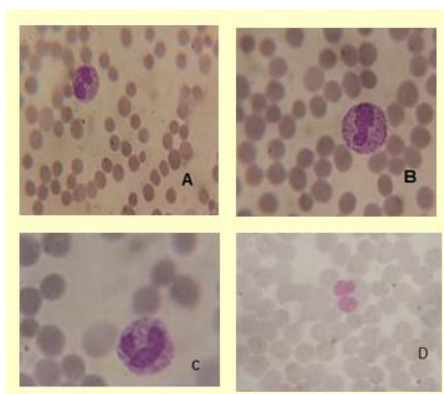


Figure 1-Microscopic images of neutrophil morphological changes. A) Hypolobated neutrophil; B and C) Toxic granules; D) Pseudo-Pelger-Huet anomaly and hypogranulation of neutrophil. All images were taken under 400X magnification.

DISCUSSION

Neutrophils are the predominant immune cells that protect the human body against bacterial, fungal, and viral infections (13). Activation of neutrophils leads to the release of neutrophil extracellular traps (NETs), which are a viscous network of nuclear chromatin, antimicrobial peptides, and enzymes that entrap microorganisms. Neutrophils produce NETs in response to inflammatory mediators and infectious pathogens, such as bacteria and fungi. During NETs formation, the nuclei of neutrophils are lost. The presence of NETs in the blood has also been reported during sepsis (14,15). The release of NETs disperses histones, DNA, and granule proteins, such as myeloperoxidase, neutrophil elastase, cathepsin G, and proteinase 3, which results in severe tissue destruction, setting up the auto-amplification loop of necrosis and

Lymphopenia in SARS-CoV-2 infection occurs due to the cytotoxic destruction of lymphocytes by cytokine storm and increased levels of IL-6, IL-8, lactic acid, and angiotensin-converting enzyme 2 on the surface of lymphocytes. This also contributes to the increased NLR ratio in COVID-19 and has a high prognostic value (18,21,25).

It has been reported that men and older individuals are at higher risk of contracting COVID-19 (16). The mean age of the patients in the present was 50.04 years, which is similar to the findings of some previous studies (16, 23).

neutrophil abnormality, followed by hypolobation, hypogranulation, PHA anomaly (20.9%), and shift to left with the presence of immature myeloid series cells. These findings In the present study, toxic

granules (48.9%) were the most common morphological are consistent with the findings of Kaur et al. (26) and Bhalchandra et al. (27).

CONCLUSION

Based on the results of our study, high ANC along with morphological changes in neutrophils could be used as a surrogate marker of COVID-19 and its severity.

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DECLARATIONS

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Ethics approvals and consent to participate

Written informed consent was taken from all subjects. Ethics approval was also obtained from the Institutional Ethics Committee (Approval code: no.IEC/MIMER/2022/784).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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