Original Article

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Pathology Section

White Blood Cells in COVID-19: A Study on Viral Induced Cytopathic Changes in the Peripheral Smear

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ABSTRACT

Introduction: Laboratory parameters are crucial in diagnosis and prognosis of Coronavirus Disease 2019 (COVID-19). It would be of interest to explore morphological changes in infected White Blood Cells (WBCs). A detailed examination of peripheral smears may shed light on pathophysiology of infected cell lines and differentiate them from those in established viral infections like dengue and infectious mononucleosis.

Aim: To study morphological changes of WBCs in peripheral smears of severe and non severe cases of COVID-19 patients.

Materials and Methods: This cross-sectional study was conducted at a tertiary care centre, Dr. Chandramma Dayananda Sagar Institute of Medical Education and Research, Ramanagara, Karnataka, India, from April 2021 to August 2021 on 120 peripheral smears of adult COVID-19 positive cases. Abnormal morphological features were graded by counting 100 cells in each of neutrophils, lymphocytes and monocyte lineage. Changes were compared and analysed between severe and non severe groups using Statistical Package for Social Sciences (SPSS) software version 26.0. A p-value <0.05 was considered as significant.

Results: The study included total of 120 cases (59 severe and 61 non severe) with a mean age of 47 years. Male to female ratio in severe and non severe categories were 1:1.2 and 1:0.6, respectively. Severe category patients (n=59) were associated with statistically significant leucocytosis (p-value=0.04), absolute neutrophilia (p-value=0.03) and higher grades of morphological changes-abnormal nuclear morphology (p-value=0.002) and Pseudo-Pelger-Huët anomaly in neutrophils (p-value=0.029), plasmacytoid lymphocytes (p-value=0.03), cytoplasmic granularity and atypical lymphocytes (p-value=0.04). Monocytes showed large coalescent vacuoles and cytoplasmic granules (p-value=0.03). Though present in non severe category (n=61), they were proportionately of lesser grades.

Conclusion: Viral cytopathic effects in WBC lines on peripheral smear had significant clinical implications on disease severity, undermining need for a comprehensive study of viral induced morphological changes in hospitalised COVID-19 patients.

Keywords: Coronavirus disease 2019, Infections, Leukocytes, Microscopy, Neutrophils

INTRODUCTION

Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), the causative agent of Coronavirus Disease 2019 (COVID-19) was first reported in Wuhan, China in December 2019 [1]. Various theories have been postulated pertaining to its pathogenesis, of which Angiotensin Converting Enzyme (ACE) receptor pathway is the prime focus [2]. Derangement of the renin-angiotensin-aldosterone axis subsequent to invasion of leukocytes by the virus activates a series of events that leads to deleterious effects in the host [3]. Activation and dysregulation of the immune system has also been strongly implicated in the pathogenesis of this virus [4].

Laboratory medicine has played an indispensable role in early detection, diagnosis and management of COVID-19. Several biomarkers have been found to correlate with disease severity such as increased C-Reactive Protein, procalcitonin, lactate dehydrogenase, bilirubin, blood urea nitrogen, creatinine and cardiac troponin [5]. Interpretation of these parameters in light of clinical presentation is crucial in prognosis of COVID-19, as the clinical manifestations overlap with other viral infections like dengue and infectious mononucleosis [6].

A Complete Blood Count (CBC) is one of the most commonly ordered test in all viral illnesses. In COVID-19, low lymphocyte count was encountered frequently, which was attributed to the deficient immune response to the virus. While lymphopenia and other quantitative abnormalities like increased Neutrophil-Lymphocyte Ratio (NLR) have been well described in literature, little is known about the morphologic changes in circulating blood cells [4]. Even in this era of automation, study of microscopic morphology of affected cell lines remains an indispensable diagnostic tool to study disease pathophysiology.

Studies have reported changes in White Blood Cell (WBC) lines of affected COVID-19 cases on examining the peripheral smears. In addition, a few have provided correlation between smear changes and disease outcomes, contributing to understanding the dynamics of viral cytopathic effects and severity [7]. It would be of interest not only to study the WBC counts but also the morphologic changes in WBCs based on severity in COVID-19 patients, which was the aim of the present study. An understanding of these morphologic changes in addition to established haematologic parameters may help patient management decisions [4].

MATERIALS AND METHODS

This cross-sectional study was conducted in Haematology Section (Central Laboratory) at Dr. Chandramma Dayananda Sagar Institute of Medical Education and Research (tertiary care centre), Ramanagara, Karnataka, India, from April 2021 to August 2021 on 120 peripheral smears of adult COVID-19 positive cases. The study was approved by the Institutional Ethical Committee (Ethical clearance number-CDSIMER/MR/0024/IEC/2021).

Inclusion criteria: All Reverse Transcription-Polymerase Chain Reaction (RT-PCR)/Rapid Antigen Test (RAT) positive COVID-19 patients above the 18 years of age admitted to the hospital with CBC and peripheral smears being available were included in the study.

Exclusion criteria: Cases of known WBC disorders and other proven haematological malignancies were excluded from the study.

Procedure

Relevant patient details were obtained from the laboratory database. Cases satisfying inclusion criteria were grouped into severe and non severe categories as per standard national guidelines [8]. Mild and moderate categories were considered as non severe group. The severe category was retained as the severe group. CBC with WBC differential performed on the first day of admission for COVID-19 positive patients was included. Patient samples were analysed on Beckmann five-part haematology analyser. The parameters were standardised by routine external and internal quality control checks done as per our laboratory protocols.

White blood cells morphology was studied on Leishman-stained peripheral smears by two pathologists independently under oil immersion. The smears were made and stained within two hours of receiving the sample to avoid Ethylenediamine Tetra Acetic acid (EDTA) induced artefactual changes. Smears from healthy individuals were analysed and used as reference for comparison, to avoid over or under diagnosing the morphological findings. The abnormal morphological changes in neutrophils, lymphocytes and monocytes were studied [Table/Fig-1]. Independent scoring of WBC morphology in conjunction with percentage of cell lineage (%) showing these changes was done in neutrophils, lymphocytes and basophils according to the criteria followed by Pozdnyakova O et al., [7]:

Neutrophils	Lymphocytes	Monocytes
Abnormal Nuclear shapes - foetus shaped/Ring shaped/C shaped	Large granular lymphocytes	Granular monocytes
Pseudo-Pelger-Huët like cells	Plasmacytoid lymphocytes	Cytoplasmic vacoulations
Toxic granules and hypergranularity	Atypical lymphocytes	Nuclear blebbing
Hypogranularity	Cytoplasmic pod formation	
Cytoplasmic vacoulations	Apoptotic lymphocytes	
Döhle/Howel Jolly like bodies	Cytoplasmic vacoulations	
Myeloid left shift		

[Table/Fig-1]: Morphological changes observed in the white blood cell lines.

- Grade 0- No changes,
- Grade 1- changes in <10% of cells,
- Grade 2- changes in 11-25% of cells,
- Grade 3- >25% of cells.

A total of 100 cells in each lineage were studied for each morphological change and graded. Morphology of basophils and eosinophils though observed, were not graded in view of their low counts on smear. The significant (>1-point) discrepancies between the pathologists examining the smears were resolved by adjudication.

STATISTICAL ANALYSIS

The data was collected, coded and analysed using statistical software, Statistical Package for the Social Sciences (SPSS) version 26.0. Baseline demographic characteristics of the study subjects were explained in terms of frequency, percentage, mean and standard deviation. Pearson's Chi-square test was used to compare categorical variables and Student's t-test to compare quantitative variables between two groups. A p-value <0.05 was considered significant.

RESULTS

A total of 120 cases (59 severe and 61 non severe) were included in the study. Age range varied from 2nd to 8th decade, with a mean age of 47 years. Male to female ratio in severe and non severe categories were 1:1.2 and 1:0.6, respectively.

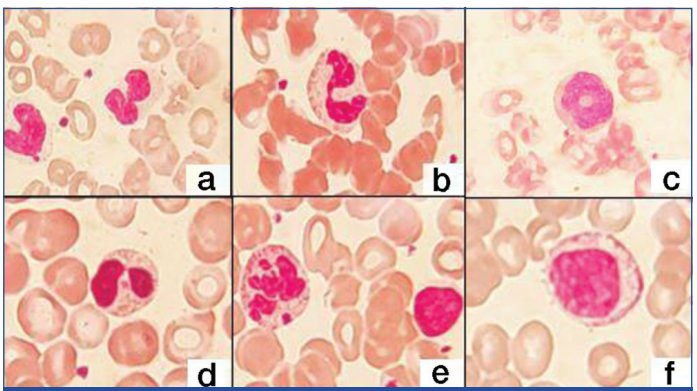
WBC quantitative parameters: Patients in the severe category were associated with statistically significant leucocytosis (p-value=0.04) and absolute neutrophilia (p-value=0.03). Though lymphopenia was seen in 61% of all cases, it was not found to be statistically significant. Absolute counts of other WBC cell lineages (monocytes, eosinophils, basophils and immature granulocytes) showed no significant association between categories.

WBC morphology: All cases showed morphological changes of WBC on peripheral smears listed in [Table/Fig-1]. However, a few of these changes were found to be of statistical significance between study groups. The table below shows the spectrum of morphological changes studied in neutrophils with grading of the cell lineages and association between study groups [Table/Fig-2]. In the neutrophils, abnormal nuclear morphology and Pseudo-Pelger-Huët anomaly were the significant changes noted [Table/Fig-3a-f]. The table below shows the spectrum of morphological changes studied in lymphocytes with grading of the cell lineages and association between the study groups [Table/Fig-4].

Plasmacytoid lymphocytes, cytoplasmic granularity and atypical lymphocytes were noted in lymphocytes which showed statistical significance between the groups [Table/Fig-5a-d]. The table below shows the spectrum of morphological changes studied in monocytes with grading of the cell lineages and association between the study groups [Table/Fig-6]. Monocytes showed large coalescent vacuoles and cytoplasmic granules, which was statistically significant between the study groups [Table/Fig-7a-c]. From the above results, it can be opined that individual morphological changes when graded, cases belonging to severe group displayed higher grade (grade 2,3).

Morphological changes in neutrophils																												
	Abnormal nuclear shapes-foetus shaped/C shaped				Pseudo-Pelger-Huët like cells				Toxic granules and hypergranularity				Hypogranularity				Cytoplasmic vacoulations				Döhle-like bodies				Myeloid left shift			
	Grade				Grade				Grade				Grade				Grade				Grade				Grade			
Clinical severity	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Non severe (n=61)	7	15	26	13	5	28	21	7	47	14	0	0	9	21	28	3	20	23	15	3	42	18	0	1	18	33	8	2
Severe (n=59)	0	6	27	26	1	16	34	8	48	11	0	0	6	27	18	8	16	23	18	2	44	14	1	0	18	29	11	1
p-value	**0.002***				**0.029***				0.56				0.12				0.83				0.47				0.79			

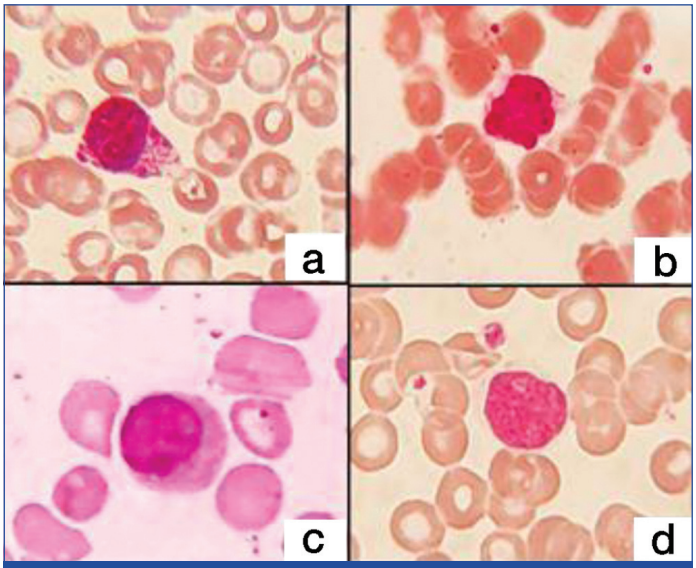
[Table/Fig-2]: Grading of viral cytopathic changes in neutrophils and comparison between the study groups.
*p-value <0.05 was considered significant



[Table/Fig-3]: a-c) Peripheral smear showing neutrophils with abnormal nuclear morphology like C- shaped nucleus, embryo-shaped nucleus and ring shaped nucleus respectively; d) Peripheral smear showing neutrophils with Pseudo-Pelger-Huët anomaly; e) Peripheral smear showing neutrophils with Döhle like bodies; f) Peripheral smear showing an immature granulocyte (1000X, Leishman stain).

Morphological changes in lymphocytes																								
	Large granular lymphocytes				Plasmacytoid lymphocytes				Atypical lymphocytes				Cytoplasmic pod formation				Apoptotic lymphocytes				Cytoplasmic vacoulations			
	Grade				Grade				Grade				Grade				Grade				Grade			
Clinical severity	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Non severe (n=61)	24	21	12	4	14	36	8	3	23	28	8	2	32	19	8	2	50	11	0	0	40	17	4	0
Severe (n=59)	13	17	25	4	15	22	20	2	27	16	16	0	24	25	8	2	52	7	0	0	40	18	1	0
p-value	**0.04***				**0.03***				**0.04***				0.59				0.34				0.40			

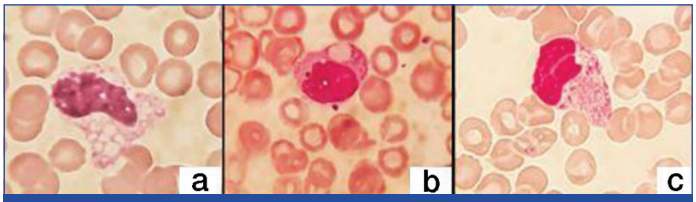
[Table/Fig-4]: Grading of viral cytopathic changes in lymphocytes and comparison between the study groups.
*p-value <0.05 was considered significant



[Table/Fig-5]: a) Peripheral smear showing larger granular lymphocytes; b) Peripheral smear showing atypical lymphocyte with irregular nuclear contour; c) Peripheral smear showing plasmacytoid lymphocyte; d) Peripheral smear showing atypical lymphocyte with moderately condensed chromatin and prominent nucleoli. (1000X, Leishman stain).

Morphological changes in monocytes												
	Granular monocytes				Cytoplasmic vacoulations				Nuclear blebbing			
	Grade				Grade				Grade			
Clinical severity	0	1	2	3	0	1	2	3	0	1	2	3
Non severe (n=61)	21	21	10	9	14	28	15	4	27	26	8	0
Severe (n=59)	11	14	19	15	6	21	20	12	18	29	11	1
p-value	**0.03***				**0.03***				0.33			

[Table/Fig-6]: Grading of viral cytopathic changes in monocytes and comparison between the study groups.
*p-value <0.05 was considered significant



[Table/Fig-7]: a) Peripheral smear showing a monocyte with large coalescing vacuoles; b) Peripheral smear showing a monocyte with a large vacuole; c) Peripheral smear showing a monocyte with granular cytoplasm (1000X, Leishman stain).

DISCUSSION

It is a well-established fact that COVID-19 has variable clinical presentations and outcomes in affected patients. Though real time RT-PCR has been touted as the gold standard diagnostic test for COVID-19, researches are underway to ascertain the prognostic efficacy of newer diagnostic tests, that can also predict the clinical outcome. The severity of this infection has been associated with several diagnostic modalities such as radiology, pulmonary function tests and SpO_2 etc. As far as laboratory medicine is concerned, there have been many studies on the assessment of CBC from automated haematology analysers in varying stages of COVID-19. There have been claims of the virus affecting haemoglobin levels, neutrophil, lymphocyte and monocyte counts. Even in this era of automation, there is no substitute to the study of microscopic features of affected cell lines in haematology laboratories. On literature search, authors came across limited studies on the cytopathic features of affected cell lines [9]. Hence, the present study was taken up with an intention to study and grade the smear findings in COVID-19 affected WBCs. Importantly, the study compares these findings between patients with COVID-19 in the severe and non severe groups and demonstrates significant differences between these two, suggesting an important role of CBC with manual smear review in patient risk stratification. Viral-induced morphologic changes in WBCs are well characterised in certain infections that can direct diagnostic workup and ensure timely therapeutic intervention. For example, in infectious mononucleosis caused by the Epstein-Barr virus, there is a significant lymphocytosis with presence of large atypical lymphocytes (Downey cells), while in human immunodeficiency virus infection, lymphocytes are morphologically unremarkable in the setting of lymphopenia [7].

A study by Pozdnyakova O et al., deciphered significant numerical and morphological changes in WBCs of COVID-19 patients. Severe category patients had significantly higher WBC counts with associated neutrophilia, similar to our study. They found that these patients were more likely to have lymphopenia which was not statistically significant in our study [7]. COVID-19 being a new infection, several studies showed that in early stages when patients have no specific symptoms, WBC count and peripheral blood lymphocytes are normal or slightly reduced, with values showing variation as disease progressed. In the study of Fan BE et al., the most common haematological findings included lymphopenia, neutrophilia, eosinopenia, mild thrombocytopenia while in the present study, WBCs parameters showed significant leucocytosis and absolute neutrophilia [10]. In a recent report from California, a mild leucoerythroblastic picture was observed in the peripheral blood film [11].

The most striking findings in WBCs of patients with COVID-19 were the morphologic changes which were observed in 100% of patients in the study, although some of them resembled changes associated with other viral or bacterial infections. In the study done by Weinberg SE et al., morphological changes reported in lymphocytes were: 1) presence of medium to large size atypical lymphocytes having loosely condensed chromatin with moderate to deep basophilic cytoplasm; 2) Atypical cells of plasmacytoid morphology with eccentric nuclei, perinuclear hoff, some mimicking immunoblasts. However, the percentage of atypical lymphocytes did not correlate with the severity of the disease in this study, unlike ours [12]. Zini G et al., noticed abnormalities of nuclear shape with increased frequency of band forms and dysmorphic cells with total absence of nuclear segmentation, consistent with Pseudo-Pelger morphology, a feature which was also seen in our study. These abnormalities mainly highlight the severe, transitory and reversible perturbation of myelopoiesis, especially in the form of accelerated and disordered granulopoiesis in patients with COVID-19 in severe

symptomatic phase. According to recent updates, such quantitative and qualitative abnormalities can be related to the cytokine storm and hyper-inflammation, which is a fundamental pathogenic factor in the evolution of COVID-19 pneumonia. Possibly, this manifest as secondary haemophagocytic lymphohistiocytosis, leading to an often fatal multiorgan failure. Authors preliminary observation in the present study could therefore, call for further studies on the involvement of myelopoiesis in pathogenesis and evolution of COVID-19 [13]. Monocytes demonstrated the most impressive vacuolisation, with numerous large coalescing vacuoles seen in all patients with severe cases showing higher percentage (89.8%) when compared to the non severe cases (77%) in the present study.

Proportion of abnormal morphologic changes when compared in lymphocytes and monocytes between severe and non severe patients with COVID-19 were significantly different. Atypical lymphocytes and plasmacytoid lymphocytes (grade >0) were more prevalent in severe patients, while cytoplasmic pseudopods and apoptotic lymphocytes were not. The Indian study by Singh S et al., also found similar morphological changes as in the present study [9]. To summarise, various morphological alterations were noted in all the WBC cell lines examined in the peripheral smear but, whether all these changes are due to the virus infecting them or are secondary to the pathogenesis of COVID-19, needs to be evaluated by larger studies.

Limitation(s)

Other viral co-infections, if present, could not be excluded. The study was undertaken as exploratory research to identify and find out morphologic changes seen in COVID-19 infected WBCs, as literature on this topic is still evolving. Serial samples from patients were not a part of the present study.

CONCLUSION(S)

The viral cytopathic effects seen in WBCs on peripheral smear have significant clinical implications on disease severity, undermining the need for a comprehensive study of viral induced morphological changes in hospitalised COVID-19 patients.

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