ORIGINAL RESEARCH

Morphological appearance of blood cells in peripheral blood smears in covid-19 disease

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ABSTRACT

Objective: To examine morphology of white blood cells on peripheral blood smears (PBSs) of confirmed COVID-19 (Corona Virus Disease-19) patients with moderate to severe disease. **Materials and Methods**: Prospective study was conducted for a period of 3 months from May 2021 to July 2021 at dedicated tertiary care COVID-19 hospital in Northern India, where Giemsa-stained PBSs from 170 COVID-19 positive patients with moderate to severe disease were examined microscopically for morphological changes in white blood cells. **Statistical analysis**: The qualitative data was analyzed using chi squared test. The confidence interval was set to 99%, the *p*-value was considered significant at the level of <.01. **Results**: On microscopic examination of PBSs, most frequent finding was neutrophilia (64.1% of total cases) and was seen in 100% of patients with severe COVID-19 disease (p value <0.01). The morphological changes seen in neutrophils were hypolobation, pseudo-pelger-huet anomaly (p value<0.01), shift to left (p value <0.01), apoptotic bodies, hypogranularity and leukoerythroblastic blood picture. Atypical lymphocytes were seen in moderate COVID-19 disease amounting to 8.2% of total cases. Vacuolated reactive monocytes were observed in 13.5% of total cases. **Conclusion**: In our study a relevant number of moderate to severe cases of COVID- 19 showed neutrophilia, pseudo-pelger-huet anomaly and shift to left on peripheral blood smear. Hence it may be assumed from the study that neutrophilia and morphological findings from PBSs in COVID-19 disease can alert the physician of an impending disease progression.

Keywords: blood cells, COVID-19, morphological changes

INTRODUCTION

Coronavirus disease 2019 (COVID-19), a highly contagious disease of viral origin first surfaced in China's Wuhan province and was brought to the notice of the World Health Organization (WHO) on 31st December 2019[(1)]. Being highly infectious, it spread across the globe in a short span of time as a pandemic with substantial loss of human lives and a detrimental effect on the economy worldwide. On the 9th of January 2020[(2)], the first human fatality due to COVID-19 occurred. In India, from 3rd January 2020 to 24th May 2023, the total confirmed cases of COVID-19 were 44,987,339 with 531,843 deaths as reported to WHO [(3)]. The causative agent of the COVID-19 pandemic is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a single-stranded RNA virus. The rate of spread of this virus is more compared to other coronaviruses known to cause severe acute respiratory syndrome (SARS)[(4)]. Since its onset in 2019, the ebb and flow of COVID-19 cases has led to COVID ' waves' with outbreaks still being bothersome. These vagaries in the number of COVID cases have been attributed to vaccinations done at the mass level, negligent attitude towards COVID appropriate measures and the emergence of new variants[(5)].

The novel coronavirus is known to target the ACE2 (Angiotensin converting enzyme-2) receptor found in human lungs and destroys them through cytokine storm leading to exaggerated inflammatory response, abnormally regulated T-cell immune response and harm to host cells. The inflammatory mediators released as a result of COVID-19 infection induce blood clotting, acute lung injury, subsequent multiorgan failure, acute respiratory distress syndrome and often death[(6)].

Thus, white blood cells leading to exaggerated inflammatory response are in the centre stage of the

pathogenesis of this disease. Quantitative hematologic parameters like leukocytosis, leukopenia, neutrophilia, neutropenia, monocytosis, monocytopenia, lymphocytosis, lymphopenia and thrombocytopenia have been studied and have been found helpful in guiding the therapy and prognosis of COVID-19 patients. A few studies exploring morphological changes in peripheral blood smears in COVID-19 infection have been undertaken and are still in the evolving phase. We conducted a study identifying qualitative haematological features in PBSs in COVID-19 patients.

MATERIAL AND METHODS

This study was performed after local ethical board approval. In this prospective study, conducted for a period of 3 months from May 2021 to July 2021, we aimed to observe abnormalities in white blood cells in COVID-19 positive patients admitted to dedicated COVID-19 tertiary care hospital. Hospitalized COVID-19 patients with moderate to severe disease with positive Polymerase chain reaction (PCR) test for COVID-19 were included in the study. Patients with a history of hematologic diseases including leukaemia, lymphoma or other comorbidities were excluded from the study.

The demographic data of the patients, including age and sex, and laboratory blood test results at admission (complete blood count) was extracted from the patients' profiles. The severity of the disease was also collected from the patients' medical profiles. Patients with moderate COVID disease had spo2 of about 90-93% on room air and respiratory rate >24/minute. Patients with severe COVID disease had arterial spo2 levels under 90% on room air and a respiratory rate of more than 30 /min [(7)]. Patients' blood samples were collected and immediately sent to the pathology laboratory in EDTA (Ethylene Diamine Tetra Acetic acid) tubes. Peripheral blood smears were made and staining was done with the Giemsa stain by an expert laboratory technician. The prepared peripheral blood smear slides were examined under light microscope. We considered following important findings in peripheral blood smears of these patients- neutrophilia, pseudo-pelger-huet anomaly, dysmorphic neutrophils, apoptotic bodies, smudge cells, shift to left, leukoerythroblastic reaction, hypogranular neutrophils, atypical lymphocytes and vacuolated monocytes. The qualitative data was analyzed using chi squared test. The confidence interval was set to 99% and accepted margin of error was set to 1%. So, the *p*-value was considered significant at the level of <.01.

RESULTS

Our study cohort comprised of 170 COVID-19 positive patients with moderate to severe disease, referred for treatment from various primary and secondary health care centres as our hospital was declared dedicated tertiary care COVID-19 referral hospital for the state of Himachal Pradesh during COVID-19 pandemic.

Out of 170 COVID-19 patients studied, disease occurred equally in both the sexes in the present study. However majority of authors have observed male preponderance for same [(8, 9, 10)]. Age of patients in present study ranged from 20- 90 years. Mean age of patients was 54 years. Similar findings were reported by Sadigh et al [(8)] Berber et al [(11)], and Luke et al [(12)].

On microscopic examination of the PBSs most frequent finding was neutrophilia (64.1% of total cases) and was seen in 100% of patients with severe COVID 19 disease (p value <0.01). The various morphological changes seen in neutrophil series include changes in nuclear lobes like hypolobation, pseudo-pelger-huet anomaly (p value <0.01), shift to left (p value <0.01), apoptotic bodies, hypogranularity, leukoerythroblastic picture. Also, few neutrophils showed dysmorphic C-shaped, fetus-like and ring-shaped nucleus. Atypical lymphocytes with large nucleus, condensed chromatin, central to eccentric nucleus and basophilic cytoplasm were seen in moderate COVID-19 disease amounting to 8.2% of total cases. Vacuolated reactive monocytes were observed in 13.5% of total cases with increased frequency in severe COVID 19 disease. (TABLE-1) (Figure1-4)

TABLE No. 1 MORPHOLOGICAL CHANGES OBSERVED IN PERIPHERAL SMEARS

S No.	ABNORMALITY	MODERATE DISEASE (157)	SEVERE DISEASE (17)	TOTAL CASES-170	Chi square	P - value	SIGNIFICANCE
1.	Neutrophilia	92(60%)	17 (100%)	109(64.1%)	11.23	0.0008	S
2.	Dysmorphic neutrophil	12(08%)	03(17.6%)	15(8.8%)	1.949	0.1627	NS
3.	Pseudo-pelger-huet anomaly	12(08%)	06(35.3%)	18(10.6%)	12.64	0.0004	S
4.	Shift to left	40(26.1%)	11(64.7%)	51(30.0%)	11.39	0.0007	S
5.	Apoptotic bodies	05(3.2%)	01(5.8%)	06(3.5%)	0.335	0.5626	NS
6.	Hypogranularity	12(08%)	01(5.8%)	13(7.6%	0.069	0.7931	NS
7.	Leukoerythroblastic picture	05(3.2%)	02(11.7%)	07(4.1%)	2.92	0.0872	NS
8.	Atypical lymphocyte	14(9.1%)	00	14(8.2%)	1.64	0.1992	NS
9.	Vacuolated monocytes	15(9.8%)	05(29.4%)	20(11.8%)	5.94	0.0148	NS

10.	Smudge cells	07(4.6%)	02(11.7%)	06(3.5%)	1.669	0.1963	NS
11.	Giant platelets	06(3.9%)	03(17.6%)	09(5.2%)	5.97	0.0145	NS

Note: p-value >.01: Not significant (NS); *p*-value <.01: Significant (S)

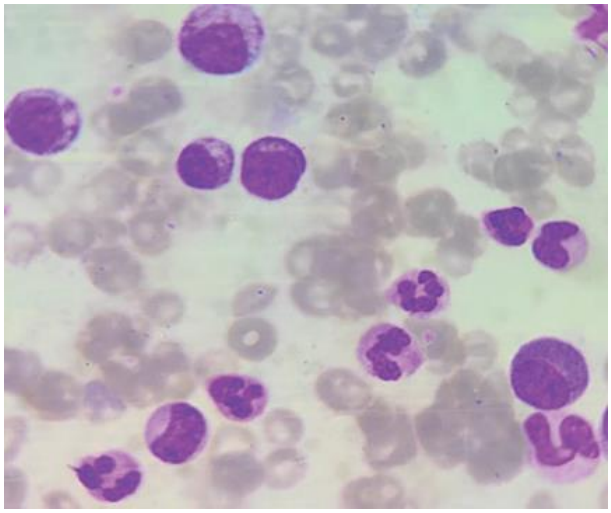

Figure 1: Neutrophilia with shift to left. 100X, Giemsa

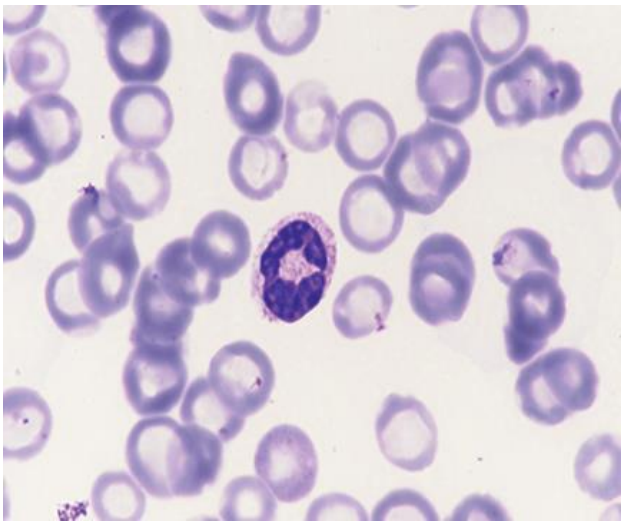

Figure 2: Ring neutrophils. 100X, Giemsa

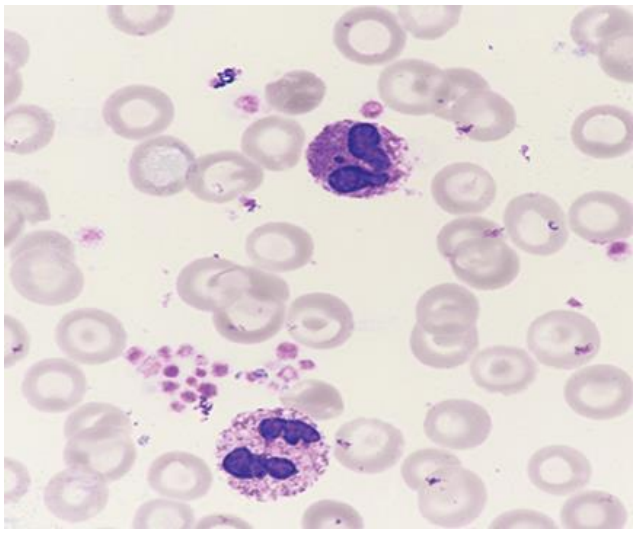

Figure 3: Neutrophils with pseudo-pelger-huetanomaly.60X, Giemsa

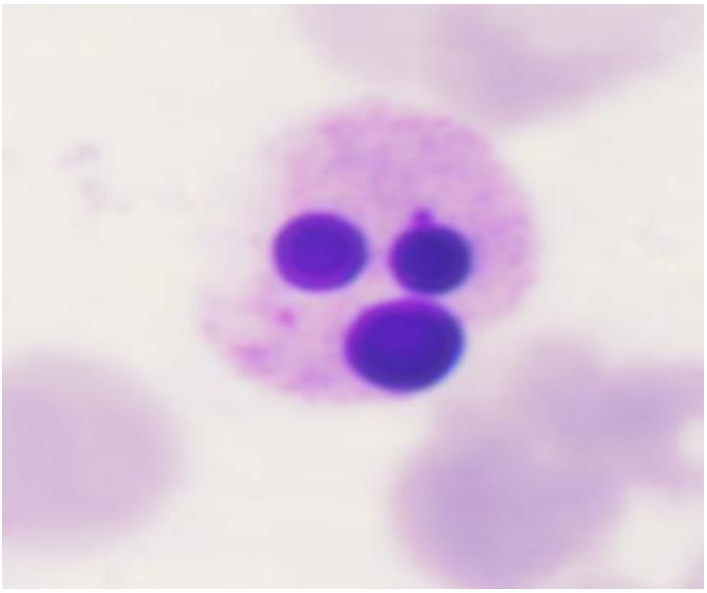

Figure 4: Apoptosis.100X, Giemsa

DISCUSSION

Viral infections are known to cause quantitative and qualitative changes in blood cells in the peripheral blood smear including COVID-19 disease. During this COVID-19 pandemic, various studies have been undertaken to understand the haematological manifestations of this disease. Worldwide studies regarding numerical haematological data outnumber the morphological analysis of peripheral blood cells and India being no exception with only few such studies documenting the morphological appearance of blood cells especially white blood cells, that too with a small sample size to the best of our knowledge. However, importance of understanding these changes have been highlighted in various studies. In our present prospective study, we analyzed various changes in peripheral blood cells.

The most frequent finding in our patients was neutrophilia, seen in 60% of moderate cases and 100% of severe cases. It may be because of the fact that we were observing patients with moderate to severe disease who had difficulty in breathing due to some element of pneumonia secondary to hyperinflammatory syndrome with cytokine storm [13]. Neutrophilia is an important finding of pneumonia thus it can serve as prognostic marker for disease progression in asymptomatic ' happy hypoxia' COVID positive patients. Similarly high neutrophil counts were observed in severe COVID disease by many other authors [14, 15,16].

Pseudo-pelger-huet anomaly and hypolobated neutrophils were seen in 10.6% of total patients however number was increased in severe cases (35.3%).Bhalchandra AR et al[9], Gabr et al [10], Berber I et al[11], Luke F et al[12] and Schapkaitz E et al[17], Nazarullah A et al[18] and Ahnach M et al[19] also reported frequent pseudo-pelger-huet cells in severe COVID disease.

Neutrophils with dysplastic features (dysmorphic nucleus, hypogranularity, apoptotic chromatin) were frequently observed in PBSs in our study. This finding was also highlighted by Bhalchandra AR et al[9], Berber I et al[11], Schapkaitz E et al[17], Singh A et al[20]and Pozdnyakova O et al[21].

Leukoerythroblastic reaction was seen in 4.1% and shift to left was evident in 30% of total patients which signify stress haematopoiesis. Schapkaitz E et a[17], Mitra et al[22] and Buoro S et al[23] also observed leukoerythroblastic blood picture infrequently in their studies. Apoptotic bodies were reported by many studies [10, 24]. Our study reported apoptotic neutrophils in 7% of smears. Usually, apoptotic neutrophils are rarely seen in peripheral smears. These findings can be consequent to inflammatory cytokine storm or hematopoietic progenitor cells infection by coronavirus.

Smudge cells were found in 3.5% of smears. Smudge cells are seen when cells are extremely fragile and thus are prone to nuclear disruption during peripheral smear making. Though mostly seen in chronic lymphoproliferative disorder, they are also seen in infectious mononucleosis, chronic inflammatory conditions or if too much pressure is applied during smear formation. Many of the laboratories are using automated analyzer which can make peripheral smears too. This could be a reason for smudge cells as artifacts. Sadigh et al [8] reported smudge cells as their predominant findings in peripheral smears while Bhalchandra A et al [9] reported it as an insignificant finding.

Atypical lymphocytes were seen only in moderate COVID disease patients (9.1%). This is in contrast to many studies where atypical lymphocytes were a significant finding. This may be because we are reporting case from tertiary care hospital where only moderate to severely sick patients were admitted and most of these patients had shortness of breath because

of underlying element of pneumonia [10, 12, 17]. Increased vacuolization of monocytes was observed in 11.8% of total cases and 29.4% of severe cases which is in concordance with Bhalchandra AR et al [9] who observed abnormal vacuolation in monocyte 27.5% of severe cases.

CONCLUSION

In our study a relevant number of cases with moderate to severe COVID-19 disease showed neutrophilia, pseudo-pelger-huet anomaly and shift to left on peripheral blood smear examination. Such abnormalities could be attributed to the cytokine storm, hyperinflammation and T-cell immune dysregulation. Hence it may be assumed from the study that neutrophilia and other morphological findings from PBSs in COVID-19 disease can alert the physician of an impending disease progression.

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