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Peripheral Blood Smear Findings in Sars-Covid-2 Infection

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KEYWORDS

Ethylenediamine tetra acetic acid, Acquired Pleger-Huet Anomaly, Corona virus Disease 19, In-patients

ABSTRACT:

Introduction

SARS-COV-2 is highly contagious enveloped single-stranded RNA virus that belongs to family Betacoronaviruses. The disease progresses from an upper respiratory tract infection with general flu-like symptoms to pneumonia and hyperinflammatory host reaction leading to acute respiratory distress and multiorgan failure. This study is to highlight the morphological changes observed in peripheral blood smears of COVID -19 positive cases.

Aims and Objectives

To evaluate the changes in Peripheral blood smears in COVID -19 positive patients like:

1. RBC morphology.
2. Platelet morphology.
3. WBC- morphological changes in granulocytes like toxic granules, vacuoles, apoptotic changes, shift to left (when immature granulocyte percent >5% & band forms >10%), Acquired Pleger-Huet anomaly (APHA) and presence of atypical lymphocytes.

Materials and Methods

Confirmed COVID-19 in-patient (IPD) cases whose samples were sent in Ethylenediamine tetra acetic acid (EDTA) vacutainers during the study period i.e, from May 1, 2020 to October 31, 2020 were included in this study.

Peripheral smears were stained with Wright stain and the manual differential count was performed along with that RBC, WBC and Platelet morphology were looked for in Peripheral blood smear.

Results

Among 650 COVID- 19 patient's. Neutrophil toxic vacuoles and granules were seen in 414 cases (63.69%), Acquired Pleger-Huet Anomaly (APHA) in 241 cases (37%), Atypical lymphocytes in 186 cases (28.61%), giant platelets in 167 cases (25.69%) & 13 cases showed leucoerythroblastic reaction.

Conclusion

We conclude that careful examination of peripheral blood smears to be done in SAR-COV2 infection. Findings like toxic granules, vacuoles, apoptotic changes, shift to left, Acquired Pleger-Huet anomaly in neutrophils and atypical lymphocytes direct it towards COVID-19 infection & are also indicators of adverse clinical outcomes. Hence Peripheral blood smear finidngs can help pathologist to alert the physicians of impending severity.

INTRODUCTION:

It is highly infectious & contagious with an enveloped single-stranded RNA virus that belongs to family of Betacoronaviruses.[1] In December 2019, Novel Coronavirus (SARS-COV-2) pneumonia was reported in Wuhan and has rapidly spread throughout China.[2] After Spanish Flu, no other illness has taken such a heavy toll on human population as SAR COV-2. Corona virus diseases- 19 (COVID-19) has spread around the world leading to worldwide morbidity and mortality with complications. In India total of 2,76,583 Cases were reported till June 10th, 2020 with a Death rate amounting to 7745.[3] In severe cases, the disease progresses from an upper respiratory tract infection with local and general flu-like symptoms to viral pneumonia and hyper inflammatory host reaction leading to acute respiratory distress and multiorgan failure.[4]

Real time- polymerase chain reaction (RT-PCR) has been

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proven as the Gold standard diagnostic test in diagnosing COVID-19 infection. However, there is still research underway to ascertain the prognostic tests which are predictive of COVID infection like : CT- SCAN, pulmonary function like SPO2 level, Lab tests like Coagulation profile and fibrinogen level.[3]. The most common findings seen in peripheral smear are lymphocytopenia, neutrophilia, eosinophilia, mild thrombocytopenia or thrombocytosis.[5]

Researches about peripheral smear findings in COVID - 19 positive patients were very few in our region and were not taken at all in our institute. This study was to highlight the qualitative changes observed in Peripheral blood smears of Lab confirmed COVID- 19 positive patients.

AIMS AND OBJECTIVES:

To evaluate the changes in Peripheral blood smears in RT-PCR proved COVID -19 patients like:

1. RBC morphology.
2. Platelet morphology.
3. WBC- morphological changes in granulocytes like toxic granules, vacuoles, apoptotic changes, shift to left (when immature granulocyte percent is >5% and band forms >10%), Acquired Pleger-Huet Anomaly (APHA) and presence of atypical lymphocytes.

MATERIALS AND METHODS:

INCLUSION CRITERIA:

All the proved Covid-19 positive IPD cases, whose fresh blood samples were received for complete hemogram during the study period were included.

EXCLUSION CRITERIA:

Radiologically or clinically suspected patients in whom RT-PCR was not positive or not done were excluded.

Patient with pre-existing hematological conditions like leukemia and delayed samples were excluded.

STUDY PERIOD AND TYPE:

This was 6 months prospective study conducted from May 1 to October 31, 2020 in our institute. Our institute is a tertiary care center as well as a COVID center with dedicated Super-specialty building to serve COVID-19 positive patients.

METHOD:

The list of all patients tested for RT- PCR was retrieved. Clinical criteria mention by the World Health Organization was adopted by our institution for COVID-19 testing. Detection of SAR-COV-2 was carried out using Reverse transcriptase polymerase chain reaction assay that had been successfully validated in our laboratory.

Fresh samples of confirmed COVID-19 cases were sent to the central laboratory of our institute in EDTA vacutainers during the study period were taken. Then with these samples's peripheral blood smears were prepared and stained with Leishman's stain. Peripheral blood smears (n- 650) were screened by two Pathologists in central lab, who counted minimum 100 WBC cells for each slide under oil immersion (100X) & RBC, WBC and Platelet morphology were looked for in peripheral blood smear.

RESULTS:

Fresh samples of 650 COVID- 19 patients were included in our study. Men (n-425) were more commonly affected than women (n -225). The age range was between 25 to 100 years. After through qualitative examination of peripheral blood smears we noted changes as stated below:

QUALITATIVE / MORPHOLOGICAL CHANGES:

RBC's morphology was normal with not much changes in Mean corpuscular volume (Mean – 82 flt).

In WBS's marked changes in neutrophil morphology were seen like toxic vacuoles and granules in 414 cases (63.69%) and in many cases neutrophils also showed apoptotic and pyknotic changes which were not quantified. APHA was seen in 241 cases which accounts for 37% of total cases which is quite significant. Atypical lymphocytes with plasmacytoid features were noted in 186 cases which accounts for 28.61% of total cases. **Table 1**

Platelets showed morphological changes like giant platelets in 167 cases (25.69%). **Table 1**

Among 650 positive cases, 13 cases showed leukoerythroblastic reaction, 10 showed myeloid – leukemoid reaction, and 39 cases showed pancytopenia. 1 case of Chronic myeloid leukemia, 1 case of chronic lymphoid leukemia & 4 case of acute myeloid leukemia were incidentally found for first time. 2 cases were

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JCHR (2024) 14(4), 983-989 | ISSN:2251-6727

Plasmodium vivax infected and 1 case had mixed infection with Plasmodium vivax and Plasmodium falciparum.

In central lab we receive samples from only in-patient & cases who were admitted in super specialty with some serious illness. Hence these findings are among bit serious cases. As it is a government college clinician didn't send sample for complete hemogram again and they followed up the case with only CBC count. On following up the neutrophilia and other blood findings decreased with clinical recovery. We could not quantify it as few samples were missed and not scanned for morphological findings. Which was drawback in our study.

TABLE 1: MORPHOLOGIC CHANGES IN NEUTROPHILS, LYMPHOCYTES AND PLATELETS

	PRESENT CASES	%	ABSENT CASES	%
SHIFT TO LEFT	122	18.76	528	81.22
APLH	241	37.07	409	62.92
TOXIC VACUOLES & GRANULES	414	63.69	236	36.3
ATYPICAL LYMPHOCYTES	186	28.61	464	71.38
GIANT PLATELETS	167	25.69	483	74.3

FIGURE 1 : SARS-COV2 VIRUS IMAGE AND ITS PATHOGENSIS. [6]

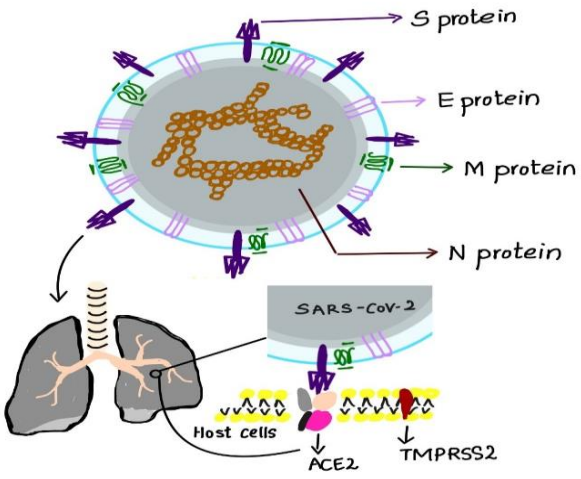

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FIGURE 2 : SARS-COV2 INFECTION - PATHOGENSIS DEPICTED IN FLOWCHART. [8]

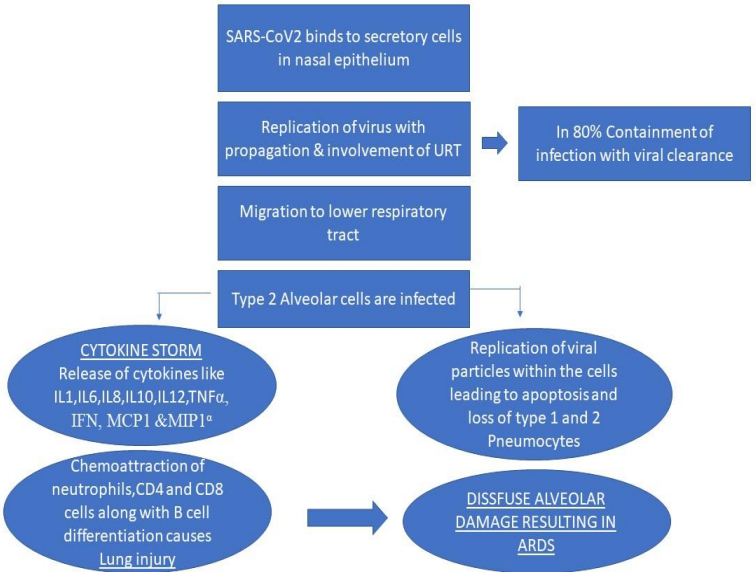

FIGURE 3: PERIPHERAL BLOOD SMEAR EXAMINATION IMAGES IN SARS-CoV2 INFECTION

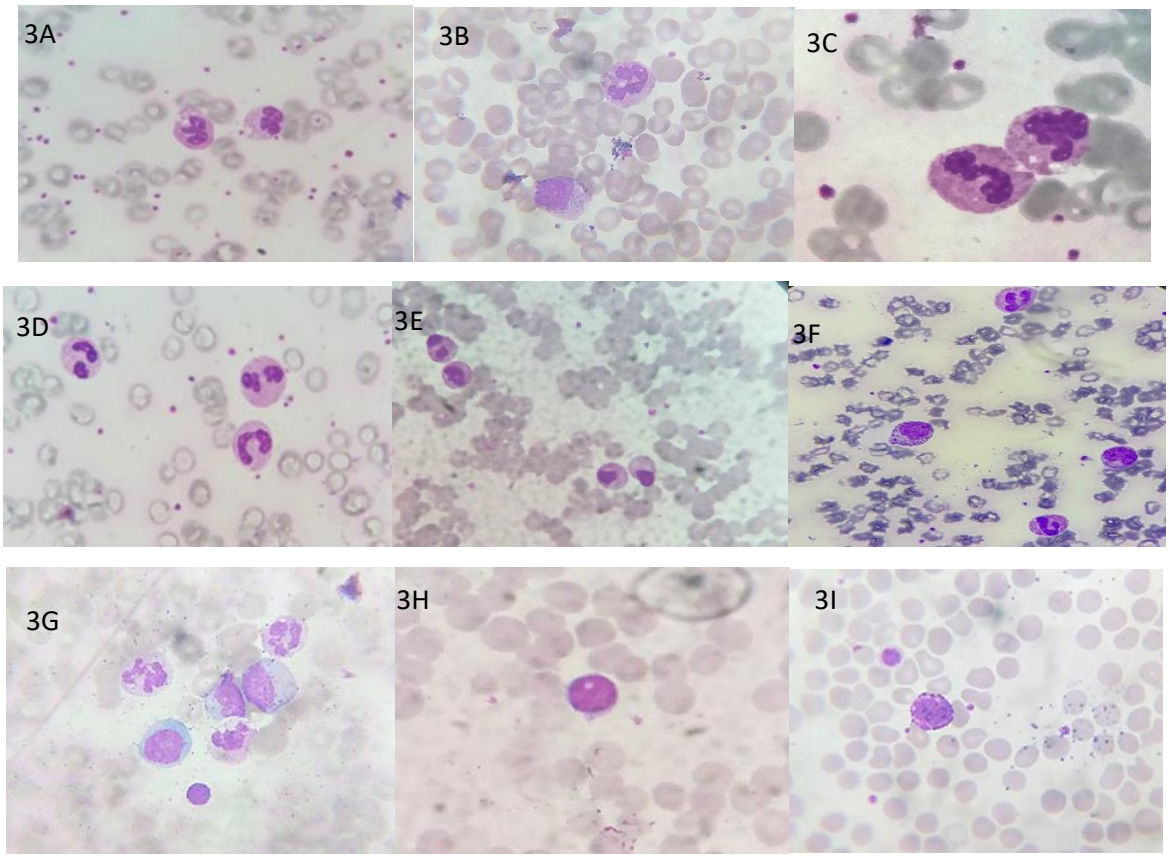

Figure 3A-Neutrophils showing toxic vacuoles, 3B-Neutrophils showing toxic granules, 3C-Neutrophils showing toxic granule's and vacuole's, 3D- Acquired Pleger-Huet anomaly of neutrophils, 3E- Neutrophils showing apoptotic changes, 3F- Left shift of granulocytes, 3G- Leucoerythroblastic reaction, 3H- Atypical lymphocytes, 3I- Platelets showing morphological changes like giant platelets

Journal of Chemical Health Risks

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JCHR (2024) 14(4), 983-989 | ISSN:2251-6727

DISCUSSION:

SARS–CoV–2 is a member of the genus Betacoronavirus in the family Coronaviridae. The family with a large genome of 26–32 kilobases, belongs to a group of enveloped positive–sense single–stranded ribonucleicacid (RNA)viruses[6]. The simplified structure of SARS–CoV–2 is shown in the **figure1**.[6] The structure of severe acute respiratory syndrome coronavirus 2 (SARS–CoV–2) comprising four different proteins: spike (S), membrane (M), envelope (E) and nucleocapsid (N) which forms the genome. To enter the cell, the virus requires the interaction of the S protein with human transmembrane angiotensin–converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2).[6]

The Pathogenesis of COVID- 19 is explained in Flow chart **figure 2**.[7] SARS-CoV-2 virus bind to endothelium and innate immune cells via Pathogen Recognition receptors and cause Cytokine storm (i.e elevated cytokine levels). This leads to Skew Hematopoietic stem cells to develop towards myeloid cells.[8]

We have studied qualitative changes of 650 peripheral blood smear samples. In our study male patients (425) were more effected with covid 19 compared to female patient (225). Other studies like Sam sadigh et al,[5] Devajit Nath et al, [9] Luke F et al [10] also showed similar results.

Qualitative / Morphological changes:

The neutrophilic morphological abnormalities concerned both nuclear changes and cytoplasmic changes including presence of many, crowded, dark & coarse granulations in the cytoplasm (similar to “toxic” granules) and presence of toxic vacuoles. In a minority of cases, cytoplasmic hypogranularity was also noted. Abnormalities of nuclear shape were striking. There was increased frequency of band forms, dysmorphic cells with total absence of nuclear segmentation which was consistent with pseudo-Pelger morphology (i.e bilobed and mono-lobated nucleus) were frequently present. These were named as Acquired Pelger-Huet Anomaly (APHA). Nazarulla A et al stated that COVID-19 cases had significant APHA with mono-lobate neutrophils and plasmacytoid lymphocytes as compared to controls.[1] Apoptotic cells had liquefied and pyknotic nucleus with loss of lobation having agranular or deep blue cytoplasm. [9]

Among the WBCs, Neutrophils showed marked changes in morphology like toxic vacuoles (Figure 2E) and granules (Figure 2F) in 414 cases (63.69%). Neutrophil morphology showed similar toxic vacuoles and granules in other studies like Nazarullah A et al[1] (8.3%), Zini G et al[4](35%), Nath D et al[9] 96% and Singh S et al[3]. In many cases neutrophils also displayed apoptotic and pyknotic changes(Figure 2G) which is comparable to studies of singh S et al[3] and zini G et al[4].

APHA (Figure 2H) was seen in 241 cases which accounts for 37% of total cases which is quite significant. Other studies which projected increase in APHA were, Nazarullah A et al[1] (50%), Luke F et al[10] (39%) and Zini G et al[4] (35%).

Left shift of granulocytes (Figure 2I) is conventionally interpreted as a sign of bacterial infection. Neutrophil kinetics in bacterial infections is thought to trigger mobilization of marrow reserves, resulting in left shift of granulocytes. SARS-CoV-2 induced cytokine release causing neutrophil migration due to bacterial superinfection is a possibility. Direct myelotoxicity caused by the virus or marrow overproduction in a background of increased peripheral cell turnover are other hypotheses.[1]

Among 650 cases shift to left were seen in 122, accounting for 18.76%. Mitra A et al[11] studied on single Covid 19 positive case which showed shift to left. Other studies like Nazarullah A et al[1] and Luke F et al [10] observed 66% and 63% shift to left in their studies respectively.

Leukoerythroblastic reactions, defined as immature erythroid and immature myeloid cells circulating in the peripheral blood it is typically associated with marrow infiltrative processes but may also represent marrow response to stressors like hypoxia, peripheral destruction/sequestration, or sepsis. The lack of any other evidence suggestive of an underlying myeloid neoplasm or malignancy causing a myelophthisic process, this may represent marrow stress and response to the viral infection.[3]

Among 650 positive cases, 13 cases showed leukoerythroblastic reaction (Figure 2J) in our study. Alia Nazarullah et al [1] & Sadigh S et al[5] also showed Leukoerythroblastic reaction in 1 and few cases respectively. Mitra A et al states that Leukoerythroblastic picture are very unusual in viral infection. [11] Leukoerythroblastic picture is rarely seen in viral infections such as parvovirus.[12] Mitra A et al described one unusual case viral infection with leukoerythroblastic picture in which neutrophilia and other blood findings got

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JCHR (2024) 14(4), 983-989 | ISSN:2251-6727

resolved after Clinical improvement. Hence it was proved that leukoerythroblastic picture can be seen in SAR- CoV 2 Infection. [11]

Atypical lymphocytes (Figure 2K) were noted in 186 cases which accounts for 28.61% of total cases. Nazarullah A et al[1], Nath D et al [9] showed 100% and 88.4% of atypical lymphocytes in their studies. Sadigh S et al[5] also shows increase in atypical lymphocytes in their study.

Platelets showed morphological changes like giant platelets (Figure 2L) were identified in 167 cases (25.69%) this was identical to studies by Nath D et al[9] (7.6%), Sadigh S and Singh S et al[5,3].

As our central lab is dedicated for receiving IPD samples & also from serious cases who were admitted in super specialty. Hence these peripheral smear findings were from bit serious cases. On following up the neutrophilia and other blood findings decreased with clinical recovery. Even in studies done by Mitra A et al and Nath D et al similar improvement was seen.[11] Since we couldn't follow up all patients with fresh blood sample examination again, we were not able quantify it.

We also observed that in other bacterial infection toxic granules and vacuoles were seen occasionally, when patient was severely infected or was in septicemia. But neutrophils with APHA and Atypical plasmacytoid lymphocytes were very rare in bacterial infection. These findings helped us in suspecting COVID infection and differentiating it from other bacterial infection. As SAR CoV-2 is virus, like any other viral infection it has lymphocytosis and atypical or reactive lymphocytes in blood, but other viruses lack neutrophil changes seen in Covid-19 like toxic vacuole's, granules & APHA like features. These were the few findings in our study which makes it unique from other studies. [1, 9,11]

CONCLUSION:

We conclude that careful examination of peripheral blood smears to be done for findings like Neutrophilic toxic vacuoles & granules, APHA like features of neutrophils, atypical plasmacytoid lymphocytes in WBC's and giant platelets. These findings direct the case towards COVID-19 infection & also can become indicators of adverse clinical outcomes. Hence morphological finding in peripheral blood smear can help a pathologist to alert the physicians of impending progression of the disease and differentiating it from other diseases.

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