Diagnostic Pathology: Open Access

Research Article Open Access

Peripheral Red Blood Cells Morphology in COVID-19 Patients

Alsadig Gassoum[1*], Sawsan AH Aldeaf[1], Tyseer AM Barri[1], Nihad Babiker[1], Altaf Taaha[1], Nedal Soliman[1], Renad Abdalla[1], Riham Ahmed[1], Mawahib Aledrissy[1], Safa Hamid[1] and Mohamed Arbab[1,2]

[1]Department of Pathology, National Center for Neurological Sciences, Khartoum, Sudan
[2]Department of Surgery, University of Khartoum, Khartoum, Sudan

[*]**Corresponding author:** Dr. Alsadig Gassoum, Department of Pathology, National Center for Neurological Sciences, Khartoum, Sudan, E-mail: sadig9@gmail.com

Received: 30-Dec-2022, Manuscript No. DPO-22-83794; **Editor assigned:** 02-Jan-2023, PreQC No. DPO-22-83794 (PQ); **Reviewed:** 16-Jan-2023, QC No. DPO-22-83794; **Revised:** 23-Jan-2023, Manuscript No. DPO-22-83794 (R); **Published:** 30-Jan-2023, DOI: 10.4172/2476-2024.8.1.211

Citation: Gassoum A, Aldeaf SAH, Barri TAM, Babiker N, Taaha A, et al. (2023) Peripheral Red Blood Cells Morphology in COVID-19 Patients. Diagnos Pathol Open 8:211

Abstract

COVID-19 pandemic has resulted in global health disaster and posed a great challenge to the suitable measures to contain the disease. The pathogenesis of the disease is still evolving. In the search for the pathogenesis of the virus, little attention has been made to the peripheral blood picture in COVID-19 patients. This study has focused on the morphology of the red blood cells as stained with Geimsa. The results showed distinct RBCs morphological changes including rouleaux formation, Significant finding of ring-like stained bodies were seen within the RBCs in 98.3%of the patients. No such ring bodies were detected in any of the control smears. In a number of smears stained particles were seen on the RBCs cell wall. Macrocytosis with central polar stomatocytes and tear shape RBCs were seen. Nuclei of neutrophils with fetus like structure and anisocytosisand mild poikilocytosis were reported the number of reticulocytes and platelets is scanty in the COVID-19 patients compared to controls. All patients' blood smears showed marked lymphocytopenia, thrombocytopenia and slight neutrophilia with nuclei fetus like structure. Direct Coombs test was listed to be positive in 15% of the patients. In conclusion peripheral blood picture seems to be easy and predictable tool to diagnose COVID-19 patients.

Keywords: COVID-19; Red blood cells; Geimsa stain; Thrombocytopenia

Introduction

Corona virus is spherical or pleomorphic, single stranded enveloped RNA, covered with club shaped glycoprotein. They are four sub types; alpha, beta, gamma and delta corona virus. The virus is composed of four main structural proteins which are the spike, membrane, envelope and nucleo capsid encoded at the 3 end of the viral genome. In addition to this, a fifth structural protein namely the hemagglutinin esterase is located in the subset of β coronaviruses which binds sialic acids on the surface glycoprotein and has acetyl-esterase activity [1]. These activities enhance the S protein mediated cell entry and virus spread through the mucosa.

In a study that involved nasal washings from volunteers, several viruses associated with common cold have been grown [2]. One such sample referred to as B814 turned out to be what we now know as a corona-virus

In late 1960, a group of virologists had studied different strains of human and animal viruses among which were mouse hepatitis virus, infectious bronchitis virus, transmissible gastro-enteritis virus of swine etc. The results revealed that, all were having the same morphology as demonstrated by electron microscope, but a new genus of viruses was found and it was named CORONA, where the term corona denoted the crown like appearance of the surface in the morphological structure of viruses [3].

Moreover, before the outbreak of SARS-CoV Corona viruses were thought to cause only mild respiratory infection. One study suggests that corona-viruses are the cause of 15%-30% of the respiratory tract infections in human every year, with more affection of infants and elderly. HC0V-NL63 virus has been isolated from a seven-month baby [4]. Consequently in 2004 in Holland, this virus has subsequently been identified in various countries, indicating a worldwide distribution. HCoV-NL63 has been shown to infect mainly children and the immune-compromised, who presented with mild upper respiratory symptoms.

The mode of transmission of SARS-CoV only occurred through direct contact with infected individuals, therefore, the outbreak was largely confined to households and health-care personnel [5].This SARS-CoV outbreak was controlled in June 2003, except for few reported cases of super spreading events. Since that time, SARS-CoV has not returned, however in 2012 a novel human corona-virus emerged in the Middle East in Saudi Arabia, known as Middle East Respiratory Syndrome (MERSA) related corona virus, also known as camel flu [6]. The symptoms ranged from mild to severe diarrhea, cough, fever and shortness of breath. During that outbreak, high mortality rate of about 50% was reported. However, the outbreak got controlled in 2013, despite sporadic cases continued to be reported throughout the rest of the year. In April 2014, there was an abrupt rise in the reported cases and deaths prompting fears of mutation being occurred in the virus making it capable of human-to-human transmission [7].

The novel corona- virus disease of 2019 (COVID-19), first appeared in Wuhan city of Hubei province in China and was declared by World Health Organization a global health emergency on 30 January 2020. As of 29 March 2020, WHO database confirms few million cases of corona globally with increasing mortality reported from more than 201

countries. However the incidence of new cases and mortality rapidly changing [8,9].

Nevertheless, taking the life cycle of a coronavirus is subdivided into four phases: entry and attachment, replicate protein expression, replication and transcription, assemble and release. Interaction between the S protein and its receptors marks initial attachment of the virus to the host cell. The interaction between the S protein and receptor is the primary determinant for a coronavirus to infect the host and it also governs the tissue tropism of the virus [10].

Rationale of the study

This study was initiated by the observation of blood smears collected from patients initially suspected as malaria cases. The blood films of some of these patients revealed distinct picture. Microscopic revision of the peripheral blood stained slides showed stained material within the red blood cells other than the plasmodium stages of malaria. The variable clinical presentation necessitated investigation for COVID-19 virus which proved to be positive in a number of the suspects. This observation called for further verification of the stained strange material in the peripheral blood picture. This study aimed at morphological description of the peripheral blood picture in COVID-19 patients.

Materials and Methods

This is an analytic study conducted at the National Centre of Neurological Sciences (NCNS), Khartoum, Sudan, during the period from January to March 2021. Blood samples were obtained from cases admitted in the center, staff and governmental isolation center for COVID-19 management. The samples were divided into two groups; COVID-19 established subjects and apparently healthy subjects. Thin blood smears were prepared from each sample and then stained with Giemsa. The stained smears were examined under simple microscope using oil immersion lens (× 100 magnifications). Morphological characteristics of the different cells in the smears were identified. The Photos from each stained blood smear were captured using digital camera. Furthermore, Direct Coombs test was performed to all blood samples.

Ethical approval was obtained from the ethical committee at the National Center of Neurological Sciences. Institutional and individual consents were obtained from all participants.

Results

A total of 80 blood samples, 60 samples from diagnosed COVID-19 patients and 20 samples from apparently normal individuals as controls were included in this study. In 90% of the COVID-19 patients, the blood smears showed; RBCs with auto agglutination and rouleaux formation. Significant finding of ring-like stained bodies were seen within the RBCs in 98.3% of the patients (Figures 1and 2). No such ring bodies were detected in any of the control smears. In a number of smears stained particles were seen on the RBCs cell wall. Macrocytosis with central polar stomatocytes and tear shape RBCs were identified. A nucleus of neutrophils with fetus likes structure and anisocytosis and mild poikilocytosis was reported. The number of reticulocytes and platelets is scanty in the COVID patients compared to controls. All patients' blood smears showed marked lymphocytopenia, thrombocytopenia and slight neutrophilia with nuclei fetus like structure (Figures 3A-3D). Direct Coombs test was listed to be positive in 15% of the patients.

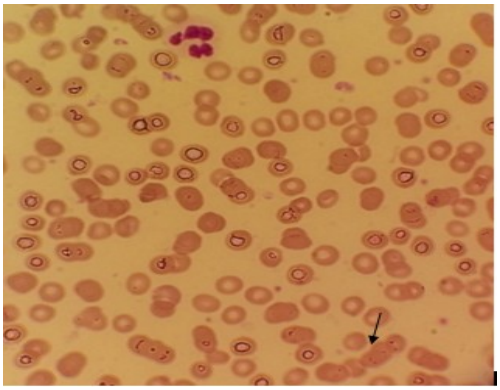

Figure 1: Geimsa stained smear showing, RBCs auto-agglutination and rouleauxformations (arrow), anisocytosis (different sizes) poikilocytosis (different shapes), and ring-like structures inside the RBCs. Magnification × 100.

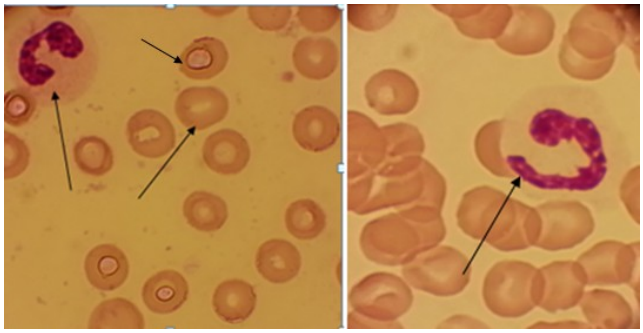

Figure 2: Black arrows indicate neutrophils with fetus-like stained nuclei, stomatocytes and ring-like structures inside the RBCs, note scattered staining on the surface of some RBCs. Magnification × 100 (Geimsa stain).

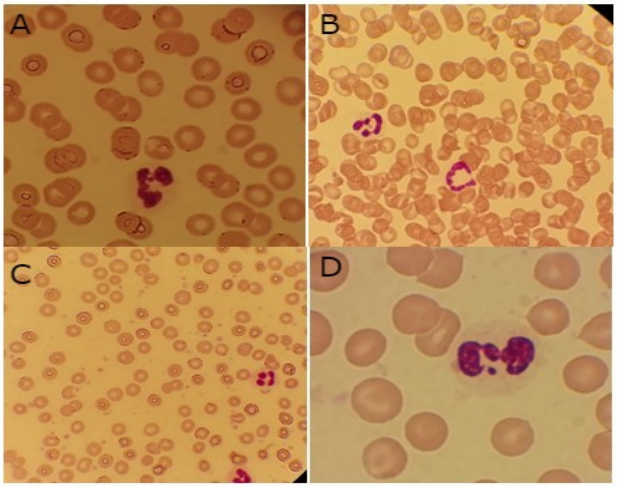

Figure 3: (A) Upper left figure shows ring-like structure material inside the RBCs and bizarre shaped neutrophil. (B)The upper right shows pearl-necklace like neutrophil lobules. (C)The lower left photo shows ring-like structure material inside the RBCs. (D)The lower right shows a neutrophil with kidney-like lobules (bow tie shape), Magnification × 100 (Geimsa stain).

In this study peripheral blood smears were studied to verify the cells morphology in patients who were diagnosed as COVID-19 positive. During this pandemic a number of diagnostic tests have been suggested to screen COVID-19 patients. Nasal swabs, CT chest and serology were all used at different levels. Little attention has been paid to the peripheral blood picture.

According to recent data, COVID-19 patients experience acute distress respiratory infection with preserved lung gas volume. One study showed that, pulmonary inefficiency which is detected in COVID-19 patients may not be caused by cell damage in the lungs only, and this postulation is supported by atypical presentation of Acute Distress Respiratory Syndrome (ARDS).

Moreover, most patients with COVID-19 who require intensive care will develop an atypical form of the Acute Respiratory Distress Syndrome (ARDS) with preserved lung gas volume. This might signify hypoxia due to physiological processes other than alveolar dysfunction, and might be responsible in disease progression and prognosis.

Discussion

Liu, et al. [10] based on recently published studies, reported in postmortem analysis evidence of pulmonary thrombosis instead of typical ARDS, and they concluded that COVID-19 does not cause pneumonia or ARDS.

Understanding the hematological manifestation in COVID-19 patients is still unclear, however one case study done in which investigated peripheral blood film, and based on morphological finding of monocytes and neutrophils, concluded that these findings would be helpful in the screening, diagnosis and management.

Other studies suggested the original pathologic viral process to begin in the lungs with subsequent general hypoxia most likely due to anemic iron dysmetabolism.

Liu et al. [10] have studied the pathogenesis of SARS-CoV-2 and obtained evidence indicating key pathogenic strategy based on the COVID 19 attacks the 1-beta chain of hemoglobin, and consequently initiates dissociation of porphyrins from iron discharging the later into the circulation. As an outcome, the ability of hemoglobin to bind with oxygen is destroyed, negatively affecting its delivery to the main organs of the body. The virus structural protein may instead bind to hemoglobin, shifting oxygen and iron.

All the above mentioned studies do not signify whether this abnormality of hemoglobin takes place within the RBCs or the complex form of hemoglobin that is circulating in the plasma.

Recently based on multi organ failure, another pathway of COVID pathogenesis is coming, which is hemoglobinopathy, and consequently two hypothesis of COVID-19 pathophysiology emerged, acute respiratory distress and attack of hemoglobin through CD147, CD26 and other receptors that are located on erythrocytes.

SARS-CoV-2 infection strictly depends upon the virus interaction with host cell's receptors and proteases. Together with the acknowledged ACE2, cyclophyllins, furins and TMPRSS2, CD147 was identified as an additional receptor on erythrocytes and other cells.

In fact, CD147 may also represent the virus entry-point in bone marrow immature cells. Finally, another receptor, namely DPP4 [CD26], was found to possibly interact with SARS-CoV-2 spikes. This molecule has been proven to play role in hematopoiesis.

To the best of our knowledge, the current study is the first that investigated RBCs morphology in patients with COVID-19 patients. The remarkable finding of ring like inclusion material within the RBCs in patients with COVID-19 needs further search. The central allocation of this material in an area rich of hemoglobin in the RBCs might reflect a metabolic interaction between presumably the COVID-19 virus and the RBCs. This assumption is supported by the clinical picture of COVID-19 which is dominated by hypoxemia. This could be explained by affinity of the virus to dissociate hemoglobin, releasing the ferritin component and subsequently disabling the RBCs as oxygen transporters.

A recent robust study revealed that corona virus proteins, particularly ORF10 has similar conserved domains to Heme Oxygenase (HO), a substantial enzyme in heme degradation pathway, in addition to a suggested role of the viral protein ORF1ab in capturing the released porphyrin. This study also promotes the notion that porphyrins are essential for virus's co-evolution, as they act as energy transporters as well as their higher permeability of the cell membranes which might facilitate corona virus accessibility into the cells and thus its infectivity.

Primarily, heme catabolism is a process of concurrent oxidation and reduction reactions. It requires both oxygen and reducing equivalents, and its end products are biliverdin, CO and iron hemeoxygenase and heme degradation. This pathway consists of Hemeoxygenase (HO), NADPH cytochrome P450 reductase and biliverdinreductase. HO is the rate limiting enzyme. It binds to heme to form ferric heme HO complex. NADPH cytochrome P450 reductase, by its electrons, converts the ferric heme iron into ferrous state. Subsequently, molecular oxygen binds the complex and yields an oxy-form complex. Following receiving of electrons and protons from reductase and water pocket, respectively, hydro peroxide intermediate is made up. Eventually, ferric alpha meso hydroxyheme is formed after hydroperoxide attack on the alpha meso carbon of the porphyrin ring. Furthermore, an oxidation reaction proceeds to produce ferrous verdoheme. Consequently, *via* hydroperoxide intermediate and biliverdin reductase, ferric iron biliverdin is produced. Lastly, the ferric iron of biliverdin is reduced to ferrous, and iron free biliverdin is obtained. It is inferred that both NADPH cytochrome P450 and biliverdin-reductase compete for the binding sites on the HO. The affinity of HO to NADPH cytochrome P450 reductase is ultimately strengthened by addition of NADPH at the expense of biliverdinreductase actions and thus subsequent biliverdin formation from porphyrins Hemeoxygenase and heme degradation.

In light of the above-mentioned data, we propose that corona virus might induce an incomplete heme degradation reaction to obtain its preferred energy transfer and transporter, porphyrin. We assume that this reaction takes place inside the red blood cells. Apparently, the red blood cells could be the preferable host for the virus to gain porphyrin, for numerous arguably reasons. Firstly, it will provide the virus with the energy and reducing agents to induce heme degradation. Virtually, the unique anaerobic glycolysis and pentose phosphate pathways' vital contributions are not limited to the energy production; however, it

extends to produce crucially important products such as NADPH and 2,3DPG. NADPH is essentially used by the RBC enzyme, mythemeglobin-reductase, to maintain heme iron in its functional ferrous state and thus to carry oxygen effectively. As the pentose phosphate pathway NADPH and glutathione function in maintaining the integrity of RBC membrane and hemoglobin normal structure against oxidative stresses.

Conclusion

We suggest that the virus might consume the RBC reducing agents-NADPH- in driving an incomplete heme catabolism reducing and oxidizing reactions and subsequent porphyrin release. Expectedly, the NADPH-depleted RBC might fail to keep its normal shape and size and even its functioning hemoglobin. This might explain the poikilocytosis and anisocytosis in our COVID-19 positive patients' peripheral blood smears. Moreover, it could promote the hypothesis that a hemoglobinopathy hemeoxygenase and heme degradation and particularly methemoglobinemia might be implicated in COVID-19 patients' hypoxia. The significant role of 2,3 DPG in regulating the oxygenation and deoxygenation states of the hemoglobin would also be disturbed. Despite the fact that increased levels of 2,3 DPG would boost the release of oxygen into tissues and reduce hemoglobin affinity to oxygen in return it would raise the level of deoxy-hemoglobin. We claim it might be a vicious cycle of continuous RBC glycolysis and heme degradation in favor of uninterrupted viral supply with porphyrin. As it is evident that abnormal configuration of red blood cell membrane proteins, especially band 3 proteins, in addition to abnormally increased levels of deoxy-hemoglobin-as we suppose it is the case in the virally invaded RBCs- would enhance further glycolysis reactions inside the RBCs, as heme degradation and eventual accumulation of porphyrins. According to our data, there is evidence which might be, this virus interacts with the hemoglobin within the erythrocyte and based on the pathogenesis of Plasmodium malaria, which enters erythrocytes through CD147 core receptor with significant role for the CD147 in several metabolic pathways. Similarly, COVID-19 may interact with hemoglobin molecule, through ACE2, CD147, CD26 on erythrocytes and other blood cell precursors; however, in our data there is lymphopenia with slight increase number in neutrophils and this may highlight the role of innate immunity, that the lymphocyte might be attacked by the virus. Taking the pattern of RBCs in our sample, the rouleauxformations may denote the clotting feature and consequences the thromboembolic phenomena and hypoxia in advance COVID patients. In conclusion the morphology of the RBCs in peripheral blood Giemsa stained smears; seem to be predictable easy tool to establish the diagnosis of COVID-19. Presence of ring form stained inclusions within the RBCs is highly indicative of COVID-19 affection. These features might change our knowledge about the pathogenesis of COVID-19.

Recommendations

Based on our finding and possible pathogenesis of this virus, further studies are needed to determine the binding receptors of the COVID-19 virus within the RBCs. During this pandemic and its disastrous sequel, use of cytotoxic drugs can be proposed to improve and enhance the micro-environment of the RBCs.

Acknowledgment

We give our deep gratitude to all patients who agreed to collect their blood samples, and to the medical staff working in COVID 19 isolation units. Thanks to the staff of the National Center for Neurological sciences, research lab, Khartoum Sudan.

References

1. Klausegger A, Strobl B, Regl G, Kaser A, Luytjes W, et al. (1999) Identification of a coronavirus hemagglutinin-esterase with a substrate specificity different from those of influenza C virus and bovine coronavirus. J Virology 73:3737–3743.
2. Tyrrell DAJ, Bynoe ML (1965) Cultivation of a novel type of common-cold virus in organ cultures. Br Med J 1:1467-1470.
3. Alanagreh LA, Alzoughool F, Atoum M (2020) The human coronavirus disease COVID-19: its origin, characteristics, and insights into potential drugs and its mechanisms. Pathogens 9:331.
4. Van Der Hoek L, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJ, et al. (2004) Identification of a new human coronavirus. Nat Med 10:368–373.
5. Cerami C, Popkin-Hall ZR, Rapp T, Tompkins K, Zhang H, et al. (2021) Household transmission of SARS-CoV-2 in the United States: living density, viral load, and disproportionate impact on communities of color. Clin Infect Dis 74:1776-1785.
6. Parry RL (2015) Travel alert after eighth camel flu death. The Times 2:22-27.
7. Al-Tawfiq JA, Zumla A, Memish ZA (2014) Travel implications of emerging coronaviruses: SARS and MERS-CoV. Travel Med Infect Dis 12:422-428.
8. Chen N, Zhou M, Dong X, Qu J, Gong F, et al. (2020) Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The lancet 395:507-513.
9. Pfefferbaum B, North CS (2020) Mental health and the Covid-19 pandemic. NEJM 383:510-512.
10. Gharizadeh B, Yue J, Yu M, Liu Y, Zhou M, et al. Navigating the pandemic response life cycle: molecular diagnostics and immunoassays in the context of COVID-19 management. IEEE Rev Biomed Eng 14:30-47.