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Clinical Significance of CBC Parameters and Peripheral Blood Morphology in the Diagnosis of Covid19 in Khartoum State 2022

A Thesis Submitted in Partial Fulfillment for the Requirements of M.Sc. Degree in Medical Laboratory Science (Hematology and immunohematology)

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Dedication

I would like to dedicate my thesis

To my parents, friends and fellow members without whom it was almost impossible for me to complete my thesis work ،

Acknowledgments

All my thanks are in the name of Allah the most gracious and the most merciful.

In this instance I extend my thanks, deep sincere gratitude and honest appreciation to my supervisor Dr Abdallah Omer Abdallah.

Department of hematology and immunohematology, Sudan university of science and technology, for the kindness .good guidance, valuable direction that has kept me on the track.

I express my deep thanks to all of my colleagues and friends of their help, encouragement .support and for all of the joyful moments we have had during this time .my thanks are also extended to Gebra Quarantine, and all staff members of hematology department –SUST. I feel indebted to many people who participated and helped me in this work.

Abstract

Hematological parameters can be affected by many diseases. Corona virus is one of recent pandemic that affect many hematological parameters. Wuhan city reported a cluster of cases of atypical pneumonia with unknown etiology in December 2019.Subsequent contrast led to identification of a “novel” coronavirus that had caused the disease. Measurement of complete blood count parameters and peripheral blood morphology is necessary for the diagnosis and medical care of covid19.

This is a case control study conducted through the period from February 2022 to April 2022 in Gebra quarantine in Khartoum state. The study population comprise two groups of adults in different ages for both sex (male 59% and female 61%) in age range from (18 to 85 years old). Two hundred subjects were recruited for this study, hundred patients with COVID-19 infection and hundred healthy volunteers as control group. The data was collected using laboratory investigation to obtain complete blood count parameters and peripheral blood morphology results. Data collected using structured questionnaire which include general information and anverbal consent wasobtained. Three ml venous blood were collected from all participants and placed in EDTA anticoagulant containers. The data analyzed by SPSS version.

The study revealed that Neutrophil, MPV, PDW, PLCR,NLR, WBCs and MCHC values of case group were significantly higher values (*P*.V 0.000),(*P*.V 0.000),(*P*.V 0.000),(*P*.V 0.000),*(P*.V 0.000),(*P*.V 0.000),(*P*.V 0.000) respectively compared to control group.

Hemoglobin, Lymphocyte, Platelet, MCV, MCH and RBCs parameters of case group were significantly lower values (*P*.V 0.000),(*P*.V 0.000),(*P*.V 0.000),(*P*.V 0.000),(*P*.V 0.000),*(P*.V 0.000) in patients with COVID-19 compared to control group.

The study concluded that COVID-19 patients share a common pattern of complete changes in morphology and blood count parameters.

In this study it is observed that there is no significant relationship between patient’s gender and COVID-19. Where there is a significant relationship between patient’s age and COVID-19.

المستخلص

يمكن أن تتأثر قيم أمراض الدم بالعديد من الأمراض. فيروس كورونا هو أحد الأوبئة الحديثة التي تؤثر على العديد من التعدادات الدموية. أبلغت مدينة ووهان عن مجموعة من حالات الالتهاب الرئوي مجهولة السبب في ديسمبر/كانون الأول 2019. وقد أدى التباين اللاحق إلى تحديد فيروس كورونا "المستجد" الذي تسبب في المرض. من الضروري قياس تعداد الدم الكامل وتشكل الدم الطرفي لتشخيص كوفيد 19 والعناية الطبية به.

هذه دراسة حالة تم إجراؤها خلال الفترة من فبراير 2022 إلى أبريل 2022 في الحجر الصحي في مركز العزل جبرة بولاية الخرطوم. وتضم مجموعة الدراسة مجموعتين من البالغين من مختلف الأعمار لكل من الجنسين (59 في المائة من الذكور و 61 في المائة من الإناث) في العمر تتراوح أعمارهم بين (18 إلى 85 عاما). تم توظيف مائتي شخص لهذه الدراسة، مئة من المرضى المصابين بعدوى كوفيد-19، ومئة من المتطوعين الأصحاء كمجموعة تحكم. تم جمع البيانات باستخدام الفحوصات المختبرية للحصول على قيم تعداد الدم الكاملونتائج تكوين الدم الطرفي. تم الحصول على البيانات المجمعة باستخدام استبيان منظم يتضمن معلومات عامة وموافقة شفوية. تم جمع ثلاثة مل من الدم الوريدي من جميع المشاركين ووضعها في حاويات المضادة للتخثر.تم تحليل البيانات بواسطة الحزمة الأحصائية للعلوم الأجتماعية.

في المرضى اعلى Neutrophilو MPV و PDW و PLCR و NLR و WBCs و MCHCكشفت الدراسة ان قيم على التوالي. (P.V 0.000)و (P.V 0.000)و (P.V 0.000)و (P.V 0.000)و (P.V 0.000)مقارنة بمجموعة الأصحاء في المرضى المصابين بفيروس كورونا Hemoglobin, Lymphocyte, Platelet, MCV, MCH and RBCsوقيم بقيم اقل مقارنة بمجموعة الأحصاء,(P.V 0.000) ، (P.V 0.000)، (P.V 0.000)، (P.V 0.000)، (P.V0.000)

خلصت الدراسة إلى أن مرضى فيروس كورونا المستجد (كوفيد-19) يتشاركون نمطا مشتركا من التغيرات الكاملة في شكل الدم الطرفي وقيم تعداد الدم.

في هذه الدراسة، يلاحظ عدم وجود علاقة كبيرة بين نوع المريض وفيروس كورونا المستجد (كوفيد-19). وان هناك علاقة كبيرة بين عمر المريض وفيروس كورونا المستجد.

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Ace2	Angiotensin converting enzyme 2
Alc.	Absolute lymphocyte concentration
AMC	Absolute monocyte concentration
ARDS	Acute respiratory distress syndrome
CBC	Complete blood count
CCL7	Chemokine ligand7
CFR	Case fatality rate
COVID-19	Corona virus disease 2019
DC	Direct current
E protein	Envelope protein
ETDA	Ethelyne diamine tetraacetic acid
HB.	Hemoglobin
He protein	Haemagglutinin esterase
Ldh	Lactate D hydrogenase
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
MCV	Mean cell volume
Mers	Middle East respiratory syndrome
MI	Myocardial infarction
MPV	Mean platelet volume
MXD	Mixed
N protein	Nucleocaspid protein
NLR	Neutrophil lymphocyte ratio
Pb	Peripheral blood
Pcr	Polymerase chain reaction
PDW	Platelet distribution width
Plcr	Platelet large cell ratio
PLT.	Platelet
Rbcs.	Red blood cell
Rna	Ribonucleic acid
Rt-pcr	Reverse transcrptase polymerase chain reaction
S protein	Spike protein
SARS-COV-2	Severe acute respiratory syndrome coronavirus
TAT	Thrombin antithrombin complex
TG	Thrombo globulin
TGF	Tissue growth factor
Tlc	Total leukocyte count
Wbcs.	White blood cell

Chapter One

CHAPTER ONE

1 INTRODUCTION

1.1Introduction:

Hematological parameters can be affected by many diseases. Covid-19 is one of the recent pandemic that affect many hematological parameters. Wuhan city, China, reported a cluster of cases of atypical pneumonia with unknown etiology in December 2019.Subsequent contrast led to identification of a "novel" coronavirus that had caused the disease. This new virus was later named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease caused by it was named as coronavirus disease 2019 (COVID-19). In India, the first COVID-19 case was reported on January 30, 2020, and more than 3.68 million positive cases had been reported by the end of August 2020.Due to the increasing patient load and the wide spectrum of clinical illness ranging from asymptomatic infection to fulminant disease. *(Jain et al., 2022)*

As the symptoms, fever is often occurring among Covid-19 cases, which can be accompanied by no symptoms. Dry cough, shortness of breath, dizziness, muscle ache, sore throat, rhinorrhea, headache, diarrhea, chest pain, vomiting, and nausea are other symptoms of Covid-19 infection. Sometimes patients had no fever, and several had no irregular radiological findings (Guan et al., 2020).

A covid-19 pandemic occurs to be an issue to the world. All health professionals have attempted to find out how to prevent, manage, and cure this pandemic.

COVID-19 patients can be classified as mild, moderate and severe. Several hematological parameters such as platelets, total white blood cell count, lymphocytes, neutrophils (along with the neutrophil to lymphocyte and platelet to lymphocyte ratio) and hemoglobin have been to describe the current state of the complete blood count changes during COVID-19 infection and to summarize the crucial role of some hematological parameters during disease progression. Decreased counts of platelets, lymphocytes, hemoglobin, eosinophils, and basophils, increased counts of neutrophils, and increased neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios have been associated with COVID-19 infection and a worse clinical outcome (IPata Dedy *et al*., 2020).

1.2 Rationale:

Many studies from China and other parts of the world showed Changes in hematological parameters associated and demonstrated with COVID-19 infection,The value of these changes can be used in forecasting, prognosis and medical care patients.No enough data regarding this issue in Sudan. Present study therefore aimed to assess the CBC parameters and morphology in Covid19 patients in Sudan.

1.3Objectives:

1.3.1 General objective

To investigate the clinical importance of CBC and peripheral blood morphology in the diagnosis of covid19.

1.3.2 Specific objectives:

1-To estimate CBC count in covid19 patients and control group.

2-To estimate peripheral blood changes in covid19 patients and control group.

3-To evaluate CBC and peripheral blood morphology in covid19 patients.

Chapter Two

CHAPTER TWO

2 Literature Review

2.1 Background

Mesoviridae and Roniviridae CoV are RNA viruses of the Coronavirinae subfamily. They belong to the family Coronaviridae and the order Nidovirales (nido Latin for "nest"). The order Nidovirales consists of the families Coronaviridae, Arteriviridae. (jie cuj et al., 2019).

The distinctive features of Nidovirales are as follows: they (1) contain very large genomes for RNA viruses, (2) are highly replicative due to the conserved genomic organization(3) exhibit multiple unique enzymatic activities,(4) extensive ribosomal reading frame switching for the expression of numerous non-structural genes. The Coronaviridae family has two subfamilies: Coronavirinae and Torovirinae. The subfamily Coronavirinae consists of Alpha-CoV, BetaCoV, Gamma-CoV and Delta-CoV based on the genomic structure (cong Y *et al*., 2017).

2.1.1Viral structure

The CoV are enveloped positive single-stranded RNA viruses with the largest known viral RNA genomes ranging in size from 8.4–12 kDa. (van der hoekL *et al*., 2004)

The viral genomes consist of 5' and 3' terminal ends. The 5' end makes up a major part of the genome and contains open reading frames that encode proteins responsible for viral replication. The 3' end contains the five structural proteins, namely the spike protein (S), membrane protein (M), nucleocapsid protein (N), coat protein (E) and the hemagglutinin esterase (HE)

Protein (Beniac DR *et al*., .2006).

The S protein mediates attachment and fusion between the virus and the host cell membrane and also between the infected and neighboring uninfected cells. They are the main inducers for the neutralization of antibodies in a vaccine. The N protein forms RNA complexes that aid in viral transcription and assembly. The M protein is the most common structural protein and also defines the shape of the virus envelope. The E protein is the most enigmatic and smallest of the major structural proteins that is highly expressed in the infected cell during the viral replication cycle. The HE protein is responsible for receptor binding and host specificity (van der hoekL et al., 2004).

2.1.2 SARS and MERS

SARS was first recognized in Guangdong province, China, in November 2002. It advanced among 30 countries, infecting 79.000 people by 2003 with a fatality of 9.5%. SARS-CoV was

traced and isolated from Himalayan palm civets found in a livestock market in Guangdong, China (cheng VC et al,.2007).

The zoonotic origin of SARS was also discovered in dogs, ferret badgers and in humans working at the same market. These market animals were therefore intermediate hosts that increased the transmission of virus to humans (Woo PC *et al*, .2005).

Thereon, in 2012, Jeddah, Saudi Arabia, a patient presented with respiratory illness consistent with pneumonia along with features of renal failure. The patient's sputum analysis was done by reverse transciptase (RT-PCR) using pan-CoV primers revealing the viral RNA to be MERS-CoV.26 As of July 2013, 91 patients were infected with MERS-CoV and had a high fatality rate of 34%. Bats and Arabian dromedary camels were identified aspotential hosts for MERS-CoV. Intermediate host reservoir species were also seen in goats, sheep and cows (Chan JF *et al* 2015).

2.1.3 Novel CoV

In view of taxonomical classification, SARS-CoV-2 (COVID-19) is one among many other viruses in the species, SARS-related CoV. However, SARS-CoV and SARS-CoV-2 vary in terms of disease spectrum, modes of transmission and also diagnostic methods (Cong Yet *etal*, .2017).

The recent report on a cluster cases having respiratory illness in Wuhan, Central China, was followed by a global spread of the disease in a very short duration of time. The samples (oral and anal swabs, blood and broncho-alveolar fluid lavage) from patients admitted to the intensive care unit of Wuhan Jinyintan Hospital were sent to Wuhan Institute of Virology. Pan-CoV PCR primers were used and these samples were positive for CoV (Meo sa *et al*, .2020).

This was followed by metagenomics analysis and genomic sequencing study. The results revealed that this virus was identical (79.6%) to the genetic sequence of SARS-CoVBJ01 leading the WHO to call it novel CoV-2019 (2019-nCoV) (Malik YS 2020).

2.2 transmission and pathogenesis

2.2.1 Zoonosis

CoV are widespread among birds and mammals with cements bats forming the major evolutionary reservoir and ecological drivers of CoV diversity. CoV causes a large variety of diseases in pigs, cows, chicken, dogs and cats. The major diseases caused by CoVs in animals are transmissible gastroenteritis virus, porcine epidemic diarrhoea virus, porcine hemagglutinating encephalomyelitis virus and murine hepatitis virus. In humans, alpha and

beta CoV have caused a variety of illness ranging from mild-self-limiting respiratory infections (HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1) to severe acute respiratory distress syndrome (ARDS). (Godet M *et al*, .1992)

Initial cases reported in Wuhan, China, are considered to be an acquired infection from a zoonotic source from Huanan wholesale seafood market which sold poultry, snake, bats and other farm animals (Li Q *et al*, .2020).

2.2.2Modes of spread

Human-to-human transmission occurs through common routes such as direct transmission, contact transmission and airborne transmissions through aerosols and during medical procedures. Cough, sneeze, droplet inhalation, contact with oral, nasal and eye mucous membranes are the common modes of spread. Viral shedding occurs from respiratory tract, saliva, faeces and urine resulting in other sources of virus spread. The viral load is higher and of longer duration in patients with severe COVID-19. Spread of COVID-19 from patients to health workers and flight attenders who were in close contact with the infected patients are also reported (Hanies *et al* ,.2020).

2.2.3 Virus–host interaction

Extensive structural analyses revealed atomic-level interactions between the CoV and the convoluted tubules), myocardium, bladder (urothelial cells) and also recently the oral mucosa. ACE2 receptors provide entry of the virus into the host cells and also subsequent viral replication. The main factors involved in viral pathogenesis of 2019-nCov are spike1 subunit protein, priming by transmembrane protease serine-2 (essential for entry and viral replication), ACE2 receptor–2019-nCov interaction and downregulation of ACE2 protein. These factors contribute to atrophy, fibrosis, inflammation and vasoconstriction resulting in host tissue injury (Xu,H *et al*,.2019).

Cross-species and human-to-human transmission of COVID-19 is mainly dependent on spike protein receptor-binding domain and its host receptor ACE2. High expression of ACE2 was identified in lung (type II alveolar cells), esophagus, ileum, colon, kidney (proximal)

2.3 clinical presentation and diagnosis

Based on numerous published studies, the median age was 56 years (range 55-65 years) and due to the high ACE2 levels in them, males were predominantly affected. Median onset of disease was 8 days (range 5-13 days) (Guan Wj *et al*., 2019).

Due to the limited availability of comorbid data, it is important to correlate with previously established susceptibility factors to SARS and MERS-CoV infection, which include smoking, hypertension, diabetes, cardiovascular disease, and/or chronic disease (Van Doremalen N *et al*., 2014).

Based on analysis by the National Health Institute in Italy, the median age at death for patients with COVID-19 was 81 years. In China, the case fatality rate (CFR) increased with age, showing a CFR of 18% for patients over 80 years (Wilson N *et al*., 2020).

This prominent target for the elderly population is attributed to underlying chronic diseases and decreased immune function. Decreased immune function has been associated with cytokine storm syndrome (elevated circulating inflammatory cytokines) and hyperinflammatory syndrome. These syndromes are triggered by viral infections and are also predictors of death in patients with COVID-19 (Mehta P *et al*,. 2020).

2.3.2 Signs and symptoms

Clinical features varied from mild disease to severe or fatal disease. The most common symptoms of COVID-19 were non-specific and mainly included fever, cough and myalgia. Other minor symptoms included sore throat, headache, chills, nausea or vomiting, diarrhea, senility and conjunctival congestion. COVID-19 was clinically classified as mild to moderate disease (no pneumonia and pneumonia), severe disease (dyspnea, respiratory rate above 30/min, oxygen saturation below 93%, PaO2/FiO2 ratio below 300 and/or pneumonia) infiltrates more than 50 % of lung field within 24-48 hours) and critical (respiratory failure, septic shock, and/or multiple organ dysfunction/failure) (singhal *et al*., 2020).

Many of the older patients with severe illness had evidence of a chronic underlying disease such as cardiovascular disease, lung disease, kidney disease or malignant tumors. (wang T et al,.2020)

2.4Laboratory Evaluation and Confirmation

Laboratory findings most consistent with COVID-19 were lymphocytopenia, elevated Creactive protein, and elevated erythrocyte sedimentation rate. Lymphocytopenia is due to necrosis or apoptosis of lymphocytes. The severity of lymphocytopenia reflects the severity of COVID-19.Procalcitonin was frequently elevated and associated with co-infection in most reported pediatric cases (Xia W *et al*., 2020).

Detection of COVID-19 is based on virological detection by RT-PCR using swabs (nasopharynx, oropharynx), sputum and feces, chest X-ray and dynamic monitoring of inflammatory mediators (e.g. cytokines) (Shi H et al., 2020).

2.4.1 Radiological Findings

The most standard patterns observed on chest CT were ground-glass opacity, ill-defined margins, smooth or irregular interlobular septal thickening, air bronchogram, crazy paving pattern, and thickening of the adjacent pleura. Chest CT is considered a sensitive routine imaging tool for COVID-19 (Wang YXJ *et al*., 2020).

2.5 MANAGEMENT:

When cluster infection is first presented, many cases have been treated with antiviral therapy, antibacterial therapy, and glucocorticoids. Observation forms the mainstay for those who have mild illness. Moderately ill patients with underlying chronic disease, immunocompromised conditions and pregnancy require hospitalization (Xu K *et al*., 2020). The antimalarial drugs hydroxychloroquine and chloroquine have shown promising results in early in vitro studies. However, the most robust and recent study in patients with COVID-19 has not provided clear evidence of the benefit of treatment with hydroxychloroquine or chloroquine. (Mehra MR *et al*. , 2020).

2.6Treatment of systemic complications of COVID-19

Extracorporeal membrane oxygenation is an excellent choice for patients with ARDS progressing to respiratory failure. Other treatment methods include high-flow nasal oxygen and endotracheal intubation. Patients with sustained refractory hypoxemia require a prone position, followed by neuromuscular blockade, inhaled nitric oxide (at 5 to 20 ppm), and also ensure optimal end-expiratory pressure by inserting an esophageal balloon (Matthay MA et al., 2020).

In shock with acute renal failure, a negative fluid balance must be achieved by dialysis. Antibiotics are used for pre- and post-exposure prophylaxis. This prevents illness from SARS-CoV-2 and also reduces the risk of secondary infection. Fluid management is important to reduce pulmonary edema. Glucocorticoids are best avoided because of their deleterious effects in viral pneumonia and ARDS. Rescue therapy by administration of an intravenous infusion of vitamin C has been suggested to attenuate vascular injury and systemic inflammation in sepsis and ARDS. (Fowler AA et al., 2019)

2.7 role of vaccines

Vaccine development for COVID-19 is ongoing, but there are several limitations. These include (1) the location for Phase 3 vaccine trials to be conducted at the site of ongoing disease transmission, (2) vaccine manufacturers to work closely with biotechnology

companies to develop effective vaccines, which will likely take at least 12-18 months, and (3) regulatory agencies should evaluate safety with a range of virus strains in more than one animal model (Du L *et al*., 2009)

The investigational vaccine is currently being developed using mRNA as a genetic platform using previous studies on SARS and MERS.16 24 The basis of an effective vaccine is immunospecific and involves the identification of B-cell and T-cell epitopes that are expressed by spike (S) and derive nucleocapsid (N) proteins from among 120 available SARS-CoV-2 gene sequences.24 Effective vaccination would play a crucial role in reducing virus spread and eliminating the virus from the host (Ahmed SF 2020) .

2.8Blood

Blood is specialized liquid connective tissue (Chauhan 2013), pumped by the heart through arteries and veins reaching all body's cells (Mehta and Hoff brand 2014). Functions Transport and distribute oxygen, nutrients, hormones and waste products, regulate PH, osmotic pressure and body temperature, control blood loss by assistance of Platelets and coagulation factors and involves in body's immune response which Mediated by leukocytes (Cheesbrough 2005).

2.8.1Constituents of blood

It consists of cells (erythrocytes and leukocyte) and cell fragments (platelets), surrounded by liquid extracellular matrix called plasma(Chauhan 2013). Plasma Forms about 55% of blood volume and contains water (95%) and many solutes, Including proteins, mineral ions, organic molecules, hormones, enzymes, products Of digestion and waste products for excretion. (Cheesbrough 2005).

Structure and Function of mature red blood cells (RBCs)

RBCs area discoid shape have specialized membrane flexibility which provide Large surface areas for gas exchange, and allow repeated passes through narrow Capillaries. RBCs lack nuclei and other organelles. These unique differences Enabling maximal cytoplasmic occupation by hemoglobin (Palis 2014; Blann and Ahmed 2014).

2.8.2Red Blood Cells Count

Assessment of the RBC is to check for anemia and to evaluate normal erythropoiesis. The number of red blood cells is determined by age, sex, altitude, diet, drug use, tobacco/nicotine use and health and disease status (Lokwani 2013).The results of an RBC count can be used to help diagnose blood-related conditions, such as iron deficiency anemia (where there are less

red blood cells than normal).A low RBC count could also indicate a vitamin B6, B12 deficiency. It may also signify internal bleeding, kidney disease or malnutrition.

A high RBC count could be caused by a number of health conditions or health-related factors, including: smoking, congenital heart disease and dehydration (NHS 2019).

2.8.3Hematocrit and red cell indices

The hematocrit is one of the most precise methods, used for red cell disorders differentiation and determination of the degree of anemia or polycythemia (Lokwani 2013). The red blood cell indices (MCV, MCH, and MCHC) provide information concerning the size and hemoglobin content of red blood cells (Ciesla 2012).

2.8.3.1MCV

MCV blood test measures the average size of red blood cells. Red blood cells carry oxygen from the lungs to every cell. Cells need oxygen to grow, reproduce, and stay healthy. If red blood cells are too small or too large, it could be a sign of a blood disorder such as anemia, a lack of certain vitamins, or other medical conditions.

red blood cells that are smaller than normal, it may be a sign of Certain types of anemia, including iron-deficiency anemia, the most common type Thalassemia, an uncommon genetic condition

Red blood cells that are larger than normal, it may be a sign of Pernicious anemia, which may be caused by A lack of vitamin B12.(medlineplus.gov, n.d.)

2.8.3.2MCH

MCH stands for "mean corpuscular hemoglobin." An MCH value refers to the average quantity of hemoglobin present in a single red blood cell. Hemoglobin is the protein in red blood cells that transports oxygen to the tissues of body

A low MCH value typically indicates the presence of iron deficiency anemia. Iron is important for the production of hemoglobin. Some of the general causes of iron deficiency include eating a diet that is low in iron, major surgery or trauma, or blood loss.

High MCH value can often be caused by anemia due to a deficiency of B vitamins,

particularly B-12 and folate.

(Seladi-Schulman, 2018)

2.8.3.3MCHC

MCHC stands for mean corpuscular hemoglobin concentration. It's a measure of the average concentration of hemoglobin inside a single red blood cell. High MCHC calculations in

Autoimmune hemolytic anemia, Hereditary spherocytosis And Severe burns low mchc can be caused by iron defiency anemia and lead poisoning. (Healthline, 2021)

2.8.4 Hemoglobin (Hb)

Hemoglobin molecule is composed of iron containing pigment called (heme) and Protein (globin) (Ramadas 2012).It binds efficiently to oxygen molecules, and Somewhat less efficiently to carbon dioxide molecules, thereby functioning in the Transport of gases through the bloodstream. (Palis 2014).Hemoglobin is measured To detect anemia and its severity and to monitor an anemic patient's response to Treatment (Cheesbrough 2005).

Medical conditions that can cause high hemoglobin levels include Polycythemia vera , Lung diseases such as, emphysema or pulmonary fibrosis , Heart disease, especially congenital heart disease

A low hemoglobin count means that the oxygen-carrying capacity of hemoglobin is reduced. Low hemoglobin levels can indicate that an individual has certain medical conditions, includingAplastic anemia, Cancer and Chronic kidney disease.

2.8.5 White Blood Cells:

The white blood cells (leucocytes) may be divided into two broad groups:

The phagocytes and the lymphocytes. Phagocytes comprise the cells of the innate immune system, which can act very quickly after an infection, whereas lymphocytes mediate the adaptive immune response, which can develop immunological memory. Phagocytes can themselves be subdivided into granulocytes (which include neutrophils, eosinophil's and basophil's) and monocytes (Hoffbrand and Moss, 2016).

White blood cells have fundamental roles in defense against invading microorganisms and recognition and destruction of neoplastic cells as well as their role in acute inflammatory reactions. Furthermore, through their phagocytic function, white blood cells are influential in clearing senescent and apoptotic cells, hence allowing tissue repair and Remodeling (Hoffbrand *et al*., 2005).

2.8.5 Neutrophils:

Neutrophils have a nucleus is divided into two to five segments or lobes, these lobes are separated by a thin strand or filament of nuclear material. The nuclear chromatin is heterogeneous with some clumping. The cytoplasm of neutrophils is very pale blue and is

packed with fine lilac-Ethical considerations Neutropenia is a condition where neutrophil count is too low, causing swelling and repeated infections. Causes of neutropenia include cancer treatment, an autoimmune disease or an infection.

Neutrophilia, also known as neutrophilic leukocytosis, occurs when neutrophil count is too high, which is often the result of a bacterial infection. To combat the infection, immature neutrophils leave your bone marrow too soon and enter blood stream.

2.8.5.3 Lymphocyte

A lymphocyte is a type of white blood cell that is part of the immune system. There are two main types of lymphocytes: B cells and T cells. The B cells produce antibodies that are used to attack invading bacteria, viruses, and toxins. The T cells destroy the body's own cells that have themselves been taken over by viruses or become cancerous (Clevel and clinic).

2.8.5.4Leukopoiesis

It is the process by which white blood cells are produced. It has three lines of cell development Lymphopoiesis. The main sites of lymphopoies are the primary lymphoid tissues (thymus for T

Lymphocyte and bone marrow for B lymphocyte) .It may also occur peripherally in the lymph nodes, spleen and peyer patches in the intestine (secondary lymphoid tissues) (Ciulla and Lehman, 2010).

Lymphocytic maturation is divided into three stages: the lymphoblast, prolymphocyte and lymphocyte (Rozenberg 2011).

2.8.5.5 Lymphopenia

Lymphocytopenia is most often due to AIDS, and recently COVID-19, or undernutrition, but it also may be inherited or caused by various infections, drugs, or autoimmune disorders.

(MSD Manual Professional Edition, n.d.)

Neutrophil to lymphocyte ratio

Systemic inflammation can be measured by using a variety of biochemical and hematological markers. Although novel disease specific biomarkers have been identified, most of which are time consuming and expensive. Observational studies have thoroughly investigated the role of C-reactive protein and total leukocyte count in different chronic conditions (Bovill et al.,1996; Flosom et al., 2002).

Recent evidence indicated that the ratio of sub types of blood cells have a significant prognostic value for cardiovascular disease. Elevated levels of neutrophil lymphocyte ratio

(NLR) were also found associated with poor survival of patients undergoing coronary artery bypass graft (Gibson et al., 2007).

By contrast, lymphocytes play vital roles in the remodeling of the myocardium following inflammation. For example, CD4+ T regulatory cells constitute a particularity-inflammatory immune regulatory lymphocyte subset which is generated in the thymus and highly enriched for T cells with auto antigen specificity (Stephenson et al., 2017).

T cells are essential for the recruitment of pro angiogenic macrophages and collateral artery formation (Tang et al., 2012). B cells are involved in monocyte recruitment through the CCL7 pathway (Stephenson et al., 2017).

The clearance of debris, activation of fibroblasts and collagen deposition for scar formation and neovascularisation (the proliferative phase) occur (Ruparelia et al., 2017; Ong et al., 2018) days after MI (Frangogiannis 2008, 2014; Prabhu et al., 2016).

The release of inflammatory and anti-inflammatory mediators IL-10, TGF and proresolving mediators (Prabhu et al., 2016; Zlatanova et al.,2016) from neutrophil or lymphocyte cells promotes neutrophil apoptosis and phagocytic up take by macrophages (Kolaczkowska et al., 2013; Horckmans et al., 2017)

2.8.6Platlelet count

A platelet count should always be performed to rule out the possibility of thrombocytopenia as the cause of bleeding symptoms. A low platelet count should be further investigated by a hematologist (Egberg and Blomback,2014).

Thrombocytopenia is defined as platelet count below 150000/cmm common cause of thrombocytopenia are hematological malignances ingestion of certain drugs, dissmented intravascular coagulation, idiopathic thrombocytopenia purpura, connective tissue disease megaloblastic anemia and a plastic anemia thrombocytosis (Platelet count >400000) occurs in Inflammation following hemorrhage and in myeloproliferative disorder (kawthalkar, 2013).

2.8.6.1Platelet indices

2.8.6.1.1Mean platelet volume (MPV)

The mean platelet volume is an indication of platelet size. Normal MPV ranges are Approximately 7 to 11 fl (Corcoran and Marchant 2002).

the MPV can be an indication of platelet turnover because younger platelets tend to be larger. A plspectrum of platelet sizes is seen in patients with rapid turnover (Corcoran and Marchant 2002).

the increase of MPV in conditions with increased .Elevated MPV levels have been identified as an independent risk factor for thrombotic diseases (Buttarello and Plebani, 2007). A low MPV can indicate exposure to certain drugs that are harmful to cells. It may also indicate marrow hypoplasia, a disorder that causes a decrease in blood cell production.

(medlineplus.gov, n.d.)

2.8.6.1.2Platelet distribution width (PDW)

A Measure of the variation in the size of platelets found in the circulating blood.

With normal range (10.0 -14.0/fl) Platelets recently released from bone marrow tend To be larger and to contain more RNA than older, smaller platelets, which discard Their endoplasmic reticulum as they mature medical dictionary, the volume is determined by a machine and a Complete Blood Profile, known as a CBC. This Reading determine if a patient's body is producing larger than average platelets,

Indicative of platelet destruction or bone marrow diseases (Chandrashekar, 2013).

high PDW means there is a great variation in size, which may be associated with vascular (blood vessel) disease or certain cancers Reduced platelet distribution width (PDW) was significantly correlated with age, carcinoembryonic antigen, tumor stage, nodule stage, and tumor-nodule-metastases stage (Zhang et al., 2017)

2.8.6.1.3 Platelet large cell ratio (P-LCR)

Means Platelet large cell ratio with normal range (13.0_43.0%) and it’s Calculated in automated blood analyzers .Increased percentage of large platelets (P-LCR) is observed in patients with Hyper-lipidaemia and suggest possible Risk of thrombosis. An increase in PLCR + MPV + PDW has been observed In autoimmune thrombocytopenic purpura PLCR is significantly decreased in patients with thromobocytopenia (Chandrashekar , 2013).

2.9 Previous studies

study from Saudi Arabia by Abozermean indices of Hb concentration (0.001), MCH (0.001), MCHC (0.001), and MCV (0.004) were all significantly lower in COVID-19 positive patients compared with negative, while the RDW was significantly higher (0.002). In contrast, the RBC count showed only a nonsignificant difference (0.113).

The mean indices were significantly lower for counts of lymphocytes (0.001), monocytes (0.001), and thrombocytes (0.001) among cases testing positive, while the neutrophil count was significantly elevated (0.001). The TWBC count (0.609) and the mean platelet volume (0.269) showed only a nonsignificant difference (0.113),

The prevalence of anemia was significantly elevated in patients with COVID-19 compared with those without (controls), respectively, at 64.2% and 35.8%. On the one hand, cases were 2.5 times more likely to be anemic; on the other, the prevalence of thrombocytopenia was significantly elevated in cases over controls, respectively, at 62% and 38%, where cases were 2.6 times more likely to be leukopaenic.

(Abozer et al.,2022).

In Kurdistan Region-Iraq Al nimer and others from total number of 204 patients were included the number of male patients was non-significantly ($\chi 2$ =0.873,P=.832) higher than the corresponding female patients There is no significant difference in the red cell indices in patients with or without concomitant diseases. The red cell indices were within the normal range. White cell indices showed the mean values are ≥ 10.000 cell/mm3, which characterized by a higher percentage of neutrophil and a lower percentage of lymphocyte in each patient group with or without concomitant diseases. The mean values of the blood platelet indices are within normal limits, and the platelet count was higher than the lower limit of normal platelet count (150,000/mm3).

(Al-Nimer MSM., et al 2021) .

Another Study from turkey done by Eren usui Comparisons made according to the RT-PCR test results revealed that while no statistically significant difference was observed between test result groups (negative or positive) regarding lymphocyte and platelet lymphocyte ratio values ($p > 0.05$), a statistically significant difference ($p < 0.05$) was found between the test result groups regarding platelet, hemoglobin, leukocyte, neutrophil, NLR and SII values. In patients with negative test results, it was found that platelet, leukocyte, neutrophil, NLR and

SII values were higher, whereas hemoglobin was found to be higher in patients with positive test results. Also, hemoglobin was found to be higher in male patients who tested positive for COVID-19 (Eren *et al.*, 2020).

Abdul warris found a significant association between the disease severity and elevation in blood parameters were observed. The WBC's and granulocyte count were significantly increased (p value <0.001) while the mean platelet count (165.0 × 10^9/L) and red blood cell volume distribution width (RDW) were decreased. In the critical group (57.86%) compared to mild group's patients (177.3%) (p = 0.83). The lymphocytes count was decreased in critical patients (1.40 × 10^9/L) compared to mild patients (1.92 × 10^9/L) (p = 0.28). A significant association was observed in platelet-lymphocyte ratio (p< 0.001), Neutrophil-Lymphocyte ratio (p = <0.001), and Lymphocyte-Monocyte ratio (0.011).

(Abdul warris et al., 2021) .

Also study from Morocco with total of 146 patients with COVID-19 the CBC parameters showed neutropenia (7.5%), hyperleukocytosis (8%), eosinopenia (47%), monocytosis (9.5%), lymphopenia (46%), and thrombopenia (10%).

(Maryame *et al*., 2020).

Abdul jappar with 12 confirmed cases as COVID-19 were analyzed. The median age of subjects involved in the present study was 50 years. Data from 8 males (66.7%) and 4 females (33.3%) were used for analysis. The CBC findings reveal lower HGB, reduced PCV, and slightly lowers RBC levels in patients with comorbid conditions. Besides, there was a significant difference in RBC, HGB, PCV, and MCV. The abnormalities of HGB, PCV, and RBC or anemia were observed in patients with comorbidities (Abdul jappar. *et al*.,2021).

In Indonesia the results showed Lower hemoglobin, hematocrit, and increased RDW.

White blood cell parameters and differential count: Lower leukocyte level, but relatively Higher in patients with severe disease, increased neutrophil, lymphopenia (and thus Causing elevated NLR and MLR), and eosinopenia up to the absence of eosinophils.

Platelet parameters showed Possible thrombocytopenia, increased PLR, and increased MPV and PDW (Kevin S Dhinata., *et al* 2021) .

Chapter Three

CHAPTER THREE

3 MATERIALS AND METHODS

3.1Study Design

This is a descriptive case control study.

3.2 Study Setting and Duration

The study conducted in Gebra quarantine, Khartoum state, Sudan in the period between February 2022 to April 2022.

3.3 Study population

Patients with COVID-19 positive confirmed by real time polymerase chain reaction test as case and Matched group of samples were collect from healthy individuals as control.

3.4 Sample size

100 samples as patients diagnosed with covid19 and 100 samples as control

3.5 Inclusion criteria

Patients with COVID-19 positive from both sexes were included.

3.6 Exclusion criteria

All patients that suffer from other diseases that may affect result of the studies are excluded.

3.7Sample collection

Venous blood were collected using sterile disposable plastic syringe after cleaning the vein puncture area with 70% ethanol from each subject, five of ml venous bloods were collected from all participants 3 ml was added to ethylene diamine acetic acid (EDTA) anticoagulant containers.

3.8 Data collection

Self administered data questionnaire was design to obtain subjects information**.**

3.9 Methodology

Principle of Electronic impedance, or low- voltage direct current (DC).

3.8.1.1 Method Test Performed

Complete blood counts CBC was done using Sysmex automated hematology analyzer xp300 Series.

3.8.1 Detection Methods

Blood sample is aspirated measured to a predetermined volume diluted at the specified ratio and then fed into each transducer. The transducer chamber has a minute hole called the aperture. On both side of the aperture there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through aperture causing direct current resistance to change between the electrodes. As direct current resistance changes the blood cell size is detected as electric pulses blood cell count is calculated by counting the

pulses and a histogram of blood cell sizes is plotted by determining the pulse sizes. Also analyzing a histogram makes it possible to obtain various analysis data (Li *et al*., 2013).

3.8.1.2 Quality Control

The reliability of this instrument and reagents is monitored by quality control, by using control blood or control materials. The stability of the measured value is monitored over a certain period of time and a quality control performed before analyzing sample, after replacement of the reagent, after maintenance,(Sysmex Corporation 2012 - 2014).

The control materials are of three types eightcheck-3WP-N (Normal), EIGHTCHECK-3WPL (Low level) and eight check -3WP-H (High level) (sysmex corporation 2012-2014). This instrument has following 2 quality control methods; choose the control method in accordance to your laboratory internal regulations, this methods are X control which use control blood (EIGHTCHECK-3WP) to monitor an instrument performance over time, and Levey - Jennings (L-J) control , which use the data from a single analysis of control blood as quality control data (Sysmex corporation 2012-2014).

Quality control process flow selects quality control method, set control blood information, perform quality control analysis, check and record quality control results (quality control chart screen) (Sysmex Corporation 2012-2014).

3.8.1.3Thin blood film

A thin film was made byplacing a small drop of blood on the centre of a grease-freemicroscopic slide. The drop of blood was then spread with a glass spreader held at an angle of 30° to obtain a thin film with a smooth tail end. Then allowed to air dry in a horizontal position and then fixed with absolute methanol for two minutes.

3.8.1.4Leishman stain

The slide was covered by Leishman Stain solution by counting the drops of Leishman stain for 3 minutes, the film was covered with distilled water for 7 minutes. The film washed carefully in tap water dried in air and examined (Rana, 2018)

3.9 Ethical approval

The study approved by the scientific ethical committee of medical laboratory science College of Medical Laboratory science Sudan University of science and technology. A written consent was obtained from Gebra hospital and also from participants after they been informed with the objectives and benefits of the study. Participants were insure that collected information will be kept confidential and will not be used for any other purpose than this study.

All guidelines for the safe collection and handling of specimens disposal of samples collected from COVID- 19 and suspected COVID- 19 patients had been followed.

4.10 Data analysis

The collected data was entered cheeked and processed by using a computer based statistical program SPSS version 24 (statistical package for social science) version (mean±STD, *P* value significant ≤ 0.05) (one way a nova test, independent sample T test).

Chi test for factors associated with COVID-19.

Chapter Four

CHAPTER FOUR

4 RESULTS

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), formerly known as 2019nCoV, has attracted tremendous attention in a short period of time as the death toll and number of confirmed cases grows unceasingly.

This study sample consisted of (200) participants of two groups. Control group and case group each group consists of (100) participants patients group with age mean of (52 ±19.6). While in control group (43 ±11.3).

Concerning gender variable, females counted (59%) of case group, while they counted (61%) of control group.

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Table (4.1) Factors Associated With Covid-19 Patients

Independent Factors	**Chi-Value**	***p*-value**
Age	99.270	0.000*
Gender	0.083	0.773

*Relationship is significant at p<0.05.

Results showed there is no significant relationship between patients gender and covid19 disease while there is a significant relationship between patients age and covid19.

Red blood cells parameters table of this study showed mean of red blood cell count, MCV, MCH, MCHC, is(3.6) (83.1) (26.6) (31.7) respectively. while in control group the mean of Red blood cell count, MCV ,MCH,MCHC is (31.8) (86.9) (28.3) (4.4) respectively. Regarding cells morphology results the red blood cells were Normocytic Normochromic cells with no changes.

Table (4.2) Red Blood Cells Parameters

Parameters	**Group**	**Mean**	***P* value**
Haemoglobin	Patients	9.3	0.000
	Control	12.4	0.000
MCH	Patients	26.6	0.000
	Control	28.3	
MCHC	Patients	31.7	0.000
	control	4.4	
RBCs	Patients	3.6	0.000
	Control	31.8	

Platelet parameters table of this study showed the mean of platelet count ,MPV, PDW,PLCR is (269.9) (12.4) (11.6) (26.8), respectively. While in control group the mean of platelet count, MPV, PDW, PLCR is (326.1) (11.4) (9.4)(20.8), respectively.
Regarding cells morphology results showed giant platelets with different sizes.

Table (4.3)Platelet Parameters

Parameters	Group	Mean	*P* value
Platelet	Patients	269.9	0.007
	Control	326.1	
MPV	Patients	12.4	0.008
	Control	11.4	
PDW	Patients	11.6	0.000
	Control	9.4	
PLCR	Patients	26.8	0.000
	Control	20.8	

WBCs parameters table of this study showed mean of total white blood cell, lymphocyte, neutrophil, NLR is (11.6) (15.5) (76.7) (9.2) respectively. While in control group the mean of total white blood cell, lymphocyte, Neutrophil, NLR is (6.5) (33.4) (57.2) (1.9) respectively.

Regarding cell morphology resultsthe morphology of WBC showed changes such as monocyte, lymphocyte and neutrophil vacuolization higher numbers of lift shifted immature granules pseudu pelger huet cells.

Hypo granular cytoplasm and hyposegmented nucleus. Atypical eosinophils containing multiple vacuoles. Rare activated lymphocytes and large Monocytes were found in some peripheral blood films.

Table (4.4) White Blood Cells

parameters	group	mean	*P* value
Lymphocyte	Patients	15.5	0.000
	Control	33.4	
Neutrophil	Patients	76.7	0.000
	Control	57.2	
WBCs	Patients	11.6	0.000
	Control	6.5	
NLR	Patients	9.2	0.000
	Control	1.9	

Chapter Five

CHAPTER FIVE

5Discussion, Conclusion And Recommendations

5.1 Discussion

The prevalence of COVID-19 cases has increased worldwide. Assessing clinical, demographic and hematological indicators is important when investigating the COVID19 outbreak. The present study investigated the role of complete blood count parameters and peripheral blood morphology for diagnosis and prognosis of COVID-19 in Sudanese patients with group of age mean of (52 ±19.6). While in control group age mean of (43 ±11.3). Concerning gender variable, females counted (59%) of case group, while they counted (61%) of control group.

Regarding white blood cells parameters Neutrophil WBCs count and neutrophil lymphocyte ratio mean level is higher in covid19 patients when compared to control group.*P* value is (0.000) (0.000) (0.000) respectively ,while lymphocyte count mean levels in case group is lower than control group with *P* value (0.000) .These findings similar to the present study

Rbcs indices RBCs count MCV and MCH mean level is lower in case group comparing to control group and the *P* value is (0.000) (0.000) (0.000) respectively.

MCHC mean level were significantly high in covid19 patients comparing to control group the *P* value is (0.000) . This result was supported by Abozer demonstrated thatHb concentration, RBC count, Haematocrit (Hct), Mean cell hemoglobin (MCH), and Mean cell hemoglobin concentration (MCHC) median values are significantly decreased in COVID-19 patients compared with the control group *(P* ≤ 0:01, *P* ≤ 0:01, *P* =0:041, and *P* ≤ 0:01, respectively) also agrees with Yuan and coworkers , who found that severe and critically ill patients had significantly decreased RBC and Hb and with another study reporting a rapid decline of Hb and RBC among COVID-19 patients. (Abozer Y. Elderdery et al., 2020).

According to platelet parameters results the mean levels of MPV, PDW, PLCR are higher in cases group than the control group and the difference was significant *P*.value (0.008), (0.000), (0.000), respectively. While platelet count mean levels in covid19 patients are lower than the control group with *P*.value (0.007). Results of current study are similar to study done by (Shankaralingappa, et al 2022) who found thrombocytopenia in COVID-19 patients with *P*value less than .05 compared to controls, and MPV, PDW, and PLCR were significantlyincreased ($p<0.05$) high in COVID-19 patients.

In comparison to controls Analysis of Variance (ANOVA) test results showed there is significant differences in means of all the studied parameters between patients and control

group at level of significance 0.05 and degree of freedom (198), *P*-values in all cases were less than 0.05.

Hemoglobin, Lymphocyte, Platelet, MCV, MCH and RBCs levels were lower in patients than in control.

On other hand, Neutrophil, MPV, PDW, PLCR, WBCs and MCHC were higher in patients than in control.

In this study Covid-19 patients had significant lymphocytopenia compared with non COVID19 patients.

Also Results showed there was significant difference in NLR between case group and control. The case group shows higher range of NLR (minimum= 1.6, maximum =71.8, mean= 9.1), while the control group shows lower range of NLR (minimum= 0.7, maximum =1.9, mean= 4.8), the difference was significant at 0.05 level of significance. So NRL with the above parameters can be used as an early warning signal for COVID-19 infection. Morphology of WBCs in this study showed many changes such as higher numbers of left shifted immature granules, pseudu pelger huet cells, hypo granular cytoplasm and hyposegmented nucleus and Large monocytes with vacuoles.

Platelets showed frequent anomalies mainly consisting of giant platelets with different sizes.

These findings are similar to study done by kevin with composite image of peripheral blood WBCs showing a spectrum of morphologic changes in coronavirus disease 2019 Segmented neutrophilia with vacuolization pseudo–Pelger-Huet nuclei and eosinophil with cytoplasmic vacuoles. , Large granular lymphocyte, lymphocyte with cytoplasmic vacuolization, atypical lymphocyte and plasmacytoid lymphocyte. Atypical monocytes with large coalescing cytoplasmic vacuoles. In contrast, monocytes in COVID-19–negative patients showed only occasional small cytoplasmic vacuoles. (Kevin S Dhinata *et al*., 2021) .

This findings also agreed with Maryame morphogical results which showed characteristics of a neutrophil granulocyte with dysmorphic morphology marked by hypogranular cytoplasm and hyposegmented nucleus. Atypical eosinophils containing multiple vacuoles, activated lymphocytes and large Monocytes were some of her morphological peripheral blood findings. Platelet morphology also showed frequent anomalies, mainly consisting of giant platelets with different sizes which are similar to present study (Maryam *et al*., 2020)

5.2Conclusions

Coronavirus disease 2019 has prominent manifestations from the hematopoietic system. Common haematological abnormalities have been identified in COVID-19 patients. Since the early stage of the disease, not only the platelets and lymphocyte but also haemoglobin, red blood cells, present a marked decrease, associating with the disease severity and clinical outcome. At the moment,An increase of neutrophils and the two markers NLR and PLR seem to correlate with progressive disease. Careful evaluation of laboratory indices at baseline and during the disease course can assist clinicians in formulating a tailored treatment approach and promptly provide intensive care to those who are in greater need.

5.3 Recommendation

1. Complete blood count is time-efficient, affordable, widely available and easily interpretable method so it’s recommended to request it for patients with COVID-19.

2.Neutrophil lymphocyte ratio as an early identification and management of severe COVID19.

3. Daily blood count with manual WBC differential to monitor for numeric values and morphological changes suggesting potential clinical changes worsening due to disease progression.

Reference

Buttarello M and Plebani M. (2008). Automated Blood Cell Counts.

Beniac DR, **A**., And Grudeski. (2006). Architecture of the SARS coronavirus prefusion spike.Am J ClinPathol, 130: p 104-116.

Bárcena M., Oostergetel GT, Bartelink W. (2009). Cryo-electron tomography of mouse hepatitis virus: insights into the structure of the coronavirion. Proc Natl Acad Sci U S A 106. P582–7.

Buttarello M and Plebani M., (2008). Automated Blood Cell Counts. Am J ClinPathol, 130: p 104-116.

Cheng VC, **L**., Woo PC,(2007) Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. Clin Microbiol Rev20 p 660–94.

Cui J, L., Shi ZL. (2019) Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 17p 181–92.

Cong Y, V., Reggiori F.(2017)The interaction between nidovirales and autophagy components.Viruses9p182.

Chan JF, Lau SK, To KK, et al. Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. Clin Microbiol Rev 2015;28:465–522.

Chandrashekar, V., (2013). Plateletcrit as a screening tool for detection of platelet quantitative disorders. Journal of Hematology, 2(1), pp.22-26.

Delmas B, Laude H.(1990) Assembly of coronavirus spike protein into trimers and its role in epitope expression. J Virol64 p 5367–75.

De Groot RJ, B., And Baric RS(2013) et al. Middle East respiratory syndrome coronavirus (MERS-CoV): announcement of the Coronavirus Study Group. J Virol;87:7790–2.

Guan Y, Z,. And He YQ.(2003)Isolation and characterization of viruses related to the SARS coronavirus from animals in Southern China. Sciencep 302:276–8.

Gorbalenya AE, B,.And Baric R.(2020)The species severe acute respiratory syndromerelated coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol p 5:536–44.

Gupta, B,. And Subhash.(2021).Patternes of complete blood counts (CBC) in patients with covid19 infection.Global journal for research analysis. 51-53.10.36106/gjra/9504399 **Godet M, L'Haridon R**, Vautherot JF, (1992). TGEV corona virus ORF4 encodes a membrane protein that is incorporated into virions. Virology p188:666–75.

Heymann DL,. And Shindo N, WHO Scientific and Technical Advisory Group for Infectious
Hazards. COVID-19: what is next for public health? Lancet2020;395:542–5.
3 Fisher D, Heymann D. Q&A: the novel coronavirus outbreak causing COVID-19. BMCMed2020;18:57.J Virol2014;88:9220–32.
Zaki AM, van Boheeman S, Bestebroer TM, (2012). Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med. P 367:1814–20.
Jain, R., G, A., P, B., S., Tilak, T. and Singh, A., (2022) . *Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio and Their Role as Predictors of Disease Severity of Coronavirus Disease 2019 (COVID-19).*
KeohaneE, M.,and Walenga Jeanine M. (2016).Rodak's Hematology Clinical Principles and Applications. 5th ed.Elsevier.
Kern W. (2002).PDQ Hematology.1st ed. Oklahoma city.B.C. Decker.
Lu R, Z,. And Li J. (2005). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet2020;395:565–74. 32 Li W, Shi Z, Yu M, et al. Bats are natural reservoirs of SARS-like coronaviruses. ScienceP 310:676–9.
34 **McIntosh K**, B, Chanock RM. Growth in suckling-mouse brain of "IBV-like" viruses from patients with upper respiratory tract disease. Proc Natl Acad Sci U S A1967;58:2268–73.
29 **Meo SA**, **A**,. And Al-Khlaiwi T. (2020) Novel coronavirus 2019-nCoV: prevalence, biological and clinical characteristics comparison with SARS-CoV and MERS-CoV. Eur Rev Med Pharmacol Sci p 24:2012–9.
Malik YS, S,. And Bhat S, (2020)Emerging novel coronavirus (2019-nCoV)-current scenario, evolutionary perspective based on genome analysis and recent developments. Vet Qp40:68–76.
Peiris JS, L, Poon LL,(2003) Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet.p361:1319–25.
Van der Hoek L, P,. And Jebbink MF. (2004)et al. Identification of a new human coronavirus. Nat Medp10:368–73
Peckham R.(2020)COVID-19 and the anti-lessons of history. Lancetp395:850–2.
Raoult D, Z, And Locatelli F (2020). Coronavirus infections: epidemiological, clinical and immunological features and hypotheses Cell Stress.

Sahu P,. (2019)Closure of universities due to coronavirus disease 2019 (COVID-19): impact on education and mental health of students and academic staff. Cureus p124 e7541. **Van Doremalen N, M,** And Milne-Price S,.(2005) Host species restriction of middle east respiratory syndrome coronavirus through its receptor, dipeptidyl peptidase Weiss SR, Navas-Martin S Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiology Biol Rev 2005;69:635–64. **Wu F**, **Z**,. And Yu B.(2020)A new coronavirus associated with human respiratory disease in China. Nature 2020;579:265–9.

El Zowalaty M,. And Jarhult JD.(2020) From SARS to COVID-19: a previously unknown SARS-related coronavirus (SARS-CoV-2) of pandemic potential infecting humans - call for a one health approach. One Healthp100_124.

WHO. Coronavirus disease (COVID-2019) situation reports. Situation report-51. 2020. (Available https://www.who.int/docs/default-source/coronaviruse/situation-reports /20200311-sitrep-51-covid-19.pdf?sfvrsn=1ba62e57_10)

Woo PC, L,.And Chu CM, (2005). Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J Virol2005;79:884–95.

Xu J, Z,And Teng T,(2020). Systematic comparison of two animal-to-human transmitted human coronaviruses: SARS-CoV-2 and SARS-CoV. Viruses p12:E244.

Elderdery, A.Y., Elkhalifa, A.M.E., Alsrhani, A., Zawbaee, K.I., Alsurayea, S.M., Escandarani, F.K., Alhamidi, A.H., Idris, H.M.E., Abbas, A.M., Shalabi, M.G. and Mills, J. (2022). Complete Blood Count Alterations of COVID-19 Patients in Riyadh, Kingdom of Saudi Arabia. Journal of Nanomaterials, 2022, pp.1–6.

Healthline. (2021). What Does It Mean to Have Low MCHC Available at: https://www.healthline.com/health/low-mchc#What-is-MCHC?.

Rana, R. (2018). Leishman Staining Technique - Principle, Preparation, Procedure & Result Interpretation | Hematology Practicals. [online] Paramedics World.

Abdul, J(2021)Diagnostic findings of Complete Blood Counts CBC for COVID-19 patients in Jordan.

Shankaralingappa, A., Tummidi, S. and Arun Babu, T. (2022). Diagnostic value of platelet indices in COVID 19 infection: a case-control study from a single tertiary care center. The Egyptian Journal of Internal Medicine, 34(1). doi:10.1186/s43162-022-00123-x.

Dhinata, K.S. (2021). Common Change of Complete Blood Count Parameters in COVID-19:

a Literature Review. Journal of Medicine and Health, 3(2). doi:10.28932/jmh.v3i2.3097.

Ahnach, M., Bouanani, N., Nejjari, S., Bendari, M., Doghmi, K. and Kettani, C.E. (2020). The critical role of complete blood count in the management of patients with COVID-19. The Pan African Medical Journal, [online] 35(63). doi:10.11604/pamj.supp.2020.35.2.23764. Procedure for Thick and Thin Blood Film Staining Technique. [online] Available at: https://martinslibrary.blogspot.com/2012/10/procedure-for-thick-and-thin-bloodfilm.html#:~:text=A%20thin%20film%20was%20made%20by%20placing%20a [Accessed 30 May 2022].

Waris, A., Din, M., Khalid, A., Abbas Lail, R., Shaheen, A., Khan, N., Nawaz, M., Baset, A., Ahmad, I. and Ali, M. (2021). Evaluation of hematological parameters as an indicator of disease severity in Covid- 19 patients: Pakistan's experience. Journal of Clinical Laboratory Analysis, 35(6). doi:10.1002/jcla.23809.

Usul, E., Şan, İ., Bekgöz, B. and Şahin, A. (2020). Role of hematological parameters in COVID-19 patients in the emergency room. Biomarkers in Medicine, 14(13), pp.1207–1215. doi:10.2217/bmm-2020-0317.

Al-Nimer MSM, Merza TA, Mohammed KYMY, Mohammed HA. Blood Cells Indices are Determinants of the COVID-19Outcome: A CrossSectional Study from Kurdistan Region-Iraq. Electron J Gen Med. 2021;18(5):em304. https://doi.org/10.29333/ejgm/11013

Zhang, X., Cui, M.-M., Fu, S., Li, L.-L., Liu, Y.-S., Liu, Z.-P., Liu, T., Wang, R.-T. and Yu, K.-J. (2017). Platelet distribution width correlates with prognosis of gastric cancer. Oncotarget, 8(12), pp.20213–20219. doi:10.18632/oncotarget.15561. **medlineplus.gov. (n.d.).** MPV Blood Test: MedlinePlus Medical Test. [online] Available at: https://medlineplus.gov/labtests/mpvbloodtest/#:~:text=A%20low%20MPV%20can%20indicate.

MSD Manual Professional Edition. (n.d.). Lymphocytopenia - Hematology and Oncology. [online] Available at: https://www.msdmanuals.com/professional/hematology-andoncology/leukopenias/lymphocytopenia#:~:text=Lymphocytopenia%20is%20most%20often %20due [Accessed 30 May 2022].

NHS (2019). Red blood cell count. [online] NHS. Available at: https://www.nhs.uk/conditions/red-blood-count/. **medlineplus.gov. (n.d.).** MCV (Mean Corpuscular Volume): MedlinePlus Medical Test. [online] Available at: https://medlineplus.gov/lab-tests/mcv-mean-corpuscularvolume/#:~:text=MCV%20stands%20for%20mean%20corpuscular.

Seladi-Schulman, J. (2018). What Is MCH and What Do High and Low Values Mean Healthline.

Cleveland Clinic. (n.d.). What are Neutrophils? What Can Cause High or Low Neutrophil Count.

Appendices

Appendix (A1)

Sudan University of science and technology faculty of graduate

Clinical Significance of CBC Parameters and Peripheral Blood Morphology in the Diagnosis of Covid19 in Khartoum State

Research questionnaire

1/ ID Number……..

2/ Name……

3/Sex. Male ☐ Female ☐

4/Duration of the disease ……..

5/Any other disease?.....

Date:……

Laboratory investigation:

CBC

WBC….....RBC……HGB……..……..
HCT……....MCV……...MCH……..…….
MCHC……..…PLT……..
LYM%..... NEUT%.....
MIX%..... . LYM#.....
NEUT#..... .. MIX#..... RDW……
MPV…….. PDW……. PLCR……

Comment :……
…….……

Appendix(2):

Sysmex Xp 300 analyzer

Appendix (A3)

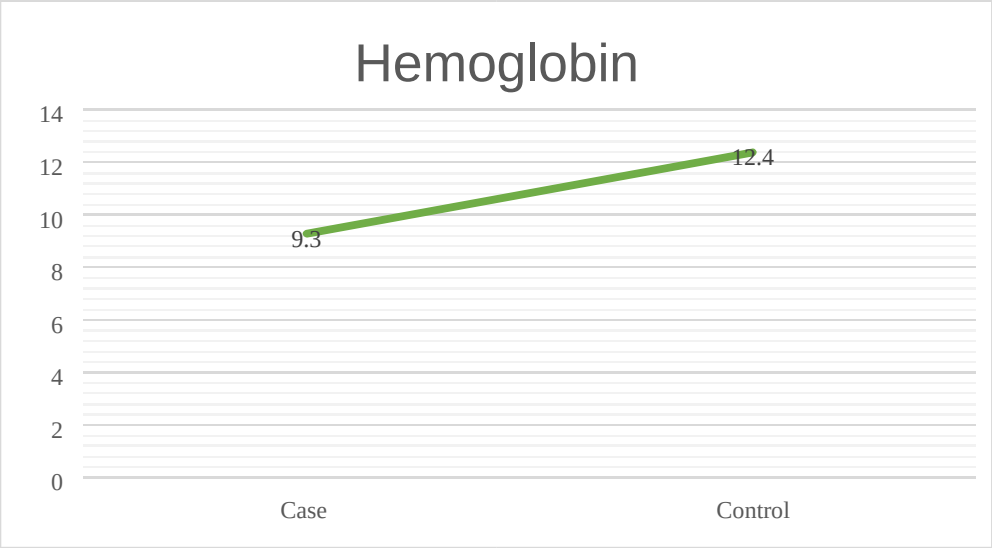

Figure 1: Hemoglobin Mean Levels in case and control group

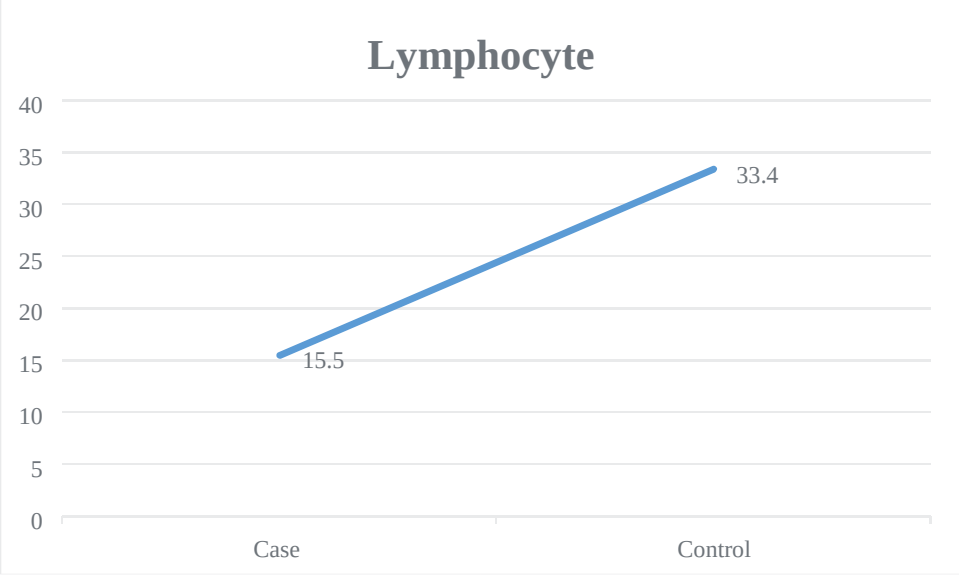

Figure 2: Lymphocyte Mean Levels in case and control group

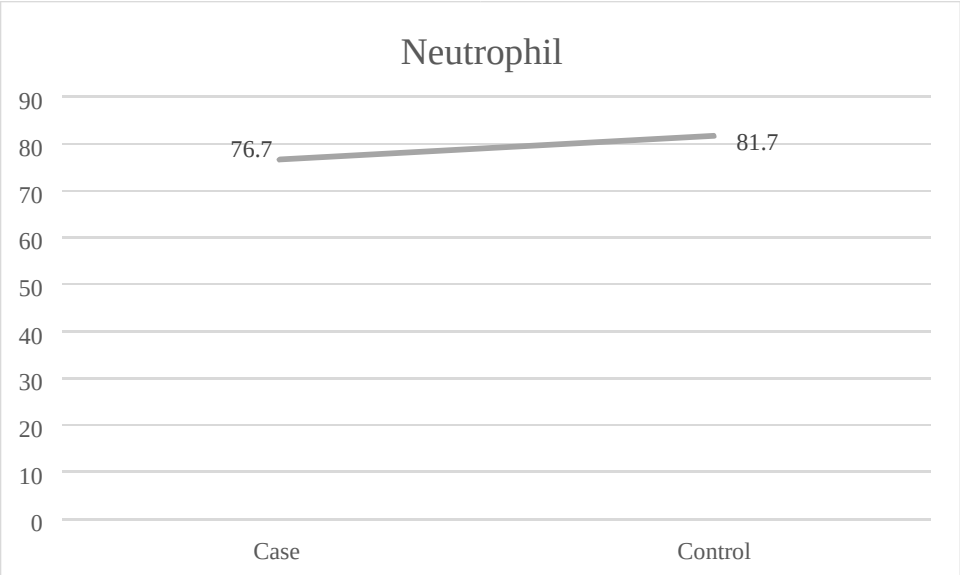

Figure 3: Neutrophil Mean Levels in case and control group

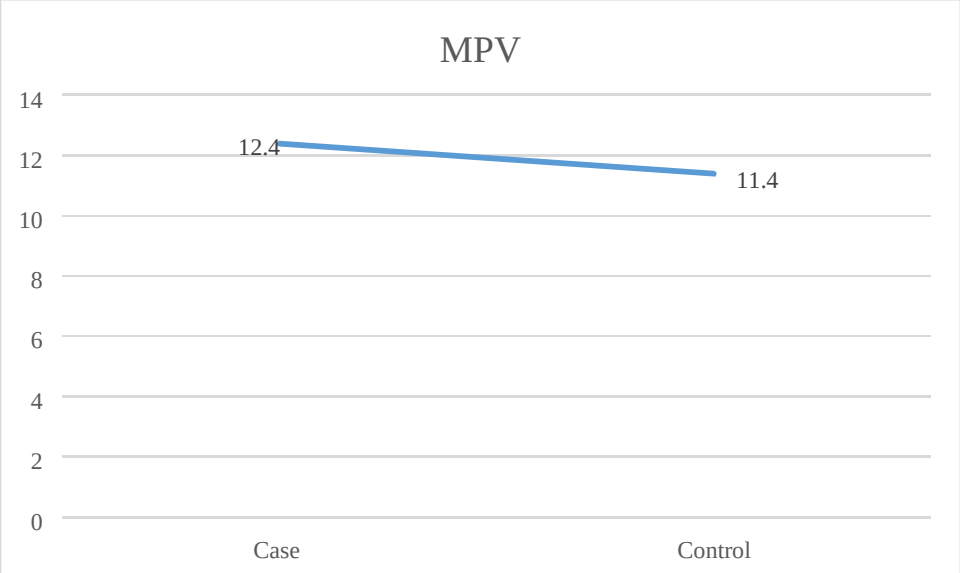

Figure 4: MPV Mean Levels in case and control group

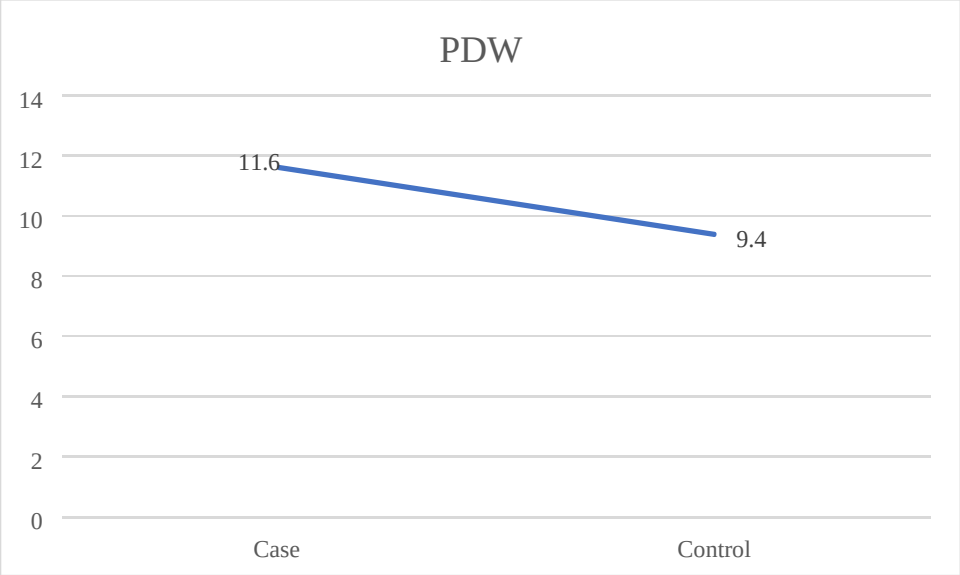

Figure 5: PDW Mean Levels in case and control group

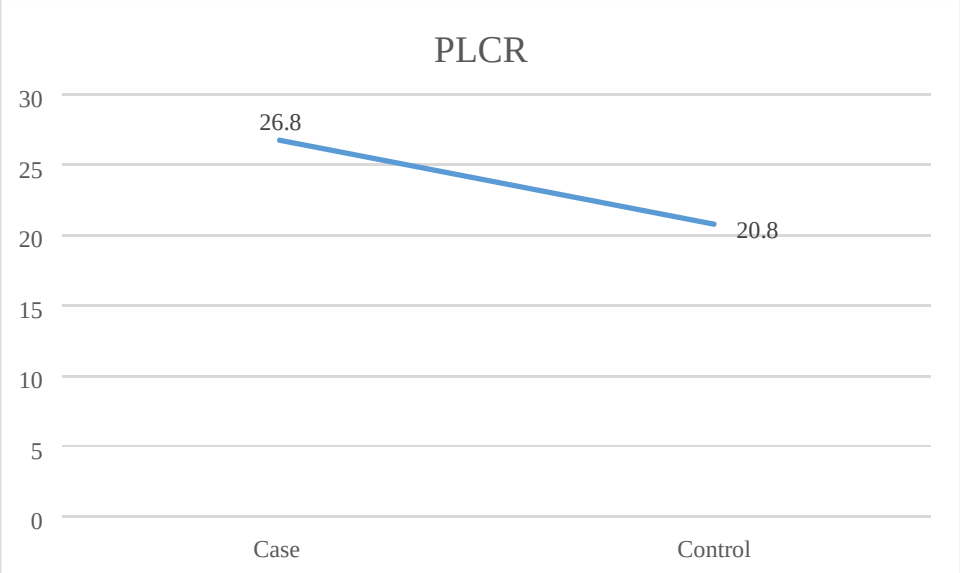

Figure 6: PLCR Mean Levels in case and control group

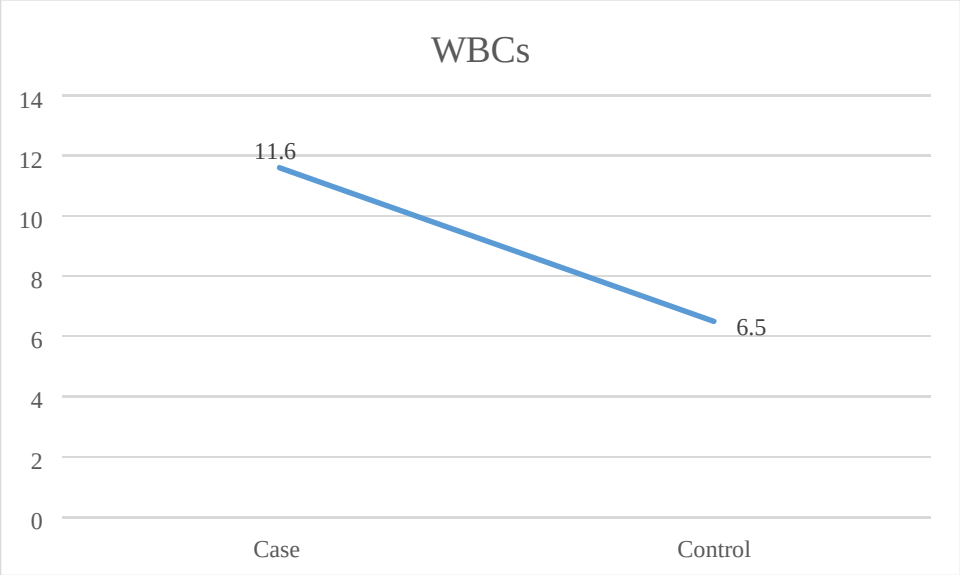

Figure 7: WBCs Mean Levels in case and control group

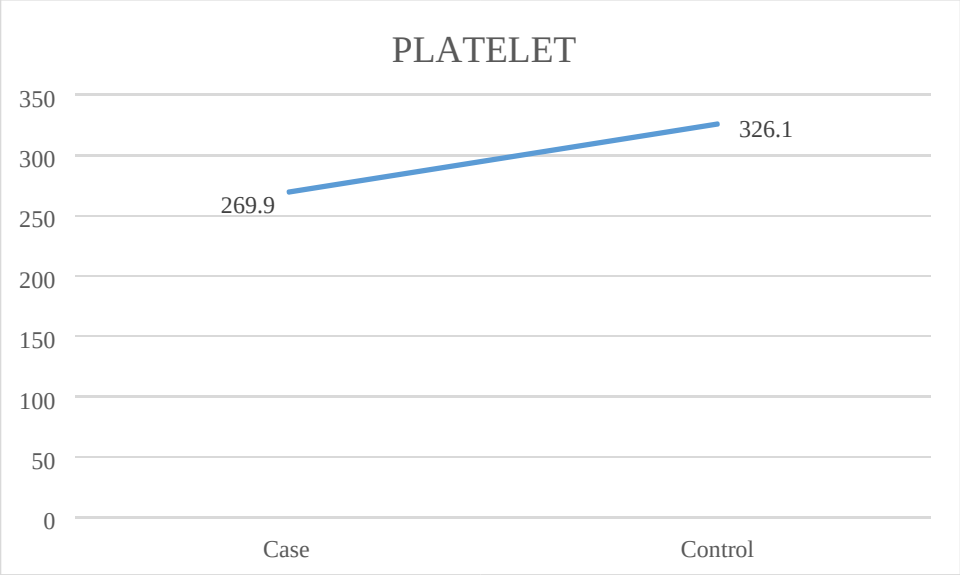

Figure 8: Platelet Mean Levels in case and control group

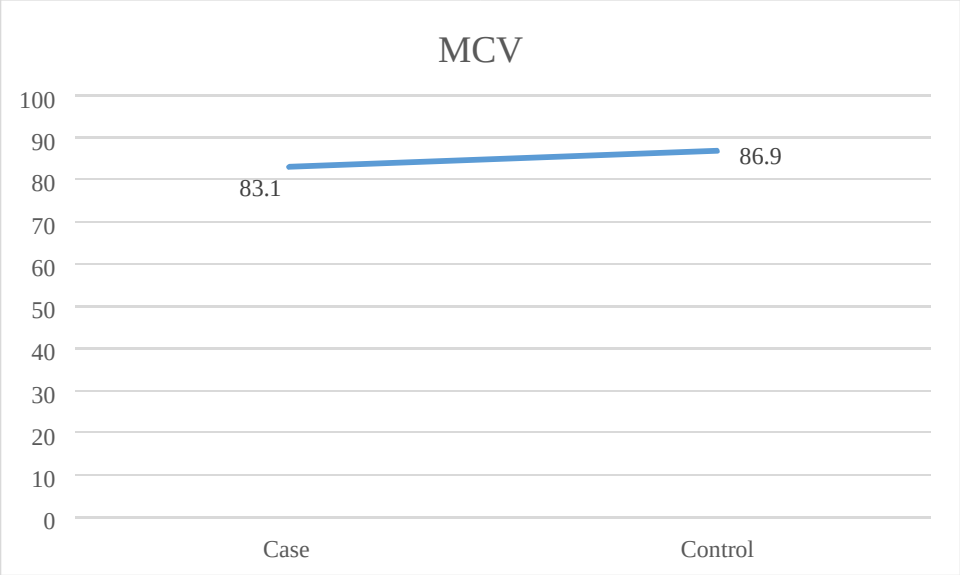

Figure 9: MCV Mean Levels in case and control group

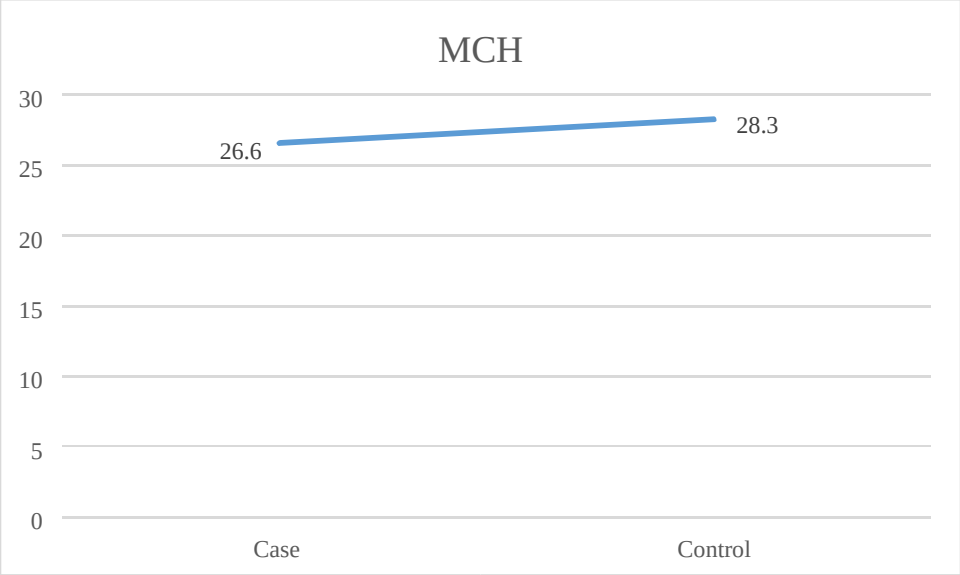

Figure 10: MCH Mean Levels in case and control group

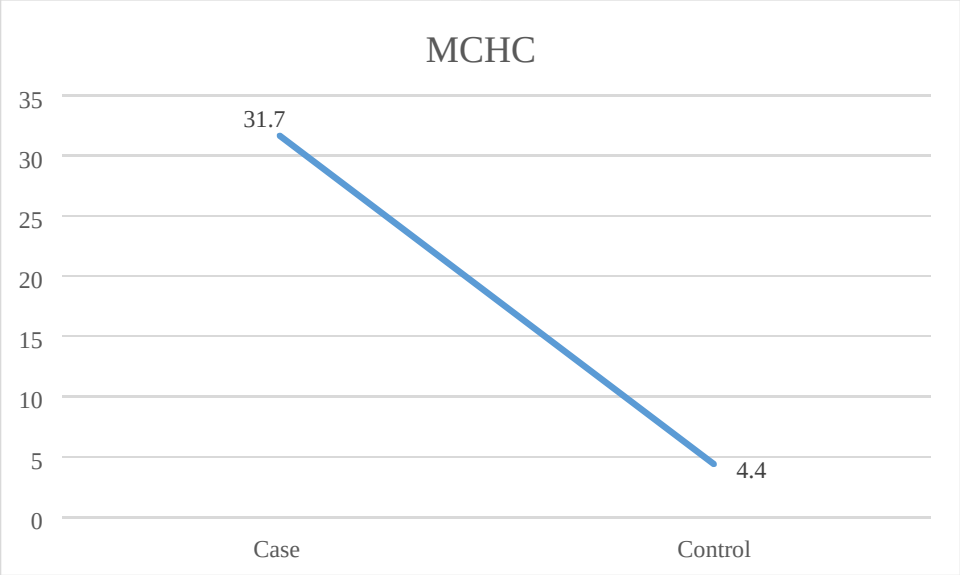

Figure 11: MCHC Mean Levels in case and control group

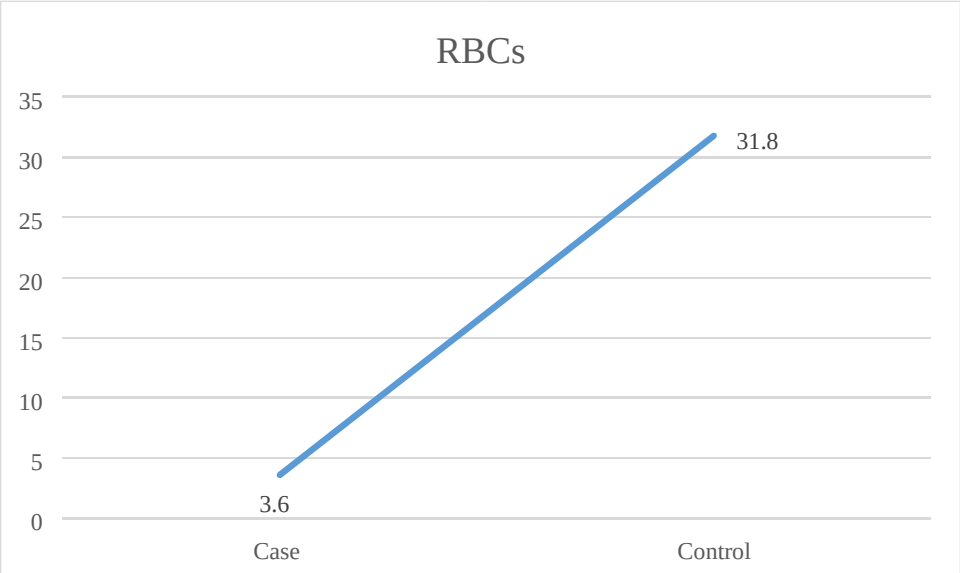

Figure 12: RBCs Mean Levels in case and control group

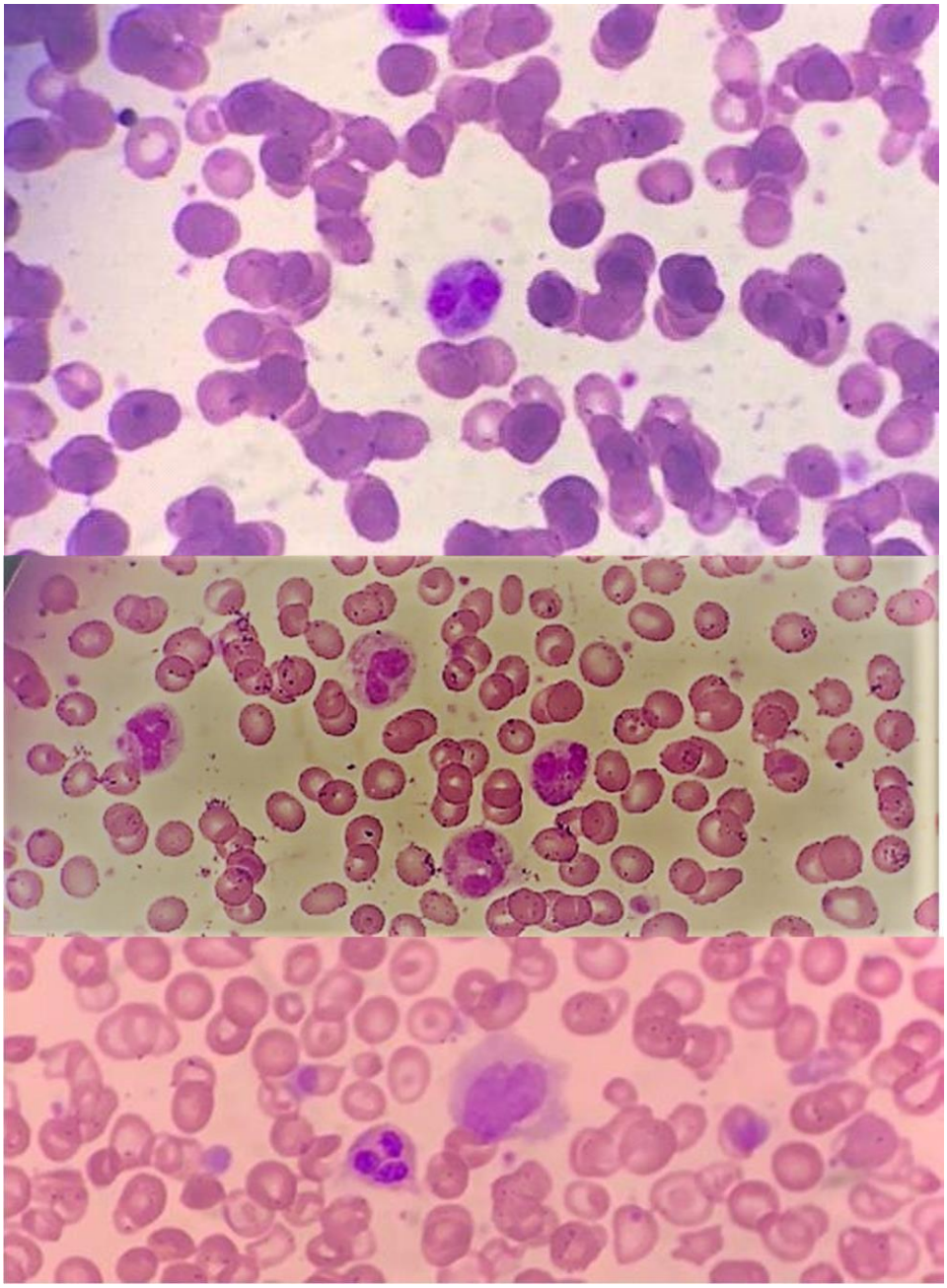

Figure 13:stained thin blood film with leishman stain from COVID-19 postive patients oil immersion 100x shows

(A) pseudo pelgr huet cell

(B) Hyposegmented hypogranular neutrophill

(C) large monocyte with vacoules