بسم الله الرحمن الرحيم

Sudan University of Science and Technology (SUST)

College of Graduate Studies

Diagnostic Utility of Neutrophil Lymphocyte Ratio and C-Reactive Protein among Sudanese Patients with COVID -19 Infection.

دراسة الفائدة التشخيصية لنسبة العدلات اللمفاويات والبروتين التفاعلي سي بين المرضى السودانيين المصابين بعدوى كوفيد-19

(A thesis submitted in fulfillment for the requirements for the master degree in medical laboratory sciences (Hematology and immunohematology)

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قال تعالي:

(..وَمَن يَتَّقِ اللَّهَ يَجْعَل لَّهُ مَخْرَجًا (2) وَيَرْزُقْهُ مِنْ حَيْثُ لَا يَحْتَسِبُ ۚ وَمَن يَتَوَكَّلْ عَلَى اللَّهِ فَهُوَ حَسْبُهُ ۚ إِنَّ اللَّهَ بَالِغُ أَمْرِهِ ۚ قَدْ جَعَلَ اللَّهُ لِكُلِّ شَيْءٍ قَدْرًا(3)

صدق الله العظيم

سورة الطلاق الآية (2,3)

Dedication

To my paved parents

To my brothers and sister

To my friends.

Acknowledgment

My great thanks and appreciation to the almighty ALLAH who help me to conduct this research. Then my thanks and appreciation extended to my supervisor **Dr. Ibrahim Khider Ibrahim** for her guidance and supports during this scientific journey, as well as his precious advice and continues assistance through the whole process of this research.

Special thanks to staff of COVID-19 isolation centers in **Omdurman and Khartoum**. My thanks and appreciation extended to all those help me to perform the research and also my thanks to Dr. Babbiker Gorish for his support. Finally, I am would like to thank all participants in this study.

Abstract

Background: Neutrophil lymphocytes ratio (NLR) and C-reactive protein (CRP) are established markers that reflect systemic inflammation in the body, and these parameters altered in patients with novel coronavirus (SARS-CoV-2) pneumonia (COVID-19).

Objectives: This study was aimed to determine the diagnostic utility of Neutrophil/lymphocyte ratio and CRP among Sudanese patients with COVID -19 infection.

Method: During the period from October 2021 to March 2022, a total of 80 subjects were involved in this study, of which 30 were cases with COVID-19 infection and the rest were 50 individuals age matched healthy controls. Among cases population 17 (56.7%) were male, while the rest 13 (43.3%) were female. About 3-5 ml of venous blood was collected from each case or control. Full blood count including was estimated by using Sysmex KX21 three parts automated hematology cell counter. NLR was calculated by dividing the absolute neutrophil count by absolute Lymphocyte count. Whole blood was used to determine CRP level by using commercially available kits manufactured by Finecare™ FIA Meter Operation according to manufacture instruction. Data was analyzed by using SPSS version 20. Independent T test was used to compare between cases and controls in the study variables.

Results: Our results demonstrated that cases have significant higher NLR (7.6±5.7 vs 1.5 ±0.85), CRP mg/l (64.9 vs 64.8 vs 6.0±0.0), TWBCs ×10^3/ul (10.5±9.3 vs 5.3±1.6), PLR (270±195 vs 142±42), RDW-CV% (15.5±2.6 vs 13.7±1.8), ANC ×10^3/ul (7.9±6.4 vs 4.5±2.5) with P- value of <0.05 for each . Cases showed a significant lower level of ALC ×10^3/ul (1.01±0.5 vs 2.75±1.25) and Hb g/dl (10.6±2.1 vs 14.5±1.5) with P-value of <0.05 for each. Our results showed that among cases group, gender have no effect on the result of all study variables with P-value of more than 0.05.

Conclusion: Cases have significant higher levels of CRP and N/L compared to controls. Among cases group, gender have no effect on the result of all study variables.

المستخلص

الخلفية: تعد نسبة الخلايا الليمفاوية العدلة (NLR) والبروتين التفاعلي (CRP) من العلامات الثابتة التي تعكس الالتهاب الجهازي ، وتتغير هذه المعلمات في مرضى الالتهاب الرئوي الناجم عن فيروس كورونا الجديد (COVID-19) (SARS-CoV-2).

الهدف: تهدف هذه الدراسة إلى دراسة الفائدة التشخيصية للعدلات / الخلايا الليمفاوية و CRP بين المرضى السودانيين المصابين بعدوى COVID -19.

الطريقة: خلال الفترة من أكتوبر 2021 إلى مارس 2022 ، شارك ما مجموعه 80 شخصًا في هذه الدراسة ، من بينهم 30 حالة مصابة بعدوى COVID-19 والباقي كان 50 كضوابط صحيين. ومن بين الحالات، كان 17 (56.7٪) من الذكور ، بينما كان الباقون 13 (43.3٪) من الإناث. تم جمع حوالي 7-10 مل من الدم الوريدي من كل حالة أو مجموعة الضوابط. تم تقدير تعداد الدم الكامل بما في ذلك باستخدام عداد خلايا الدم الآلي Sysmex KX21 المكون من خمسة أجزاء. تم حساب نسبة N / L٪ بقسمة عدد العدلات المطلق على عدد الخلايا الليمفاوية المطلق. تم استخدام المصل المنفصل لتحديد مستوى بروتين سي التفاعلي في المصل باستخدام أطقم متوفرة تجاريًا تم تصنيعها بواسطة Finecare ™ FIA Meter Operation وفقًا لتعليمات التصنيع. تم تحليل البيانات باستخدام الإصدار 20 من SPSS. تم استخدام اختبار T المستقل للمقارنة بين الحالات والضوابط في متغيرات الدراسة.

النتائج: أظهرت نتائجنا أن الحالات تحتوي على NLR (7.6 ± 5.7 مقابل 1.5 ± 0.55) ، CRP mg /l (64.9 مقابل 64.8 مقابل 6.0 ± 0.0) ، TWBCs أعلى بكثير × 10 ^ 3 / ul (10.5 ± 9.3 مقابل 5.3 ± 1.6) ، PLR (270 ± 195 مقابل 142 ± 42) ، RDW-CV ٪ (15.5 ± 2.6 مقابل 7.13 ± 1.8) ، ANC × 10 ^ 3 / ul (7.9 ± 6.4 مقابل 4.5 ± 2.5) مع قيمة P <0.05. أظهرت الحالات مستويات أقل بكثير من ALC × 10 ^ 3 / ul (1.01 ± 0.5 مقابل 2.75 ± 1.25) و Hb g / dl (10.6 ± 2.1 مقابل 14.5 ± 1.5) مع قيمة P <0.05. أظهرت نتائجنا أنه من بين مجموعة الحالات ، لم يكن للجنس أي تأثير على نتيجة جميع متغيرات الدراسة مع قيمة P تزيد عن 0.05.

الخلاصة: الحالات لديها مستويات أعلى بكثير من نسبة CRP و N / L مقارنة بالضوابط. علاوة على ذلك من بين مجموعة الحالات ، ليس للجنس أي تأثير على نتيجة جميع متغيرات الدراسة.

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CHAPTER ONE

Introduction

Chapter one
Introduction and objectives

1.1. Introduction

Coronavirus 2019 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The nature of this virus is zoonotic (transmitted between animals and humans). However, the particular animal as the source of transmission of COVID-19 is still unknown.' The first COVID-19 case was found in Wuhan City, Hubei Province, China, marked by the first reported case of mysterious pneumonia on December 31, 2019. On January 30, 2020, the World Health Organization (WHO) declared the incident as a Public Health Emergency of International Concern (PHEIC), and on March 11, 2020, WHO declared COVID-19 as a pandemic. This virus spread rapidly and to various countries in a short time (Chen et al., 2020). To August 2022, there have been 596,873,121 confirmed cases of COVID-19, including 6,459,684 deaths, reported to WHO (WHO., 2022). Since first case of COVID-19 reported in Sudan on 13 March 2020 and by 11 November 2020, there are 14,401 confirmed cases with 1,116 deaths and 9,535 recovered cases. The highest number of confirmed cases were reported in Khartoum State 10,393 (72.2%); followed by Gezira state 1,214 (8.4%). Regarding the total number of the death per the state; Khartoum state has contributed the most in the country total deaths related to COVID-19 with 415 cases of death (37.18%); followed by Gezira state and North Darfur and the least one was Blue Nile state (0.08%) (Ahmed et al., 2021). Common symptoms of COVID-19 infection include acute respiratory problems such as fever, cough, and shortness of breath. Severe cases of COVID-19 can cause pneumonia, acute respiratory syndrome, kidney failure,

and even death. The average incubation period is 5-6 days, with the most extended incubation period of 14 days (Chen et al., 2020).

Inflammation caused by the transmission of infectious diseases plays a vital role in the development of the virus. A severe inflammatory response contributes to a weak adaptive immune response resulting in an immune response imbalance that inhibits interactions with blood cells. Interaction with blood cells is critical in inflammation, immune response, hemostasis, and oncogenesis.' Neutrophil to Lymphocyte Ratio (NLR) can describe systemic inflammation used in various diseases such as acute coronary syndrome, cancer, chronic kidney disease, and others!' Another laboratory marker of inflammatory response is C-Reactive Protein (CRP), which has been identified as a predictor of clinical complications and severity. C-reactive protein is a marker of acute phase inflammation that has been found to be associated with disease severity and response therapy. Several studies stated that NLR and CRP are markers of acute inflammation, used in various diseases to see prognosis and mortality (Chen et al., 2020; Guan et al., 2019; Naess et al., 2017; Bozbay et al., 2014).

1.2. Rationale

COVID – 19 infection may produce various hematological changes, early studies have shown that lymphocytopenia is common among patients with COVID-19 (Wang et al., 2020. Accordingly, the neutrophil to lymphocyte ratio (NLR) in peripheral blood has been suggested to be useful in discriminating between types of infection and predicting the outcome of infection (Naess et al., 2017). C-reactive protein (CRP), as a classic inflammatory biomarker, is one of the most sensitive acute-phase reactants and is virtually absent from blood serum in healthy people, CRP levels can increase dramatically after bacterial and viral infections, inflammation, and severe trauma, elevated CRP levels were also observed in COVID-19 patients (Guan et al., 2019; Huang et al., 2020). However, the diagnostic performance of NLR and CRP in COVID-19 remains elusive.

In Sudan, only few data are published about the diagnostic and prognostic utility of NLR and CRP among COVID -19 patients and most of the previous studies are focused on the lymphopenia and other hematological markers. Therefore, this study is aimed to study the diagnostic utility of Neutrophil/lymphocyte ratio and CRP among Sudanese patients with COVID -19 infection.

1.3. Objectives

1.3.1. General objective

To study the diagnostic utility of Neutrophil/lymphocyte ratio and CRP among Sudanese patients with COVID -19 infection.

1.3.2. Specific objectives

- To measure the NL ratio, CRP, TWBCs, ANC, PLR, RDW-CV, ALC and Hb among cases and controls.
- To compare between the cases and controls in the level of NL ratio, CRP, TWBCs, ANC, PLR, RDW-CV, ALC and Hb .
- To correlate between the cases gender and the level of NL ratio and CRP.

CHAPTER TWO

Literature review

Chapter two
Literature review

2.1. Covid 19 infection

2.1.1. Definition

Coronavirus disease 2019 (COVID-19) is a contagious disease caused by a virus, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The first known case was identified in Wuhan, China, in December 2019. The disease quickly spread worldwide, resulting in the COVID-19 pandemic (Page et al., 2021).

2.1.2. Transmission

COVID-19 is mainly transmitted when people breathe in air contaminated by droplets/aerosols and small airborne particles containing the virus. Infected people exhale those particles as they breathe, talk, cough, sneeze, or sing. Transmission is more likely the more physically close people are. However, infection can occur over longer distances, particularly indoors (Wang et al., 2021; Greenhalgh et al., 2021; Bourouiba., 2021).

2.1.3 Signs and symptoms

The symptoms of COVID-19 are variable depending on the type of variant contracted, ranging from mild symptoms to critical and possibly fatal illness. Common symptoms include coughing, fever, loss of smell (anosmia) and taste (ageusia), with less common ones including headaches, nasal congestion and runny nose, muscle pain, sore throat, diarrhea, eye irritation, and toes swelling or turning purple, and in moderate to

severe cases breathing difficulties. People with the COVID-19 infection may have different symptoms, and their symptoms may change over time (Grant et al., 2020; Pardhanetal et al., 2020).

2.1.4. Epidemiology

To August 2022, there have been 596,873,121 confirmed cases of COVID-19, including 6,459,684 deaths, reported to WHO (WHO., 2022). Since first case of COVID-19 reported in Sudan on 13 March 2020 and by 11 November 2020, there are 14,401 confirmed cases with 1,116 deaths and 9,535 recovered cases. The highest number of confirmed cases were reported in Khartoum State 10,393 (72.2%); followed by Gezira state 1,214 (8.4%). Regarding the total number of the death per the state; Khartoum state has contributed the most in the country total deaths related to COVID-19 with 415 cases of death (37.18%); followed by Gezira state and North Darfur and the least one was Blue Nile state (0.08%) (Ahmed et al., 2021).

2.1.5. Prevention

Preventive measures to reduce the chances of infection include getting vaccinated, staying at home, wearing a mask in public, avoiding crowded places, keeping distance from others, ventilating indoor spaces, managing potential exposure durations, washing hands with soap and water often and for at least twenty seconds, practicing good respiratory hygiene, and avoiding touching the eyes, nose, or mouth with unwashed hands. Those diagnosed with COVID-19 or who believe they may be infected are advised

by the CDC to stay home except to get medical care, call ahead before visiting a healthcare provider, wear a face mask before entering the healthcare provider's office and when in any room or vehicle with another person, cover coughs and sneezes with a tissue, regularly wash hands with soap and water and avoid sharing personal household items. Moreover Covid 19 vaccination has been started to reduce the distribution of disease (Anderson et al., 2020).

2.2. N L Ratio

In medicine neutrophil to lymphocyte ratio (NLR) is used as a marker of subclinical inflammation. It is calculated by dividing the number of neutrophils by number of lymphocytes, usually from peripheral blood sample. Neutrophil to Lymphocyte ratio was first demonstrated as useful parameter after a correlation of a relationship between the neutrophil lymphocyte ratio to reactions of the immune response was noted. A study in 2001 was conducted by the Department of Anesthesiology and Intensive Care Medicine, St. Elizabeth Cancer Institute in Bratislava by Zahorec which suggested the routine used of the ratio as a stress factor in clinical ICU practice in intervals of 6-12 and 24 hours. The first study to demonstrate that pretherapeutic NLR can be used as a predictor of chemotherapy sensitivity to thoracic esophageal cancer was demonstrated by Hiroshi Sato, Yasuhiro Tsubosa, and Tatsuyuki Kawano in a 2012 study published in World Journal of Surgery journal. NLR can be used as a prognostic marker for COVID-19 given the significant difference of NLR between those died and recovered from COVID-19 (Huang et al., 2017; Yin et al., 2016; Azab et al., 2014).

2.3. CRP

2.3.1. Definition

C-reactive protein (CRP) is an annular (ring-shaped) pentameric protein found in blood plasma, whose circulating concentrations rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T cells (Thompson et al., 1999).

2.3.2. Function

CRP binds to the phosphocholine expressed on the surface of bacterial cells such as pneumococcus bacteria. This activates the complement system, promoting phagocytosis by macrophages, which clears necrotic and apoptotic cells and bacteria. This so-called acute phase response occurs as a result of increasing concentrations of IL-6, which is produced by macrophages as well as adipocytes in response to a wide range of acute and chronic inflammatory conditions such as bacterial, viral, or fungal infections; rheumatic and other inflammatory diseases; malignancy; and tissue injury and necrosis. These conditions cause release of interleukin-6 and other cytokines that trigger the synthesis of CRP and fibrinogen by the liver. CRP binds to phosphocholine on micro-organisms. It is thought to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages (opsonin-mediated phagocytosis), which express a receptor for CRP. It plays a role in innate immunity as an early defense system against infections (Lau et al., 2005; Pepys and Hirschfield., 2003).

2.3.3. Serum level

In healthy adults, the normal concentration of CRP varies between 0.8 mg/L and 3.0 mg/L. However, some healthy adults show elevated CRP at 10 mg/L. CRP concentrations also increase with age, possibly due to subclinical conditions. There are also no seasonal variations of CRP concentrations. Gene polymorphism of interleukin-1 family, interleukin 6, and polymorphic GT repeat of the CRP gene do affect the usual CRP concentrations when a person does not have any medical illnesses. The plasma half-life of CRP is 19 hours and is constant in all medical conditions (Knight., 2015; Helal et al., 2012).

2.3.4. Clinical Significance

When there is a stimulus, the CRP level can increase 10,000-fold from less than 50 μg/L to more than 500 mg/L. Its concentration can increase to 5 mg/L by 6 hours and peak at 48 hours. Therefore, the only factor that affects the blood CRP concentration is its production rate, which increases with inflammation, infection, trauma, necrosis, malignancy, and allergic reactions. Other inflammatory mediators that can increase CRP are TGF beta 1, and tumor necrosis factor alpha. In acute inflammation, CRP can increase as much as 50 to 100 mg/L within 4 to 6 hours in mild to moderate inflammation or an insult such as skin infection, cystitis, or bronchitis. It can double every 8 hours and reaches its peak at 36 to 50 hours following injury or inflammation. CRP between 100 and 500 mg/L is considered highly predictive of inflammation due to bacterial infection.

Once inflammation subsides, CRP level falls quickly because of its relatively short half-life (Bray et al., 2016).

2.4. Previous studies

Study done by Sukrisman et al., in 2021 in Indonesia to evaluate hematologic profiles with inflammation markers in COVID-19 patients and to determine the correlation of neutrophil-lymphocyte ratio (NLR) with disease severity. They did a cross-sectional study involving hospitalized COVID-19 patients confirmed with a positive SARS-CoV-2 PCR test in Dr. Cipto Mangunkusumo Hospital. Lymphocyte count, NLR, C-reactive protein (CRP) and ferritin were evaluated in severe and non-severe COVID-19 cases at hospital admission. Data was analyzed using Spearman correlation. They found that there were 41 patients aged 20 to 79 years with COVID-19; 33 (80.5%) were no severe, and 8 (19.5%) were severe cases. They found that there is a statistically significant difference in WBC, relative neutrophils and lymphocytes, NLR, and CRP between non-severe and severe cases. There is a strong correlation between NLR and CRP ($r = 0.738$; $p < 0.001$). Our findings show that NLR and absolute lymphocyte count, but not ferritin, play a role in differentiating between non-severe and severe COVID-19 cases. They conclude that in COVID-19 cases, a strong correlation between NLR and CRP might suggest the use of NLR to differentiate between non-severe and severe cases, especially in a remote healthcare facility.

Study done by Rotty et al., 2020 in Indonesia they aimed to assess the utility of neutrophil-to-lymphocyte ratio (NLR), as reliable inflammatory biomarkers for

Indonesian COVID-19 patients; by analyzing the correlation of NLR level with CRP and D-dimer plasma level. They conducted cross-sectional study in Professor Dr. R.D. Kandou Hospital, Manado involving RT-PCR confirmed and hospitalized COVID-19 patients. Lymphocyte count, NLR, CRP, and D-dimer were examined in severe and non-severe COVID-19 cases at hospital admission. Correlation test was done using Spearman correlation test. Their results demonstrated that A total of 40 COVID-19 patients were included in the analysis, with 50% having mild disease and other half having severe disease. The NLR, CRP, and D-dimer were significantly higher in severe COVID-19 group. Significant correlation was found between NLR and CRP ($p = 0.001$ and $r = 0.506$) and also with D-dimer level ($p = 0.000$ and $r = 0.570$) in differentiating severity of COVID-19. They conclude that NLR is correlated with CRP and D-dimer level; therefore, NLR may serve as reliable, cost-effective, and practical inflammatory biomarker for differentiating severe and non-severe COVID-19 cases

Another study done by Mousavi-Nasab et al., in Iran 2020 they aimed to investigate neutrophil-to-lymphocyte ratio (NLR) with C-reactive protein to identify potential clinical predictors and analyze differences among severe and non-severe COVID-19 patients. They did a population of patients with COVID-19 referred to Loghman Hospital in Tehran was analyzed. The baseline data of laboratory examinations, including NLR and CRP levels, was collected in their study. Pearson analysis was used to assess the independent relationship between the NLR with disease severity and CRP levels. COVID-19 cases comprised 14 (20%) patients with severe disease and 56 (80%)

with non-severe infection. The mean values of WBC, NEU, LYM, and NLR of the severe patients were significantly higher than those of the non-severe patients. Forty-six patients (65.7%) had NLR >1, and the remaining patients had NLR <1. Plasma CRP levels were higher in severe cases than in non-severe cases, and this difference was significant. The results showed that NLR was positively correlated with CRP levels (R=0.23) and negatively correlated with WBC (R=-0.38). CRP (AUC = 0.97, 95% CI: 0.95-0.99) and NLR (AUC = 0.87, 95% CI: 0.81-0.93) had a very good accuracy in predicting the severity of COVID-19 disease.

Study done by Yang et al., in 2020 to assess the evidence that indicated the key role played by virus-triggered inflammation in the 2019-novel coronavirus disease (COVID-19) which emerged in Wuhan City and rapidly spread throughout China. Age, neutrophil (NEU)-to-lymphocyte (LYM) ratio (NLR), lymphocyte-to-monocyte (MON) ratio, platelet-to-lymphocyte ratio (PLR), and C-reactive protein (CRP) of 93 patients with laboratory confirmed COVID-19 were investigated and compared in their study . The receiver operating characteristic curve was applied to determine the thresholds for five biomarkers, and their prognostic values were assessed via the Kaplan–Meier curve and multivariate COX regression models in their study. Their results demonstrated that the median age was 46.4 years old, and 37cases were females. A total of 27.8% of patients had been to Wuhan, and 73.1% had contacted with people from Wuhan. Fever (83.8%) and cough (70.9%) were the two most common symptoms. Elevated NLR and age were significantly associated with illness severity. The binary logistic analysis identified

elevated NLR (hazard risk [HR] 2.46, 95% confidence interval [CI] 1.98–4.57) and age (HR 2.52, 95% CI 1.65–4.83) as independent factors for poor clinical outcome of COVID-19. NLR exhibited the largest area under the curve at 0.841, with the highest specificity (63.6%) and sensitivity (88%).

A literature search was conducted by Simadibrata et al., on 23 July 2020 to retrieve all published articles, including grey literature and preprints, investigating the association between on-admission NLR values and severity or mortality in COVID-19 patients. A meta-analysis was performed to determine the overall standardized mean difference (SMD) in NLR values and the pooled risk ratio (RR) for severity and mortality with the 95% Confidence Interval (95%CI). A total of 38 articles, including 5699 patients with severity outcomes and 6033 patients with mortality outcomes, were included in their study. Their results demonstrated that severe and non-survivors of COVID-19 had higher on-admission NLR levels than non-severe and survivors (SMD 0.88; 95%CI 0.72–1.04; I2 = 75.52% and 1.87; 95%CI 1.25–2.49; I2 = 97.81%, respectively). Regardless of the different NLR cut-off values, the pooled mortality RR in patients with elevated vs. normal NLR levels was 2.74 (95%CI 0.98–7.66).

CHAPTER THREE

Materials and Methods

Chapter three
Material and Method

3.1. Study design:

This study is acase control-based study.

3.2. Study area:

This study was carried out in Khartoum state, within two COVID-19 isolation centers, one in Khartoum city and another in Omdurman.

3.3. Study duration

This study was conducted during the period from October 2021 to March 2022.

3.4. Study population:

COVID-19 confirmed patients in the isolation centers were included as cases in this study, while age matched healthy individuals were involved as controls in this study.

3.4.1. Inclusion criteria

Cases and age matched controls were included in this study.

3.4.2. Exclusion criteria

Any case that have leukemia or lymphoma or inflammatory disease other than COVID-19 (for cases only) or Immunodeficiency was excluded from this study, because the mentioned defects will affect on the result of NLR and CRP level as well.

3.5. Sample size and sampling technique

Simple random sampling technique was used to involve a total of 80 subjects in this study, of which 30 were cases with COVID-19 infection and the rest were 50 age matched healthy controls.

3.6. Sample collection:

Taking all aseptic precautions, 3-5 mL venous ED blood was collected from each case or control antecubital vein. Total amount of blood collected was mixed with EDTA anticoagulant in a vial for complete blood count (CBC) estimation. Whole blood was used for CRP estimation according to the manufacturer instruction.

3.7. Laboratory method

3.7.1. CBC and NLR estimation

Full blood count including Hemoglobin (Hb), Platelet (PLT) count, RDW%, White cell count (WBCs) and differential was estimated by using Sysmex KX21 three part semiautomated hematology cell counter. NL ratio % was calculated by dividing the absolute neutrophil count by absolute Lymphocyte count. PLR was also calculated by dividing the PLT by Lymphocyte count.

3.7.2. CRP level assessment

Whole blood was used to determine CRP level by using commercially available kits manufactured by Finecare™ FIA Meter Operation according to manufacture instruction.

3.7.2.1. Principle

The Finecare™ CRP Rapid Quantitative Test is based on fluorescence immunoassay technology. The Finecare™ CRP Rapid Quantitative Test uses a sandwich immunodetection method, when sample is added to the sample well of the Test Cartridge, the fluorescence-labeled detector CRP antibody binds to CRP antigen in blood specimen. As the sample mixture migrates on the nitrocellulose matrix of test strip by capillary action, the complexes of detector antibody and CRP are captured to CRP antibody that has been immobilized on test strip. Thus, the more CRP antigen is in blood specimen, the more complexes are accumulated on test strip. Signal intensity of fluorescence of detector antibody reflects amount of CRP captured and Finecare™ FIA Meter shows CRP concentrations in blood specimen. The default results unit of Finecare™ CRP Rapid Quantitative Test is displayed as XXXmg/L from Finecare™ FIA Meter. The working range and the detection limit of the CRP Test system are 0.5～200 mg/L and 0.5mg/L

3.8. Data collection

Data was collected by using structural questionnaire and clinical data was obtained from sample analysis.

3.9. Data analysis

The data was statistically analyzed by software SPSS version 20. Results were be presented as tables and figures.

3.10. Ethical considerations

Before conducting the research, verbal approval has been obtained from isolation centers administration and then samples were taken from the study participants after the understand the goal and aim of study and their verbal approval.

CHAPTER FOUR

Results

Chapter four
Results

This study was involved a total of 80 participants of which 30 were cases and 50 were controls. Among cases population 17 (56.7%) were male, while the rest 13 (43.3%) were female (Figure 1). Our results demonstrated that cases have significant higher TWBCs ×10^3/ul (10.5±9.3 vs 5.3±1.6), NLR (7.6±5.7 vs 1.5±0.55), PLR (270±195 vs 142±42), RDW-CV% (15.5±2.6 vs 13.7±1.8), ANC ×10^3/ul (7.9±6.4 vs 4.5±2.5), CRP mg/l (64.9±64.8 vs 6.0±0.0) with P- value of 0.005,0.000,0.001,0.002,0.007,0.000 respectively (Table 1). On the other hand, cases showed a significant lower level of ALC ×10^3/ul (1.01±0.5 vs 2.75±1.25) and Hb g/dl (10.6±2.1 vs 14.5±1.5) with P value of 0.000 for both (Table 1). Our result demonstrated that there was insignificant difference between the cases and controls in the level of PLT count with P-value of 0.320 (Table 1). Furthermore, our results showed that among cases group, gender have no effect on the result of all study variables with P-value of more than 0.05 (Table 2).

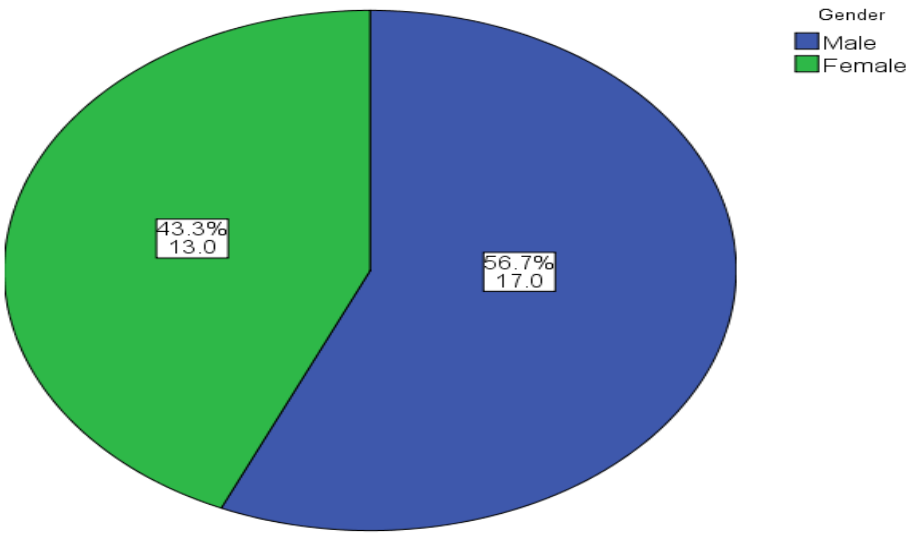

Figure 1. Show distribution of cases according to their gender

Table 1. Variables mean comparison between cases and controls (P-value is significant at level equal to or less than 0.05)

Variable	Types of participants	Mean±SD	P-value
TWBCs ×10^3/ul	Cases	10.5±9.3	0.005
	Controls	5.3±1.6	
NLR	Cases	7.6±5.7	0.000
	Controls	1.5±0.55	
PLR	Cases	270±195	0.001
	Controls	142 ± 42	
RDW-CV %	Cases	15.5±2.6	0.002
	Controls	13.7±1.8	
ANC ×10^3/ul	Cases	7.9±6.4	0.007
	Controls	4.5±2.5	
ALC ×10^3/ul	Cases	1.01±0.5	0.000
	Controls	2.75±1.25	
PLTs count ×10^3/ul	Cases	268±171	0.320
	Controls	300±150	
Hb g/dl	Cases	10.6±2.1	0.000
	Controls	14.5±1.5	
CRP mg/L	Cases	64.9±64.8	0.000
	Controls	6.0±0.0	

Table 2. Variables Mean comparison between cases genders (P-value is significant at level equal to or less than 0.05)

Variable	Gender	Mean ± S.D	P-value
TWBCs ×10^3/ul	Male	10.6 ± 9.6	0.981
	Female	10.5 ± 9.2	
ANC ×10^3/ul	Male	7.1 ± 3.8	0.506
	Female	8.9± 8.9	
ALC×10^3/ul	Male	1.1 ±0.5	0.754
	Female	1.0 ±0.4	
NLR	Male	7.6 ±6.0	0.995
	Female	7.6 ± 5.3	
PLTs count ×10^3/ul	Male	260 ±145	0.787
	Female	278 ±205	
Hb g/dl	Male	11.0 ±2.1	0.268
	Female	10.1 ± 2.1	
PLR	Male	277 ±205	0.820
	Female	261 ±188	
RDW-CV %	Male	15.6 ± 2.6	0.790
	Female	15.3 ±2.7	
CRP mg/L	Male	79.2 ± 67.9	0.212
	Female	48.2 ± 59.2	

CHPATER FIVE

Discussion, conclusion and recommendation

Chapter five
Discussion, Conclusions and Recommendations

5.1. Discussion

COVID – 19 infection may produce various hematological and inflammatory changes. Early studies have shown that lymphocytopenia and high titer of CRP is common among patients with COVID-19 (Wang et al., 2020). This study was aimed to determine the diagnostic utility of N/L and CRP among Sudanese patients with COVID-19. In this study the results demonstrated a highly significant higher levels of N/L among cases compared to healthy control (7.6±5.7 vs 1.5±0.55), Several studies agreed with our finding and their results showed a cutoff of elevated NLR of 0.8, 2.9, 3.17, 7.38 ± 6.94 and even as high as 11.75 as described in a metanalysis (Yang et al., 2020; Simadibrata et al., 2021; Rotty et al., 2019). Thus, we can pointed that NLR may provide the clinicians with rapid, easy, and economical means to suggest early diagnosis of COVID-19 infection.

In this study CRP was significantly higher among cases group compared to the controls (64.9 vs 64.8 vs 6.0±0.0 mg/l). This finding is in agreement with the finding of Rotty et al., in Indonesia in 2022 who demonstrated higher levels of CRP among COVID-19 patients 23.73 mg/l with sever cases more much higher 34.21 mg/l. Also, higher CRP levels were detected in several other studies and more prominently among sever COVID-19 cases raging from 75.0±27 mg/l (Mousavi-Nasab et al., 2020) to 274 mg/l (Li et al., 2020). As the first line of innate host defenses for clearance of viral infections, CRP might be linked to the overproduction of inflammatory cytokines in severe COVID-19

patients (Li et al., 2020). This finding along with previous studies reports have proven that elevated CRP is a functional biomarker that influences the progression of pneumonia in COVID-19 patients.

Neutrophilia, except for patients with bacterial infections or superinfections, correlates with hyperinflammatory state and cytokine storm, an integral part of the pathogenic mechanism of COVID-19. In this study there were significant higher TWBCs ×10^3/ul (10.5±9.3 vs 5.3±1.6) along with ANC ×10^3/ul (7.9±6.4 vs 4.5±2.5) among cases when compared to controls. Similar finding has been reported by Eslamijouybari et al., in 2020 in Iran cases vs controls TWBCs ×10^3/ul was (7.6±7.8 vs 6.4±1.5) and ANC ×10^3/ul was (5.3±4.2vs 3.7±1.2). Moreover, a meta analysis done by Soraya and Ulhaq in 2020 found that Leukocytes and neutrophils were significantly higher in severe than in non-severe COVID-19 infected patients, and both leukocyte and neutrophil counts increased in COVID-19patients. Thus, as COVID-19 progresses, the number of circulating neutrophils gradually increases; thus, neutrophilia has been identified as a marker of severe respiratory disease and a poor outcome (Zhang et al., 2020). Confirmation of these findings might lead to targeting neutrophils and their recruitment mediators to reduce the severity of COVID-19.

Our result demonstrated that there was insignificant different between the cases and controls in the level of PLT count. However, a significant thrombocytopenia has been demonstrated by Eslamijouybari et al., in 2020 in Iran. On the other hand, in the current study PLR was significantly higher among cases group compared to controls (270±195 vs

142±42). Similar finding has been reported by Eslamijouybari et al., in 2020 in Iran. LR is currently known as an inflammatory marker. This is not surprising because Various studies have assessed the association of PLR with viral infections (Meng etal., 2016). In this stuy RDW-CV% (15.5±2.6 vs 13.7±1.8) was significantly higher among cases group. In a previous study, higher levels of RDW were identified in patients with severe compared to moderate symptoms (Wang et al., 2020).

In the current study a significant lower level of ALC ×10^3/ul (1.01±0.5 vs 2.75±1.25) was seen among cases. Similar finding has been reported by Eslamijouybari etal., in 2020 in Iran that ALC ×10^3/ul was (1.5±0.4 vs 2.4±0.74). Lymphopenia might be caused by virus attachment or indirectly by immune injuries from inflammatory mediators. Moreover, exudation of circulating lymphocytes into inflammatory lung tissues might also lead to lymphopenia. The reduction of lymphocyte subset count in COVID-19 patients were investigated across twenty peer-reviewed studies for reporting lymphocyte subset counts and COVID-19 disease severity. CD4+ T cell, CD8+ T cell, B cell, Natural killer (NK) cell, and total lymphocyte cell counts all showed a statistically significant reduction in patients with severe/critical COVID-19 disease compared to mild/moderate disease (Huang et al., 2020).

In this study cases have significant lower levels of Hb g/dl concentrations (10.6±2.1 vs 14.5±1.5). Wang *et al.* reported reduced haemoglobin concentration (< 110 g/L) in 19% of the hospitalized patients. while Huang *et al.* in 2020 0reported reduction in haemoglobin concentrations in 38% of the study population admitted to the hospital.

Anaemia could be the result of a sideroblastic-like anaemia pattern arising from alterations in iron metabolism, while increased ferritin could be indicative of a strong inflammatory reaction in COVID-19 or related to viral entry into the human body and its impact on iron metabolism (Wang et al., 2020).

In the current study, our results showed that among cases group, gender have no effect on the result of all study variables with P-vlue of more than 0.05. Similar report has been documented by Urbano et al., in 2022. This point may need more investigations with inclusion of more sample size to be rolled out.

5.2. Conclusion:

CRP and NLR were significantly increased among COVID-19 patients.

5.3. Recommendation

- N/L and CRP should be used as easy and economical means to suggest early diagnosis of COVID-19 infection.

- Further studies includes underlying diseases of patients should be done to determine the monitoring utility of N/L ratio and CRP levels.

- further investigations with inclusion of more sample size should be done draw the role of gender in the development of hematological changes among COVID-19 patients.

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Appendix

Appendix I