BIOCHEMICAL MARKERS ANALYSIS FOR SARS-COV-2 INFECTED PATIENTS IN KAKAMEGA COUNTY, KENYA

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN INFECTIOUS DISEASES IN THE SCHOOL OF HEALTH SCIENCES, KENYATTA UNIVERSITY

JULY, 2024

DECLARATION

This thesis is my original work and has not been presented for the award of a degree or any other award in any other University.

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DEDICATION

I dedicate this work to family members. Thank you so much and may God bless you abundantly.

ACKNOWLEDGEMENT

I thank God this far. May I acknowledge all the participants who played a role in making this study. I would like to sincerely thank Dr. Nelson Menza and Dr. Iddah Ali of Kenyatta University and Masinde Muliro University of Science and Technology respectively for their guidance throughout this study. May I also thank Kenyatta University Ethical Review Committee for ethical approval of this study. To all the patients who participated in the study, God bless you all.

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LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	-	Acquired Immune Deficiency syndrome
ALT	-	Alanine Amino Transferase
AST	-	Aspartate Amino Transferase
ANOVA	-	Analysis of Variance
ARD	-	Acute respiratory Disease Syndrome
CT	-	Computed Tomography
COVID	-	Coronavirus disease
COVID-19	-	Corona Virus Disease 2019
DIB	-	Difficult in Breathing
DBIL	-	Direct Bilirubin
ESR	-	Erythrocyte Sedimentation Rate
FBC	-	Full blood count.
ID	-	Infectious Disease
GGT	-	Gamma –Glutamyl Transferase
HDL-C	-	High Density Lipoprotein Cholesterol
LDH	-	High Lactate Dehydrogenase
LDL-C	-	Low Density Lipoprotein Cholesterol
LFTs	-	Liver Function Tests
MMUST	-	Masinde Muliro University of Science and Technology
MOH	-	Ministry Of Health.
PCR	-	Polymerase Chain Reaction
PTB	-	Pulmonary Tuberculosis
RDT	-	Rapid diagnostic test

RT-PCR	-	Reverse Transcription polymerase chain reaction
SARS-COV-2	-	Severe Acute Respiratory Syndrome Corona virus 2
SARS	-	Severe Acute Respiratory Syndrome
TAT	-	Turnaround Time
TNF	-	Tumor Necrosis Factor
U/E	-	Urea/Erythrocytes
WBC	-	White blood cells
WHO	-	World Health Organization
2019-Ncov	-	Novel Corona Virus

ABSTRACT

Aims and objectives: Severe Acute respiratory syndrome corona virus 2(SARS-CoV-2) is a type of coronavirus that first emerged in Wuhan, China late 2019. Once this virus infects any person, it causes Coronavirus disease 2019 (COVID-19) capable of causing death. Since then, hundreds of thousands of deaths have been reported as a result of COVID-19 globally. It is critical to detect the people with the potential of becoming very sick with COVID-19 early enough in order to save their lives by testing blood for certain chemicals known as biomarkers. This study aimed to determine biochemical markers in SARS-COV-2 infected patients in Kakamega County, Kenya. The Selected biochemical markers LFT, RFT, electrolytes, blood glucose level C-reactive protein (CRP), lactate dehydrogenase (LDH), were also evaluated for correlation with disease severity.

Materials and methods: A cross-sectional study was conducted on 350 patients with COVID 19 attending Kakamega County general teaching and Referral Hospital. Ethical approval was sought from Kenyatta University Ethical and research committee and permission was obtained from Kakamega county general teaching and referral hospital administration. Socio-demographic, clinical characteristics of covid-19 among admitted patients in Kakamega Country Referral hospital was collected using a questionnaire. Five (5ml) of venous blood was collected and analyzed using standard hematological parameters profile, assessment of liver, renal and cardiac functions tests and evaluation of High-sensitivity C-Reactive protein.

Results: A total of 350 patients were enrolled in the study. Majority of the participants were male (52.9%, 185/350) while 165(47.1%) were female. Out of the 350 patients recruited, 296 (84.6%) were Covid-19 positive while 44 (15.1%) were negative. This indicated a high prevalence of 84.6% of Covid-19 among patents who attended the facility during the study period. Data presentation was done using tables and figures and analyzed using formula of Le and Boen, Chi square and Correlation tests.

Conclusions: Out of the 350 patients recruited, 296 (84.6%) were Covid-19 positive while 44 (15.1%) were negative. This indicated a high prevalence of 84.6% of Covid-19 among patents who attended the facility during the study period. Biochemical markers of the liver (ALT, AST), Total bilirubin, blood protein were elevated among Covid-19 positive patients, while albumin was low among Covid-19 negative patients.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

Coronavirus an infection affecting humans which emerged in 21st century *(May et al., 2020*). There were more than 188,128,952 confirmed cases of COVID-19, which included 4,059,339 deaths (2.2% mortality) globally report (Wang *et al*., 2020). This disease (SARS-CoV-2) resulted in great fatality and eventually serious financial hardship worldwide. Much of the studies so far done on COVID-19 symptoms and hematologic framework of patients upon admission do not provide information on medication (*Wang et al., 2020*), and notably there was need for abit of research on compelling variations with the victim disease course (*Liu et al., 2020).*

There were notable series of clinical symptoms on patients with Corona virus disease. This includes extreme heat, hack, sorehead, retching, puking, loss of appetite, looseness of the bowels, labored breathing, multisystem organ failure (*Chen et al., 2020).* A large group of Corona virus victims presented with moderate manifestation of the infection and recovered *(Liu et al., 2020),* However a small group of infected people sequentially advance to complexity, notably septicemia, stalling, flatulence, asystole, nephropathy, brain disorder, and ultimately succumb to the disease (*Chen et al., 2020).* Research is currently underway into new expressions such as loss of smell and taste. *(Chen et al., 2020).* With cognition of immense spread and contagious sequence, WHO announced the illness to being catastrophic and of community well-being interest on March 31, 2020. During the onset of the disease pandemic, death rate was recorded at 2-5 %, far greater among the elderly (*Chen et al., 2020).* Incidence of death among the infected people

confirmed in Wuhan city was recorded at 7% in the onset early days (*Gautier & Ravussin, 2020).*

Initial detection of acute disease exposure would assist health care givers expedite appropriate remedial measures and aid curb death rate *(Lu et al., 2020).* Preliminary present data on laboratory-criteria on coronavirus patients highlighted alterations in the victims' chemical compounds, which were white blood cell count, and D-dimer status *(Liu, Martins-Filho et al., 2020).*

A further research work asserted alterations in chemical compounds among COVID-19 cases, including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and Interleukin-6 (*Henry et al., 2020).* Similarly, more studies recorded lymphopenia, hyperglycemia gamma-glutamyl transferase (GGT), elevated lactate dehydrogenase (LDH) more among SARS-COV-2 cases. (*Gao et al., 2020; Bonilla-Aldana et al., 2020).* Notably, laboratory analysis of 77 COVID-19 fatalities and 852 COVID-19 cases equally showed elevation in renal and cardiac associated biomarkers. (*Zhang et al., 2020*). Laboratory findings in 143 SARS-COV-2 incidences indicated elevated levels of cardiac, renal, as well as liver associated biomarkers as well as reduced ranges in albumin openly associated to serious disease progression (*Wang et al., 2020*).

1.2 Statement of the Problem

Kakamega county general teaching and referral hospital receives patient from the vast former western province that is densely populated, this was an indicator for horizontal

dissemination of the disease. The developing nations with limited resources have greatly felt the impact since their healthcare systems were not prepared for such a widespread of the disease. With the increase in infection and the changing nature of the virus, there emerged versions of the virus that have not been experienced previously which necessitates immediate and expedient intrusion. Different types of vaccines have been developed however there is a drift due to the emergence of superinfection in people previously immunized. Molecular tests such as polymerase chain reaction (PCR), act as a gold standard for the diagnosis of COVID-19 infection. The requirement for advanced equipment and the lack of trained personnel to perform reverse transcriptase polymerase chain reaction (RT-PCR) pose challenges. Therefore, biomarkers (ALT, AST, CRP, D.,bil, T.bil, WBCs, RBCs, CRPs, D-dimer, electrolytes (Na+,K+,CL,), blood glucose, can be used to predict the occurrence and the severity of the disease hence act as a prognosis tool for COVID-19 disease.

1.3 Justification of the Study

As the SARS-COV-2 pandemic evolved, medical search patterns decreased significantly and now included all patients, including those taking tuberculosis drugs and antiretroviral drugs (ARVS), which was previously achieved. National health milestones appear irrecoverable. It is taken to avoid infection with the Novel corona Virus disease (COVID-19), therefore, in order to reduce the medical burden, it is necessary to improve the quality of COVID-19patients and strengthen advance detection, treatment, and care which will lead to the transformation and relief of this important entity due to the spread of infection. And by its nature, the SARS-COV-2 pandemic spreads throughout the

population, with many cases showing no symptoms of the disease, necessitating accelerated investigation and testing. The greatest impediment to amidst other factors is the method of acquiring samples at the moment that is incursive making it uncomfortable, equally analyzing procedure is associated with taking more TAT and a reduced value in observation and taking care of the larger asymptomatic Covid-19 populations.

The undertaking in this work was of significant assistance in timely diagnosis and analyzing to be of much assistance in detection procedures, cure, and care of verified cases. Timely identification was of great help as well as crucial in determining public health strategies like sequestration, motion check, as well as decision of restrain duration. This study aimed to use biochemical markers which include Creatinine, liver function tests (LFTs), Electrolytes sodium potassium chloride (Na+, K+, Cl+), Urea, C-reactive protein (CRP), and cholesterol, Low Density Lipoprotein(LDL), High Density Lipoprotein (HDL) Aspartate Aminotransferase(AST and Alanine Aminotransferase (ALT) in detection of covid-19 employing non-invasive sample acquisition approach with a shortened TAT time.

1.4 Research Questions

i. What is the prevalence and clinical characteristics of asymptomatic covid-19 infected patients in Kakamega, Kenya?
ii. What are the biochemical markers abnormalities in asymptomatic covid-19 infected patients in Kakamega, Kenya?

iii. What is the association between the biochemical markers and the severity of the disease in asymptomatic covid-19 infected patients, in Kakamega Kenya.?

iv. What is the diagnostic performance of target parameters in asymptomatic covid-19 cases in Kakamega Kenya?

1.5 Study Objectives

1.5.1 General Objective

To determine biochemical markers analysis for SARS-COV-2 infected patients in Kakamega County, Kenya.

1.5.2 Specific Objectives

i. To determine the prevalence and clinical characteristics in asymptomatic covid-19 infected patients in Kakamega, Kenya.

ii. To determine the biochemical markers abnormalities in asymptomatic covid-19 infected patients, in Kakamega, Kenya

iii. To determine the associations between biochemical markers and the severity of the disease in asymptomatic covid-19 infected patients, in Kakamega, Kenya.

iv. To determine the diagnostic performance of targeted parameters in asymptomatic covid-19 infected patients, in Kakamega, Kenya.

1.6 Significance of the Study

Data obtained would facilitate evidence based decision making and management of COVID-19 infections. Prompt diagnosis was critical and crucial in determining public

health measures such as restricting movement during quarantine and determining the duration of confinement. Molecular tests such as polymerase chain reaction (PCR), act as a gold standard for the diagnosis of COVID-19 infection. The requirement for advanced equipment and the lack of trained personnel to perform reverse transcriptase polymerase chain reaction (RT-PCR) pose challenges. Therefore, biomarkers (ALT, AST, CRP, D.,bil, T.bil, WBCs, RBCs, CRPs, D-dimer, electrolytes (Na+,K+,CL,), blood glucose, can be used to predict the occurrence and the severity of the disease hence act as a prognosis tool for COVID-19 disease.

CHAPTER TWO: LITERATURE REVIEW

2.1 Epidemiology of Covid-19 infection

Early Covid-19 infection got highlighted to have occurred at the city of Wuhan, Hubei province, China towards late 2019. Since then the disease spread across the world culminating into a pandemic (*Ciotti et al., 2020*). As of first quarter of 2020 the largest number of COVID-19 was recorded in Italy, United States, Spain, France, Iran and Germany (*WHO, 2020).* The initial ascertained COVID-19 infection among African nations was reported in Egypt on 14th February, 2020. Notably tallied ascertained around the zone accelerated transcending beyond 5000 cases as of March 31, 2020 (*Ferguson et al., 2020).* There were a few African nations by March 31 that had not reported any confirmed infection; Comoros, Lesotho, Malawi and South Sudan. Currently, there are 414, 525,183 Covid-19 cases across the world. About 5, 832,333 of the total cases have had fatal outcomes (*WHO, 2022).*

Covid-19 is a viral infection whose known mode of transmission is contaminated air (*Azimi et al., 2020*). The virus has been reported to be viable while suspended in the air for 3 hours but if it lands on a hard surface, it can survive to a maximum of 72 hrs, (*Van Doremalen et al.,* 2020). The spread of this disease is via nasal droplets from infected to healthy individuals. As such, the spread of the virus has majorly been facilitated by the interaction of people while carrying out their daily activities. Countries with relatively large populations of close contact people such as China, Europe, and America constitute a fundamental platform for the virus to spread. It thus parallels the fact that currently,

European and American regions have the highest number of cases at 167,611,393 and 143,864,770 accordingly (*ECDPC, 2022).*

South-East Asia follows in third position with 54,599,070 cases while the Eastern Mediterranean has reported 19,600,215 cases as of 16th February 2022. Interestingly, the African region has the least number of cases reported at 8,259,115 as of 16th February 2022 *(WHO, 2022).* However, these values cannot be taken with absolution as some states are not members of WHO and therefore do not provide data on their Covid-19 cases. Furthermore, the diagnostic capacity is relatively low in some regions pointing to a possibility of underreporting *(Chitungo et al., 2020*).The African region has a total of 52 countries out of which 47 registered Covid-19 incidences(*Centers for Disease Control & Prevention* (*CDC, 2021*). Out of the reported 8,259,115 cases, 166,912 have resulted in death while; 7,402,340 have recovered (*CDC, 2021; WHO, 2022).*

Compared to other regions, Africa, in general, has not been severely impaired by the outbreak in terms of death rate. South Africa has been the most hit country reporting the highest cases currently at 3.6 million (SAVOP, 2022). The East African region has almost 1.3 million cases with Ethiopia leading at over 450,000 (*Faria, 2022).* Kenya follows in second position with 322,517 cases while Uganda and Rwanda in third and fourth position with 162,932 and 129,311 cases respectively (*HDE, 2020).*

Kenya became the initial country that reported a Covid-19 case in East Africa (*Medical Xpress, 2020).* This happened on 13th March, (*WHO, 2020).* By 8thof the month more

than 45,000 incidences and 1,000 COVID-19 fatalities had been reported as well established expansion of infected people across the 47 counties as attested by the Ministry of Health *(MoH, 2022).*

Amidst total cases reported country wide as of 16th February 2022, 5,633 cases have been fatal while, about 302,918 have recovered. As of 20th February, 2022, the positivity rate (R) stands at 0.5% (*MOH, 2022).* Kakamega is one of the regions situated at Kenyan western province. Last situational report on Covid-19 done on 22nd April, 2021 indicated that Kakamega had cumulative 31 reported cases (*MOH, 2022).* Kakamega is densely populated with a population of 1.6 million people and thus potential for horizontal dissemination is relatively high. The hot and wet climatic conditions in the county however may not support thriving of the virus outside the body of a host (*Pawar et al., 2020*) as shown in Figure 2.1 below.

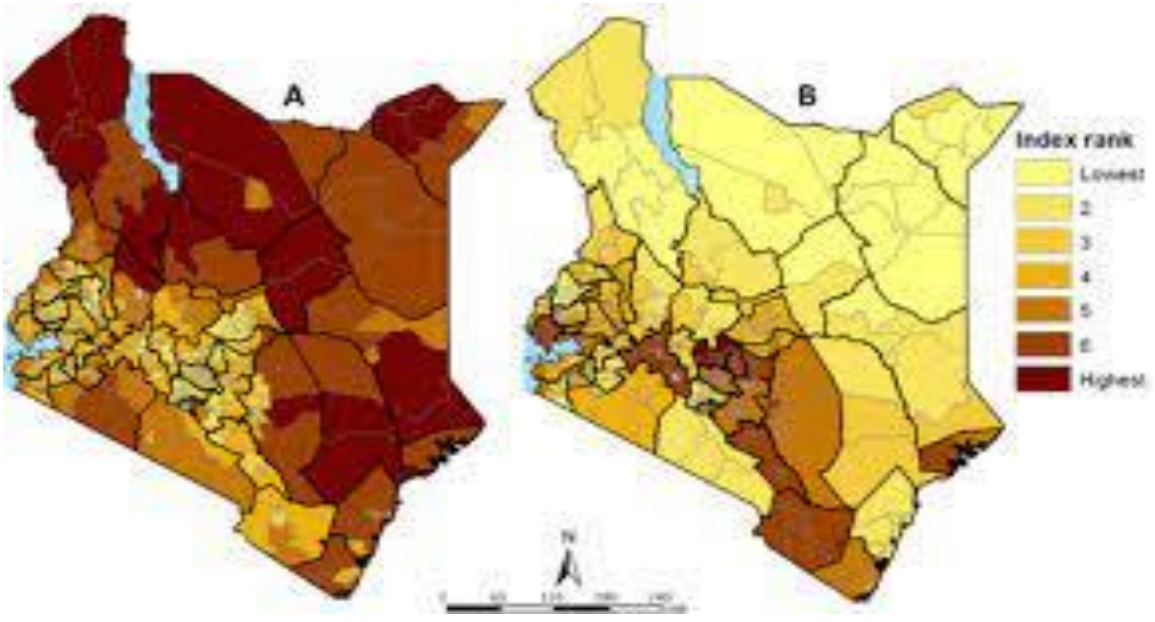

Figure 2.1: Distribution of SARS-Covid- 19 cases across the counties

(*Source: MOH, 2022).*

2.2 Clinical manifestations of Covid-19 cases

Dissemination of SARS-COV-2 has been shown occurring in the asymptomatic phase *(UKRI, 2020; Liet al., 2020).* Moreover, the infection affects both children and adults but children often have no or mild symptoms. Elderly people, on the other hand, are the worst hit with severe COVID-19 symptoms, especially in a setting of morbid conditions *(Dai et al., 2020; Singh-Grewal et al., 2020).* Children have also been implicated to be a source of transmission to the elders as they either have no or mild symptoms and viral transmission is greatest during the asymptomatic phase *(Yoon et al., 2020).* A study done by Yu *et al*. demonstrated that the infection in both patients presenting the signs and those not presenting with any sign of the disease to be similar *(Yu et al., 2020).*

Majority of the population of asymptomatic patients has been shown to comprise individuals with three characteristics: Most of them were young, of the female gender, and did not have any co-morbidity at the time of infection *(Kim Dai et al., 2020).* Asymptomatic individuals usually do not manifest any signs and symptoms and laboratory diagnosis shows no abnormalities but a PCR test of a nasal swab shows the presence of the viral genetic material *(Kim et al., 2020).* They thus remain an active and potential source of spreading the infection to the general population if not tested earlier enough (*Bai Rothe et al., 2020).*

Confinement of this disease takes about two weeks with an average of 4 to 5 days after exposure *(McIntosh, 2022).* During this time, the virus uses the host cell machinery to grow and manufacture new virion particles that in turn infect other cells. The initial

symptomatic manifestations that have been reported are headache, myalgia and cough, diarrhea, loss of taste and sore throat *(Wang et al., 2020).* If the condition is not addressed, the infection progresses to a serious form in which pneumonia ensues. The pneumonia is characterized by dyspnea, fever, bilateral lung infiltrates and cough *(Yang et al., 2020).* The symptomatology of this infection has is indicated being more serious among middle aged and older adults. This may be attributed to existence of at least one co-morbid condition in most adult *(Richardson et al., 2020).*

Most young adults, adolescent and children typically acquire asymptomatic form of the disease even after incubation period has elapsed. The symptomatic cases reported in most children are mild and resolve spontaneously *(Levin et al., 2020).* If not treated, Covid-19 has severe complications. These complications can be classified as respiratory failure, cardiovascular complication, thromboembolic complications, neurologic problems, inflammatory and secondary infections (McIntosh, 2022).

ARDS is the initial and frequent complication often occurring after onset of dyspnea. If not promptly managed, ARDS is highly fatal. Venous thromboembolism, myocardial injury, shock, arrhythmias are common thromboembolic and cardiovascular complications *(Bialek et al., 2020).* Involvement of the nervous system especially the brain leads to stroke, seizures, and encephalopathy and movement disorders. Secondary infections such as Aspergillosis, *Streptococcus pneumoniae*, and *Staphylococcus aureus* infections were commonly reported as coinfections with Covid-19 disease *(Bialek et al., 2020).* The systemic manifestation of the disease is associated with abnormalities in the

laboratory panel. These changes include lymphopenia, elevated LDH, inflammatory markers and aminotransferases *(McIntosh, 2022).* The changes correlate to tissue injury being experiment during progression of the disease.

2.3 Biochemical Markers Abnormalities in Covid-19 Infected Patients

Corona virus uses the ACE-2 receptor found at the top of epithelium of the respiratory system to gain access into cells. They replicate within such cells and spread first within the respiratory system then into the systemic circulation *(Sarhan et al.,* 2021*).* The said receptor is located in various tissues including, small intestines, liver, kidney and heart. Systemic circulations ensure the virus gets access to these tissues where they again infect the cells, replicate and form new virions. The cumulative effect is activation of the immune system and changes in the landscape of the biochemical markers in the body (Liu *et al*., 2021).

A biochemical marker is a compound produced during the initiation, pathogenesis, and pathology of a disease process *(Robb et al., 2016; Samprathi & Jayashree, 2021).* It is paramount to identify critical cases early enough, to decrease fatality and improve the recuperating period. Surveillance of chemical compounds in SARS-COV-2 patients is crucial for determining infection austerity, advancement also observing remedial arbitration. Biochemical variations such as decreased albumin, elevated biochemical markers of the liver as well as the heart, were key signs for ICU admission among 140 COVID-19 patients (Liu *et al*., 2020). Increased procalcitonin levels has been observed in terminally ill patients and therefore reported to a prognostic marker patients with COVID.

A comparison of biochemical compounds between survivors and non-survivor showed that elevations in white blood cells, neutrophils, urea, creatinine, kinase, high-sensitivity cardiac troponin, lactate dehydrogenase, D-dimer and IL-6 increased the risk of death in patients with COVID. (*Wang et al., 2020).* Serum concentration of D-dimer, c-reactive protein and procalcitonin have been reported to increase 2-fold in severely ill patients compared to mildly ill patients (Martin-Filh *et al.,* 2020).

Biochemical findings in patients with COVID-19 include decreased albumin and increased levels of AST, ALT, total bilirubin, BUN, creatinine kinase, lactate dehydrogenase, myoglobin, creatinine kinase MB and cardiac troponin I. These include significant increase in liver enzymes, renal biomarkers and CRP levels. A patient with a severe form of the disease was reported (*Liu et al., 2020*). In a study that investigated serum cholesterol levels in 597 patients with the new coronavirus infection (mild patients; 171 patients, severe patients with the new coronavirus infection and total cholesterol levels were found to be lower than in normal patients. In three groups, remarkable results were obtained with gradual reductions in LDL-C and TC levels. HDL cholesterol levels were significantly reduced in severe cases compared to levels in mild and severe cases. (*Wei et al., 2020).* LDL - C and TC levels were inversely correlated with CRP protein and positively correlated with patient's lymphocyte counts (*Wei et al.,* 2020*).*

The pathophysiology of the novel coronavirus infection COVID-19 involves a complex combination of coagulation immune responses and inflammatory responses. Biomarkers

are classified into different classes such as coagulation, cardiac and inflammatory immunological and inflammatory. Hematological, biochemical, and immunological as shown in the table below (*Samprathi & Jayashree, 2021*).

Table 2.1: Various Classes of biomarkers: coagulation, cardiac, inflammatory, hematological, biochemical and immunological

Biomarker class	**Biomarker example**
Inflammatory	Cytokines, chemokines, CRP, LDH
Electrolyte	Hypocalcemia, hypokalemia, hyponatremia
Cardiac (Li et al., 2020)	BNP, Cardiac troponin
Hematological	Neutrophilia lymphopenia elevated neutrophil: lymphocyte ratio elevated or decreased platelet levels
Hepatic	AST, ALT, Albumin, bilirubin
Fats	LDL, HDL levels.
Renal	Creatinine
Coagulative	d-dimer, prothrombin time, fibrinogen
Muscle	Myoglobin, creatine-kinase

Sources: (*Amgalan & Othman, 2020; Huang & Pranata, 2020; Terpos et al., 2020; Zeng et al., 2020).*

During pathophysiology of Covid-19, changes in these biomarkers can reflect as initiation, progression or severity of the infection and subsequently used for prompt diagnosis and treatment (*Leulseged et al., 2021*). Since Covid-19 causes multi-system organ failure, the clinical picture portrayed by the biomarkers is quite complex and whether it correlates to initiation, progression, or prognosis of infection is yet to be fully elucidated. Currently, such biomarkers are majorly used to assess the severity of infection rather than being used as a tool to diagnose patients. Moreover, symptomatic patients are the ones that majorly showcase changes in such biomarkers (*Samprathi & Jayashree, 2021).* The occurrence of changes of such biomarkers in asymptomatic patients is yet to

be fully explored. However, it may paint a picture of a mild form of changes of biochemical markers in symptomatic patients.

2.4 The Association between Biochemical Markers and Severity of Covid-19 infected patients

Initiation of Covid-19 starts with the virus binding to the ACE-2 receptor to gain cellular entry. This receptor is an enzyme that in normal physiology cleaves angiotensin II to inactive by-products. During the infection, the receptor is internalized and an excess of angiotensin II remains in circulation. Excess angiotensin II activates the inflammatory cascade by binding to the angiotensin I receptors resulting in the production of pro inflammatory cytokines: interleukin-6, TNF-alpha, and interleukin 1 (*Keddie et al., 2020*). These cytokines are responsible; for the cytokine storm seen in Covid-19 patients (Hodges *et al*., 2020). Moreover, diabetic patients have been shown to express more ACE-2 proteins on the surface of their cells compared to healthy individuals thus they are at a higher risk and more likely to be infected and acquire a severe form of the disease (*Yu et al., 2020).*

Initial 6-9 days of inpatients, notable elevated serum, LDH and CK was recorded among patients among the seriously ill group as compared to those in the mild group. There was notable changes in CRP levels day 3 and drop on the 6^{th} and 9^{th} in both mild and seriously ill category of patients. COVID-19 m RNA clearance ratio was significantly correlated with decreased serum CK and LDH levels (*Mardani et al., 2020*). Patients with COVID-19 have been found to have high blood sugar levels(7.4 mmol/L) which may be

due to an underlying disease that causes hyperglycemia, similar results have been reported in which blood sugar levels are elevated in severely ill(9.91mmol/L) patients compared to mildly ill patients (7.07mmol/L) (*Rodriguez-Morales, AJ et al., 2020*). Similar results of elevated level of glucose in severe patients compared to the mild cases.

Daily blood analysis of 207 patients who underwent RTPCR testing, after emergency hospitalization with symptoms of COVID-19 showed that plasma WBC, CRP, and AST were significantly lower in plasma WBC, CRP, and AST between patients with a positive genetic test and those without ALT, and LDH levels were found to be statistically significant. Those who tested negative using RT-PCR as the gold standard almost 70% of patients could be classified as COVID positive or negative based on hematological parameters (*Ferrari et al., 2020*). Five studies with a total sample size of 1415 COVID-19 patients showed hypokalemia, hyponatremia and hypocalcemia in patients with severe disease *(Lippi et al., 2020).* Hypokalemia is known to exacerbate acute respiratory distress syndrome and acute cardiac injury, which are common complications in COVID-19, especially in patients with underlying lung or heart disease. Hypokalemia may potentially contribute to unraveling pathogenic mechanisms underlying COVID-19 and hence may have clinically significant implications for patient management (*Lippi et al., 2020; Liu et al., 2020*).

Increased plasma angiotensin II concentration has been described in patients with COVID-19, possibly acting as a mediator of acute lung injury, as earlier confirmed in SARS-CoV animal models (*Kuba et al., 2005; Pan et al., 2020*). Hypokalemia also

provides a pathophysiologic clue, as SARS-CoV-2 binds to its host receptor, angiotensin converting enzyme 2 (ACE2), a likely reduction in ACE2 expression, leads to increased angiotensin II. This can cause increased potassium excretion by the kidneys, ultimately leads to hypokalemia in COVID-19 cases (Kuba *et al*., 2005). Gastrointestinal losses with diarrhea and nausea present in as many as 34.0% and 3.9% of COVID-19 patients respectively, may also contribute to hypokalemia and other electrolyte imbalances (*Wu et al., 2020).*

Primary infection of lung tissues produces more virion particles that gain systemic entry to cause multi-organ damage. The main mode of viral spread and shedding is through the killing of the host cells and thus releasing intracellular contents into the extracellular space. This may or may not destruct the electrolyte concentration of the extracellular fluid. The virus also affects hepatocytes that express ACE-2 receptors. This leads to cytolysis of hepatocytes during viral shedding that is associated with the changes in the ALT, AST levels ultimately destructing the ALT: AST ratio. Moreover, studies have shown that infections with SARS-CoV-2 are associated with increased levels of triglycerides in circulation (*Hilser et al., 2021; Masana et al., 2021*).

These triglycerides together with the inflammatory process of the virus affect the integrity of vascular endothelium leading to vascular inflammation and thrombosis (*Catanzaro Li et al., 2020).* Such pathological processes are associated with neutropenia and elevation of the d-dimers. Cardiac and kidney injury also results in elevation of their specific biomarkers (cardiac: myoglobin, troponin and creatine kinase; kidney: creatinine,

albumin) due to the cell lysis induced during viral shedding. In general, Covid-19 has a heterogeneous spectrum of biomarkers panel due to its multi-system involvement. As such, the use of biomarkers for presumptive diagnosis of Covid-19 is best placed at a panel of biomarkers rather than using a single biomarker.

2.5 The Diagnostic Performance evaluation of target Parameters in Covid-19 Patients

Changes in the behavior of biomarkers during Covid-19 infection can be used for presumptive laboratory diagnosis of the disease subject to definitive nucleic acid-based tests. There is however paucity of information relating to which type of biomarkers to be used and whether they are consistent and can be standardized to be used for performing diagnoses (*Figliozzi et al., 2020).* While the pathogenesis of SARS-COV-2 is similar in every person, factors such as comorbidities and age affect the extent to which a particular biomarker will be expressed and in what amount. In return, this may give a wrong indication of the severity of the infection that ultimately affects therapeutic modalities. In addition, studies have shown that most of the asymptomatic patients do not have co-morbidities and are mostly younger (*Kim et al., 2020).* As such, the use of biomarkers for presumptive diagnosis can be directed towards the younger population and if a confirmatory test is required, a nucleic acid test is done. This will reduce the burden of testing and also the cost of definitive testing (*Figliozzi et al., 2020*).

Studies have majorly focused on evaluating the diagnostic performance of other methods of diagnosis Covid-19 including the use of saliva, radiography, nucleic acid tests, and

ELISA tests (*Yüce et al., 2021).* When it comes to the use of biomarkers, there is limited information that may be attributed to the complex nature of biomarkers associated with Covid-19 *(Kermali et al., 2020; Letelier et al., 2021*). Scholars have however found out those biomarkers such as CRP, interleukin-6, d-dimer, platelet count, and neutrophil to lymphocyte ratio demonstrate promising use for presumptive diagnosis (*Ghahramani et al., 2020*). CRP is one of the first biomarkers whose changes reflect pathophysiology, especially in the setting of Covid-19.Interleukin 6 is the main cytokine associated with Covid 19 cytokine storm while d-dimer is elevated in response to clotting occurring due to thrombosis of vascular systems.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Area

This study was carried out at Kakamega County General Teaching and Referral Hospital. Kakamega County is in the former western province of Kenya. It has a population of 1,867,579 and an area of 3,033.8 $km^{2.}$ (*Kenya census, 2019).* Western Kenya block cumulatively had 20,281 confirmed COVID-19 cases with a majority in urban settings (*Cultural Practices Resilience in the Wake of COVID-19 among Communities in Western Kenya Research Journal in Advanced Humanities, n.d.).* The hospital serves patients from all over western Kenya. It has been vital in the testing and management of Covid - 19 patients.

3.2 Study Population

Patients with COVID 19 attending Kakamega County General Teaching and Referral Hospital. They were categorized into three groups to assess disease severity according to (*WHO 2020)* as moderate, severe and critical.

3.3 Study Design

A descriptive cross-sectional study design was adopted among patients with Covid 19 attending Kakamega County General Teaching and referral hospital.

3.3.1 Inclusion Criteria

1. Asymptomatic cases for COVID-19 aged 18 years and above
2. Asymptomatic cases for COVID-19 with or without co-morbidities

3.3.2 Exclusion Criteria

1. Asymptomatic cases for COVID-19 aged under 18 years

2. Cases reporting respiratory conditions not related to COVID-19

3. Refusal to give informed consent.

3.4 Sample Size Determination

A Fishers *et al* (2008) was used to calculate the sample size using an estimated proportion of COVID-19 infection in the population of 65% (Randolph & Barreiro, 2020).

$$N = \frac{z^2 P(1-P)}{D^2}$$

N = required sample size,

Z = standard normal deviation=1.96 $N = \frac{1.96^2 X\ 0.65(1-0.65)}{0.0025}$

P = prevalence rate of asymptomatic covid-19 =0.65

D = desired degree of accuracy at 95% confidence level=0.05

3.846 x 0.2275 divide by 0.0025 = 350

n = 350

Therefore, 350 voluntary participants were recruited in the study.

3.5 Sampling Technique

This study adopted purposive sampling technique among patients with Covid-19 attending Kakamega County General Teaching and Referral Hospital.

3.6 Data Collection

Socio-demographic, clinical characteristics of covid-19 among admitted patients in Kakamega Country Referral hospital were collected. A structured questionnaire enabled the acquisition of information of the participants' demographic and clinical data.

3.7 Sample collection

At most 5ml of venous blood was collected from each Covid patient in a plain red top vacutainer tube with no additive. The rest of the whole blood was collected in a serum separating gel tube to obtain blood serum for further laboratory tests (*WHO, 2020*). All laboratory tests for blood samples were carried out on the same day of collection. DIRUI BCC automated hematology analyzer and 36000 automated chemistry analyzers manufactured in China were used to determine hematological and biochemical markers. Quality of test results was maintained by running commercially prepared three-level quality control (low, normal and high) reagents before running the patient's sample. During laboratory analysis, standard operational procedures (SOP) were strictly followed with integrity of samples and reagents regularly checked. Samples were stored in sterile cups with viral transport medium, and kept at 4 °C.

3.8 Laboratory Analysis

3.8.1 Hematological profile

A haemogram test (HMG), also called a whole blood test was performed on blood sample. The parameters (WBCs, RBCs, Hb,) were measured using automated hematology analyzer (DIRUI BC3600) which was validated and quality controlled done

prior to analysis of blood samples collected at EDTA vacutainers. Five (5 ml) of blood was collected in a test tube with the anticoagulant, ethylene diamine tetra acetic acid (EDTA). The hematological parameters analyzed included white blood cell count (WBC) and their subsections (neutrophils, monocytes and lymphocytes), red blood cell (RBC) count and hemoglobin (Hb) concentration. According to the WHO standards values anemia is diagnosed when the haemoglobin level is estimated <12.0 g/dl in adult women and <13.0g/dl in adult men (WHO2019).

In addition, leukopenia was defined as a total white blood cell count <3.6 x109/l and thrombocytopenia was defined as platelet count <150,000/µl. Cytopenia was defined as the presence of anemia, leukopenia or thrombocytopenia, whereas dicytopenia was characterized as the presence of two forms of cytopenia (*WHO 2019*).

3.8.2 Assessment of Selected liver, renal and cardiac functions

Selected liver, renal and cardiac functions were assessed according to the procedure of Liu *et al.* (2020). The liver function tests included, aspartate aminotransferase (AST), alanine saminotransferase (ALT), gamma-glutamyl transferase (GGT), total bilirubin, direct (conjugated) bilirubin and albumin, alkaline phosphatase (ALP). Blood serum samples were processed and analyzed on a fully automated clinical chemistry analyzers (DIRUI CST-180 automated analyzer) which was validated and quality controlled done prior to analysis of blood samples collected at plain vacutainers. The study results were obtained and reported (see chapter 4). Test interpolation which include normal ranges,

3.8.2.1 ALT

ALT stands for Alanine transaminase, an enzyme found mostly in the liver. An ALT test measures the amount of ALT in the blood. When liver cells are damaged, ALT is released into the blood stream. High ALT levels of in the blood may be sign of liver damage or disease. ALT test principle; in the reaction, ALT catalyzes the reversible transamination of L-alanine α-ketoglutarate to pyruvate and L-glutamate followed by reduction of pyruvate to lactate in the presence of lactate dehydrogenase (LDH) will be done simultaneous oxidation of NADH to NAD.

3.8.2.2 Alkaline Phosphatase (ALP)

The alkaline Phosphatase (ALP) Test measures the level of ALP in the blood which comes from the liver and bones and it's one of the tests included in a comprehensive metabolic panel. High levels of ALP in the body can be a sign of liver disease or certain bone disorders. This is done according to the following principle.

During the reaction of magnesium ions, P-Nitrophenyl phosphate hydrolysed to phosphatase to form phosphoric acid and P-Nitrophenyl. The release rate of P-Nitrophenol is proportional to ALP activity and can be measured photometrically. Elevated ALP activity is associated with two of diseases: Those that affect liver function and those that affect the activity of osteoblasts in bones. In liver disease, increased ALP activity is generally considered a sign of bile duct obstruction.

Aspartate Aminotransferase (AST) Aspartate aminotransferase (AST) is an enzyme that is found primarily in the liver, but it's also in muscles and other organs in your body. When cells that contain AST are damaged, AST is released into the blood An AST blood test measures the amount of AST in your blood.

The test is done in accordance to the following principle Aspartate aminotransferase (AST/GOT) catalyzes the transfer of the amino group from aspartate to oxoglutarate to form glutamate and oxaloacetate. The latter is reduced to malate by malate dehydrogenase (MDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance due to oxidation of NADH to NAD+, which is proportional to the activity of AST present in the sample.

3.8.2.3 Total Bilirubin

A bilirubin blood test measures the level of bilirubin in the blood. Bilirubin is a yellowish pigment found in bile, a fluid made by the liver. Bilirubin passes through the liver and is eventually excreted out of the body. Higher than normal bilirubin levels can indicate various types of liver or bile duct problems. Increased bilirubin levels may be caused by an increased rate of destruction of red blood cells.

Test principle. Bilirubin is measured by diazotization and the formation of red azobilirubin, which has absorbance maximum at 525nm.Direct bilirubin (diglycuronide)

is measured in aqueous media, while total bilirubin (direct and indirect) is measured by the action of strong solubilizing agents with catalytic effect.

3.8.2.4 Total Protein

Total protein and albumin/globulin (A/G) ratio test measures the total amount of protein in the blood. There are two main types of protein in the blood: **Albumin**, prevents blood from leaking from blood vessels. It also supports the movement of hormones, medicines, vitamins, and other important substances throughout the body. Albumin is made in the liver.

3.8.2.5 Globulins

This test also compares the amount of albumin and globulin in the blood. The comparison is called the albumin/globulin (A/G) ratio. Measurement of total protein are used in the diagnosis and treatment of liver diseases, kidney, or bone marrow diseases, and other metabolic disorders .Abnormal results in total protein levels or A/G ratio are an indicative of severe liver, kidney or bone marrow disease.

Test principle: Proteins forms strong purple-blue complexes with copper salts in an alkaline media. Contains iodide as an antioxidant. The intensity of the color formed is proportional to the total protein concentration in the sample.

3.8.2.6 Gamma Glutamyl transaminase

A gamma-glutamyl transferase (GGT) test measures the amount of GGT in the blood. **Test principle** enzyme rate methods are used to determine the GGT activity in serum or plasma. In the reaction, the GGT catalyzes the transfer of a gamma-glutamyl group from the colorless substrate, gamma-glutamyl-pnitroaniline, to the acceptor, glycylglycine producing the colored product, p-nitroaniline. The system monitors the rate of change in absorbance at 410 nm over a set period of time. The rate of change in absorbance is directly proportional to the activity of GGT in the sample.

3.8.3 Rapid Test Polymerase chain reaction (primers used)

Blood analysis of patients was also performed according to standard procedures: real-time reverse-transcriptase polymerase chain reaction (RT-PCR) test for SARS-CoV-2, after being admitted to the emergency room with COVID-19 symptoms using the procedure of (*Ferrari et al. 2020*). Using RT-PCR as the gold standard, the test was done to confirm positivity of patients to COVID-19 infection. Biospeedy® SARS-CoV-2 Variant Plus kit, Bioeksen with the following primers was used. In this kit, 6-carboxy-fluorescein (FAM), phosphoramidite (Hex), 6-carbocyl-X-Rhoddamine (ROX) and, carboxylic acid (Cy5) channels were utilized for ORF1ab

ORF1ab	CTAGGACCTCTTTCTGCTCA ACACTCTCCTAGCACCATCA
S	CCCTGTTGCTATTCATGCAG CCCTATTAAACAGCCTGCAC
N,	CCTCTTCTCGTTCCTCATCA CCTGGTCCCCAAAATSTTCCT

In this kit, 6-carboxy- fluorescein (FAM), phosphoramidite (Hex), 6-carbocyl-X-Rhoddamine (ROX) and, carboxylic acid (Cy5) channels were utilized for ORF1ab respectively. Based on the kit protocol 2.5 µl patient samples with VTM was added to a 7.5 µl ready kit mixture to achieve a 10 µl PCR mixture in total. Thermal cycle parameters of RT-PCR amplification was set as follows: 52°C for 3 min for reverse transcription, 95°C for 10 s for holding, then 35 cycles of 85°C for 1 s and 60°C for 1 s for denaturation, annealing, and extension, respectively.

3.9 Ethical approval and Research Permit

Ethical approval was sought from Kenyatta University Ethical and Research Committee while Research permit was obtained from the National Commission of Science Technology and Innovation (NACOSTI). All study participants gave an informed consent to participate and codes were used when recording the results instead of patients names to ensure confidentiality.

3.10 Data Analysis

Data presentation was done using tables and figures. The prevalence of covid-19 infected patients was calculated using the formula of Le and Boen, 1995 ($\frac{O}{P} X\ 100\%$), Where 0 =the number of individuals with the disease, P = the population at risk of having the infection during the study period. The clinical characteristics i.e. severity of the disease and biochemical markers abnormalities in covid-19 infected patients was analyzed using Chi square tests. The associations between biochemical markers and the severity of the disease in covid-19 infected patients were tested using correlation test. Diagnostic

performance of targeted parameters in covid-19 infected patients was analyzed using Chi square tests.

CHAPTER FOUR: RESULTS

4.1 Prevalence and clinical characteristics of covid-19 infected patients in Kakamega, Kenya

Among the 350 respondents, prevalence of Covid-19 infected patients attending Kakamega County Referral Hospital between October and November 2022 was established. Out of the 350 patients recruited, 296 (84.6%) were Covid-19 positive while 44 (15.1%) were negative. This indicated a high prevalence of 84.6% of Covid-19 among patents who attended the facility during (Figure 4.1).

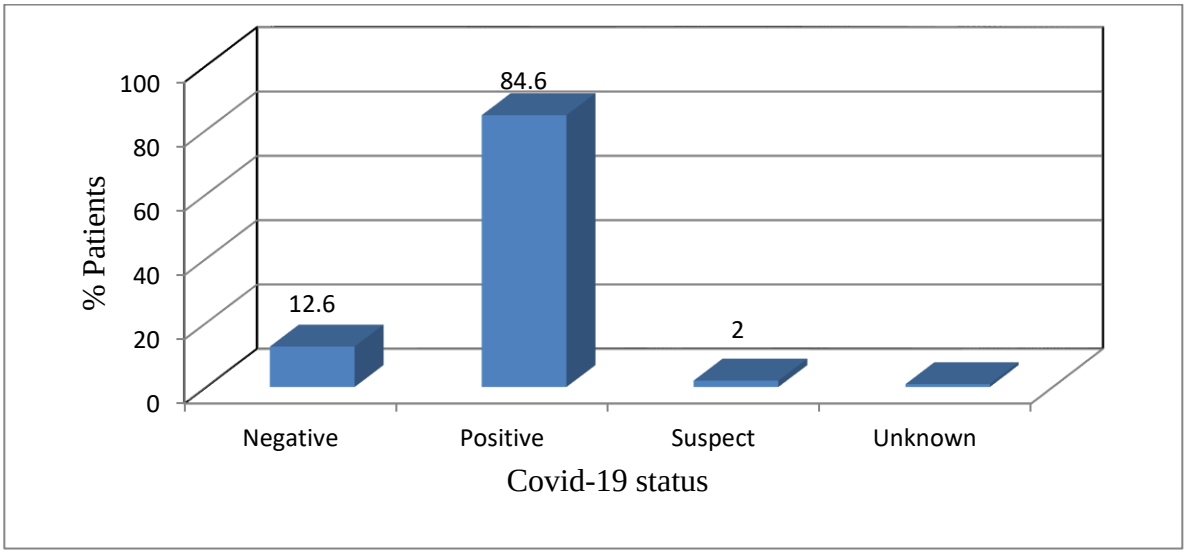

Figure 4.1: Prevalence and clinical characteristics of Covid-19 infected patients

4.1.1 Prevalence of covid-19 according to age of patients

When a comparison using One-Way analysis of variance was used to show the average ages of the positive patients, the result showed that Covid – 19 positive patients were in the average of 58.5 years while those who were Covid-19 negative were 49.8 years of age. This result therefore showed there was a significant difference in ages of the Covid-

19 positive patients (F -= 3.494, P = 0.016). It was further established that Covid-19 affected a wide range of ages, Table 4.1 below.

Table 4.1: Prevalence of covid-19 according to age of patients

Covid-19 status	Mean age	SE	Range of age	F -value	P value
Negative	49.8	3.01	17 – 74		
Positive	58.5	1.03	18 – 99		
Suspect	59.8	7.8	30 – 75		
Unknown	68.0	0.0	68 – 68	3.494	**0.016**

4.1.2 Clinical characteristics of covid-19 infected patients

4.1.2.1 Clinical diagnosis/presentations of Covid-19 positive patients

Some of the clinical diagnosis reported for COVID- 19 patients indicated was that of diabetic mellitus, Hypertension, Pneumonia or PTB (Table 4.2). Based on the clinical diagnosis, 6.1% of the Covid-19 positive were clinically diagnosed as diabetes mellitus, 2.7% as hypertension, 1.0% as pneumonia, clinical diagnosis was significantly associated with prevalence of diabetics in the samples (χ^2 = 27.288, P = 0.024), Table 4.2 below.

Table 4.2: Clinical diagnosis of Covid-19 positive patients

Comorbid	Diabetic mellitus	Hypertension	Pneumonia	PTB	
Positive (N = 296)	18 (6.1%)	8(2.7%)	3(1.0%)	-	-
Negative (N=44)	0(0.0%)	0(0.0%)	0(0.0%)	3(6.8%)	
χ^2 =			27.288		
P – value			**0.024**		

4.1.2.2 Prevalence of diabetics among the Covid-19 patients

Out of the population of 350, diabetic condition was found in 36 patients (10.3%). Most of the diabetic patients (8.5%) were also Covid-19 positive. However, these were 30 of the population showing a significant association between Covid-19 infection with diabetic conditions ($\chi^2 = 27.550$, P = 0.0001) as shown in Table 4.3.

Table 4.3: Association between prevalence of Covid-19 and diabetic condition of the patients

	Covid-19 status				
Diabetic condition	**Negative**	**Positive**	**Suspect**	**Unknown**	**Total**
Not diabetic	41(11.7%)	266(76 %)	7 (2 %)	0 (0.0%)	314
Diabetic	3 (0.9%)	30 (8.5%)	0 (0.0%)	3 (0.9%)	36
χ^2 =			27.550		
P – value			**0.0001**		

4.2 Biochemical markers abnormalities in covid-19 infected patients, in Kakamega, Kenya

4.2.1 Liver, renal and cardiac functions

Biomarkers of the liver; Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, direct (conjugated) bilirubin and albumin results revealed that ALT and albumin levels were significantly lower in positive Covid-19 samples where compared to Covid-19 negative samples. Using other biochemical markers, there was no significant difference noted in the Covid-19 samples (P > 0.05). It was however noted

that Covid-19 positive patients had lower AST and direct bilirubin levels, the differences were not significant (P > 0.05) as shown in Table 4.4.

Table 4.4: Average levels of the biochemical markers among Covid-19 patients

	Covid -19 status					
Biochemical marker (Mean± SE)	**Negative**	**Positive**	**Suspect**	**Unknown**	**F-value**	**P-value**
ALT	53.6 ±8.65b	38.77±1.52a	49.44±9.71ab	54.0± 0.00b	3.162	0.025
AST	61.51± 5.61a	58.95±3.20 a	50.49 5.28 a	52.0 ± 0.0 a	0.112	0.953
Total Bilirubin	10.93± 1.21 a	11.77±0.69 a	14.92 2.70 a	8.20 ±0.0 a	0.332	0.802
Direct Bilirubin	10.59 ±2.19 a	7.44± 0.52 a	3.60 0.79 a	1.70 ± 0.0 a	2.202	0.088
Albumin	42.06±.82b	34.17±0.75a	39.77± 2.54ab	58.01± 0.00b	2.842	0.038

Mean values in the same row denoted by different letters are significantly different at (P≤ 0.05).

4.2.2 Total Protein levels in the samples

The level of total protein determined in the blood samples of positive Covid – 19 patients was higher (63.5 mg/l) compared to that of Covid-19 negative samples (59.04 mg/l). However, the variations established was significantly lower than the levels in samples that were unknown Covid 19 status (81.5mg/l), 81.50± 0.00b, P value, 0.001 as shown in Table 4.5.

Table 4.5: Levels of total protein in patients' samples

Biochemical marker (Mean± SE)	Covid -19 status				F-value	P-value
	Negative	**Positive**	**Suspect**	**Unknown**		
Total protein	59.04 ±1.92a	63.53± 0.72a	74.14± 5.68ab	81.50± 0.00b	5.697	0.001

Mean values denoted by similar letters are not significantly different at (P≤ 0.05).

4.2.3 Gamma –Glutamyl Transferase levels

The levels of Gamma Glutamyl Transferase enzyme in the blood revealed that, Covid -19 positive samples had elevated levels (105.81units/L) compared to Covid-19 negative samples (88.39 units/L). However, when analyzed with ANOVA, the difference was not significant (P value, 0.137) as shown in table 4.6.

Table 4.6: Gamma –Glutamyl Transferase levels

Biochemical marker (Mean± SE)	**Covid -19 status**				**F-value**	**P-value**
	Negative	**Positive**	**Suspect**	**Unknown**		
GGT (units/L)	88.39± 6.27	105.81 ±3.19	109.29± 8.45	139.00±0.00	1.854	0.137

4.2.4 Alkaline phosphatase levels in the blood

The ALP levels in Covid-19 positive blood samples were higher (87.53 units/l) than the levels in determined negative Covid-19 samples (75.19 units/L). Using one-way ANOVA, the result showed no significant difference in the levels (F = 0.775, P = 0.509), Table 4.7.

Table 4.7: Levels of Alkaline Phosphatase in blood samples

	Covid -19 status					
Biochemical marker (Mean± SE)	**Negative**	**Positive**	**Suspect**	**Unknown**	**F-value**	**P-value**
ALP (units/L)	75.19±7.13	87.53±3.90	62.66± 8.23	81.80± 0.00	0.775	0.509

4.3 The associations between biochemical markers and the severity of the disease in asymptomatic covid-19 infected patients in Kakamega, Kenya

The number of days of the patients was an important indicator since it informs on whether there is an association between the COVID status of a patient and the time they spent at the hospital hence severity of the disease. The numbers of days spent by patients in the hospital were grouped into an ordinal scale with four (4) categories. The patients who spent 4 days or less at the hospital, those who spent between 5 to 9 days, those who spent 10 to 13 days and those who were admitted for more than 14 days. A large proportion of the patients (33.33%) spent 4 days or less at the hospital and 31.75% of the patients reported that they were in the hospital for between 5 to nine days. About 19.05% of the patients spent 14 days or more at the hospital while 15.87% were admitted for between 10 and 13 days as shown in Figure 4.2 below.

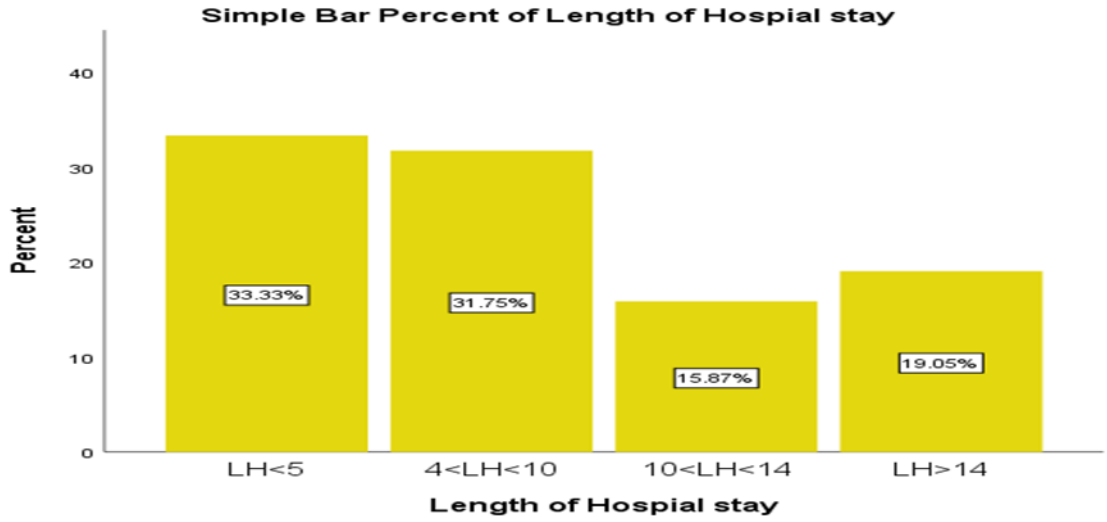

Figure 4.2: Association between severity of COVID -19 disease based on time patient stayed at the hospital

4.4 Diagnostic performance of targeted parameters in Covid-19 infected patients, in Kakamega, Kenya

Covid- 19 diagnosis was carried out using RT-PCR as the Gold standard method. Other methods used involved blood slide sample examination, BS, D-Dimer, ESR, FBC, Gene expert, HBSaq, HBV, K-3-92, RBC, U/E-{protein, UREA method. Performance of the various Covid-19 diagnosis techniques were therefore evaluated based on the percentage out of the Gold standard.

The performance was therefore computed using a formula;

$$Y = \frac{N}{Z} x100$$

Where N = Number of Covid-19 positive samples detected by the diagnostic, Z = Number of the Confirmed samples from the method using RT- PCR method.

All the samples identified as Covid-19 positive by red blood cell counts (RBC) and all those samples identified as positive using Urea test were confirmed using RT-PCR technique (Table 4.8). Only 33.3% of the samples that were purposively identified to be Covid-19 positive using D-dimer were confirmed by RT –PCR while 56.9% of the positive samples according to ESR technique were confirmed to be Covid-19. This showed that the two methods i.e. RBC and Urea were the good performing techniques to use in Covid-19 testing apart from the gold standard method (Table 4.8).

Table 4.8: Diagnostic performance of targeted parameters in Covid-19 infected patients

Covid -19 Diagnosis	**Covid-19 Positive samples**	**Covid -19 PCR confirmed Positive (296)**	**Performance (%)**
Blood slide	14	0	0.0
BS	45	40	88.9
FBC	95	70	73.7
D-Dimer	15	5	33.3
ESR	116	66	56.9
HBS aq	5	0	0.0
Gene expert	5	0	0.0
RBC	10	10	100
HBV	5	0	0.0
U/A-Protein	5	0	0.0
Urea	5	5	100

4.4.1 Performance of saliva test

Out of the 350 samples tested, 314 samples were found to be Covid 19 positive. Result of the test was validated by the RT-PCR test. This showed that only 314 samples were tested both by saliva rapid test and PCR test while the rest 36 samples were not tested using RT-PCR method but were tested using saliva test (Table 4.9).

Table 4.9: Performance of the saliva test method

Covid -19 Diagnosis	**Covid-19 Positive samples**	**Covid -19 PCR confirmed Positive**	**Performance (%)**
Saliva test	130	117	90.0%

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Sociodemographic characteristics of covid-19 infected patients attending Kakamega hospital recruited in the study

In this study, 350 covid-19 infected patients attending Kakamega County referral hospital were recruited in the study. Out of the 350 participants recruited in the study, 296 (84.6%) were confirmed having Covid-19 positive while (44) 15.4% were negative. The participants age of was between 17 and 99 years. Based on gender, most patients who admitted at Kakamega County referral hospital with asymptomatic COVID-19 were males (185/52.9%) while 165 (47.1%) were females. This is in agreement with other studies done by Wang D et al., 2020; Huang et al., 2020; Chang D et al., 2020 in which majority of the patients (54%) were males. This male dominance is not fully understood and may require further investigation. Other explanations to why men were associated with severe outcomes compared with women in response to COVID-19 infection may involve differences in immunologic reaction and the lack of protective effect of estrogen signaling seen in females (*Mack M., et al.*2017)

5.1.2 Prevalence and clinical characteristics of covid-19 infected patients in Kakamega, Kenya

5.1.2.1 Prevalence of Covid-19 according to age of patients

COVID-19 is an ongoing pandemic and the virus is still spreading worldwide (*Ciotti et al., 2020).* As stated by World Health Organization (WHO) reports, more than 110 million cases have been confirmed globally and 2.44 million deaths (*WHO, 2021*). Out of

the 350 patients recruited, 296 (84.6%) were Covid-19 positive while 44 (15.1%) were negative. This indicated a high prevalence of 84.6% of Covid-19 among patents who attended the facility during the study period. This could be associated with the fact that Kakamega County has a population of 1.6 million people and thus the potential for horizontal dissemination is relatively high.

5.1.2.2 Clinical characteristics of Covid-19 infected patients

Some of the clinical diagnosis reported for COVID-19 patients indicated was that of diabetic mellitus, HIV/ AIDS, Hyperglycemia, hypertension, pneumonia or PTB (*Liu, Wang.et. al2020)*. Based on the clinical diagnosis, 6.1% of the Covid-19 positive were clinically diagnosed as Diabetes mellitus, 2.7% as Hypertension, 1.0% as Pneumonia, 1.0% as Hyperglycemia, 0.7% as HIV/ AIDS. This agrees with several studies done elsewhere that have shown age, comorbidities, and abnormalities of various clinical biomarkers can be essential to understand disease severity (*Liu, wang.et. al 2020*). The study population had an underlying medical condition (s). Huang et al, 2020 reported similar findings (32%), however, Wang et al, 2020 reported much higher values (46.4%). About a quarter (25.3%) had hypertension and 13.7% had diabetes mellitus as coexisting medical conditions. These figures closely reflect the prevalence of these conditions in the general Kenyan population. These differences could be as a result of sample size and sampling differences.

Contrary to reports from other studies by Wang et al., 2020, in which majority of patients presented with fever (>90%) and cough (59–76%), nearly half (49%) of the patients in

this study were asymptomatic at the time of diagnosis with only 6.8% having fever or reporting a history of fever and less than a quarter (23%) having cough at the time of diagnosis. This significantly lower proportion of patients with symptoms at the time of diagnosis could be the result of the approach adopted by Kenya in identifying cases of COVID-19. After the first few imported cases of COVID-19 in Kenya and the institution of mandatory quarantine of all travelers arriving into Kenya, all the quarantined travelers were mandatorily tested for the SARS-COV-2 virus irrespective of clinical presentation. It is plausible to expect that a proportion of this group may have contracted the disease while in transit through major travel hubs and international airports in Asia, Europe and Northern America which by then were major global COVID-19 epicenters. Therefore, though these individuals tested positive, they were likely to be presenting much earlier in their disease process.

In addition to the routine surveillance, the country also adopted an enhanced surveillance approach where all contacts of cases were identified and tested (*WHO2020)*. Community members of these cases were also identified and tested whether they had symptoms or not. This made it possible for individuals to be diagnosed and isolated before the onset of symptoms in a lot of cases. This has the potential of reducing the number of contacts made by these individuals and thus reducing the rate of spread of the virus. Further studies might be required to determine more precisely how long it takes for a confirmed case of COVID-19 to fully recover in Kenya.

5.1.3 Biochemical markers abnormalities in Covid-19 infected patients in Kakamega, Kenya

This study observed that biomarkers of the liver; Alanine aminotransferase **(ALT),** aspartate aminotransferase (AST), total bilirubin, direct (conjugated) bilirubin and albumin, result revealed that ALT and albumin levels were significantly lower in positive Covid -19 samples when compared to Covid-19 negative. The amount of total protein (TP) determined in the blood samples of positive Covid-19 patients (63.5 mg/l) was higher compared to that of Covid-19 negative samples (59.04 mg/l).This was an indicator that the viral load was significantly high However, the variations established was significantly lower than the levels in samples that were unknown Covid-19 status (81.5mg/l). It was however noted that Covid-19 positive patients had lower AST and direct bilirubin **(DBil)** levels, the differences were not significant samples. We discovered a marked increase in levels of cholesterol and triglycerides in diabetic and nondiabetic COVID-19-infected patients *(Sarhan et al., 2021).* The most notable comorbidities with COVID-19 in our study were diabetes (6.1%) and hypertension (2.7%). Coronaviruses bind to their target cells via angiotensin-converting enzyme 2 (ACE2), which is widely expressed in the kidney, intestine, and epithelial cells of the lung *(Robb et al., 2016; Samprathi & Jayashree, 2021)*

In this study, many other biochemical indicators showed a significant difference between COVID-19 infected and COVID-19 non-infected. The abnormalities seen in the above mentioned indicators suggested that SARS-COV-2 infection may harm hepatic, renal, myocardial, and other human body organs ((Liu *et al*.,

2020). These findings are in agreement to the earlier research on patients with COVID-19 disease. However, some clinical indicators reported controversy in the present work compared to earlier work, including-D-dimer and platelet count in patients with COVID-19. The change in findings may be due high mortality among patients with COVID-19 in initial days and a lack of complete information on the status of D-dimer in all patients with COVID-19. Controversial presentation of clinical characteristics among patients with COVID-19 need further research on large sample across multiple centers to reach on a specific conclusion (*Keddie et al., 2020*).

5.1.4 Associations between biochemical markers and the severity of the disease in asymptomatic Covid-19 infected patients, in Kakamega, Kenya

The current COVID-19 pandemic is challenging the healthcare capacities and system resilience but also attracting attention on the importance of identifying patients with an increased risk of severe disease. Our study showed biological features (biochemical marker) related to severity of COVID-19 the study observed marked changes in the biochemistry profile (*Wei et al., 2020*). This is as a result of the activation of the innate and adaptive immune response, results from the release of chemokines and other cytokines inducing the inflammatory response (Keddie et al., 2020) this includes the increase of acute-phase proteins such as CRP, LDH, ferritin and fibrinogen which were significantly elevated at admission. Specific liver enzymes, such as ALT, AST, GGT and ALP showed no significant changes at admission for both groups, while LDH was significantly increased in most patients.

Most commonly a hallmark of liver disease, LDH is also a marker of lung injury. Comparable study findings were found by *(Mardan et al.,)*. Significant changes were seen in the blood cell count. Most patients suffering from SARS-CoV-2 show a deregulation of their immune system. Severity biomarkers of the disease such as CRP was consistently increased during hospitalization. This observation confirms its usefulness as an early inflammatory biomarker. These findings were also observed by *(Lippi et al., 2020)* and other studies which concluded that CRP was associated with disease progression and predicted early severe COVID-19.

Ferritin was also higher in the severe group in comparison to other group. Evidence is increasing in the literature for ferritin as an early marker of severity for COVID-19 patients, which confirms our results. Studies suggested that the prognostic effect of cardiac biomarkers in severe COVID-19 patients could be related to direct and indirect effects on the heart. SARS-CoV-2 binds with ACE2, resulting in high levels of angiotensin 2 and restricted synthesis of angiotensin 1-7(*Kuba et al.,2005*) The latter exerts anti- inflammatory effect to protect tissue while angiotensin 2 plays an opposite role and facilitate the secretion of hs-TnT and NT-proBNP, thus, cardiac biomarker levels are associated with the severity of infection. The study also observed a severe lymphopenia in the hospitalized group. When an absolute lymphopenia was not present at the onset of disease, a relative one could be observed. Neutrophils are known to be contributors to lung inflammation and are also identified as a key element in the outcome of viral infections (*Keddie et*

al., 2020).

However, despite their beneficial effect on the initiation of the adaptive response, a pathogenic role for neutrophils in the development of ARDS (acute respiratory distress syndrome) has been proven. Eosinopenia correlates with the presence of absolute lymphopenia and appears to correlate with severity of the disease. Lastly, changes in coagulation parameters were also observed suggesting a vascular component to SARS-COV-2 patients, we examined hemostasis parameters which are classical markers of DIC (disseminated intravascular coagulation) are platelet count and D-dimer concentration. It is known that infections can cause the activation and acceleration of the coagulation mechanisms in the human body. This leads to benefits because the coagulation system also has an immune function in cases of severe infection. This explains why in COVID-19 patients parameters such as D-dimers are elevated (*Catanzaro Li et al., 2020)* this mechanism could explain normal levels or slightly increased levels even in the presence of peripheral consumption. The D-dimer levels are always increased showing peripheral coagulation activation and fibrinolysis. Both parameters showed a correlation with the severity of the disease and its prognosis, e. g. D-dimers can be increased 25-fold in severe patients hence development of DIC, which on its own has a high mortality rate.

The study also determined the severity of the disease based on time patient stayed at the hospital. The number of days of the patients was an important

indicator since it informs on whether there is an association between the COVID status of a patient and the time they spent at the hospital. The current study showed that a large proportion of the patients (33.33%) spent 4 days or less at the hospital and 31.75% of the patients reported that they were in the hospital for between 5 to nine days. About 19.05% of the patients spent 14 days or more at the hospital while 15.87% were admitted for between 10 and 13 days. This can be explained by the fact that It should be noted that hospital length of stay (LoS) of COVID-19 patients can be influenced by other factors such as disease prognosis, comorbidities, availability, and accessibility to health services, so this disparity between continents can be muddled by various factors such as major comorbidities, different treatment protocols, different care protocols, availability of resources, available beds, and so on.

Furthermore, elderly patients, particularly those 65 and older with comorbidities and infections, have a higher rate of admission to the critical care unit (ICU). The most common comorbidities among COVID-19 patients were hypertension, diabetes, and cardiopathy (*Liu et al., 2020)* and they were hospitalized for a longer period of time. Comorbidity is one of the key causes for the varying lengths of hospitalization in different studies, and the average length of hospital stay is reported to be longer depending on the number of patients studied. Additionally, willingness to pay may influence hospital duration of stay in different countries or continents based on resource availability. Willingness to pay is associated with mortality/morbidity risk reductions by incorporating

several highly relevant aspects during an epidemic, namely, healthcare capacity constraints, dynamic aspects of prevention (i.e., interventions aimed at flattening the epidemic curve), and distributional issues due to high heterogeneity in the underlying risks.

In countries with abundant resources, patients are more eager to pay for hospital treatments, therefore hospital equipment is sufficient to keep patients in the hospital until they are fully recovered, and hospital lengths of stay are indeed longer. While in low-resource countries, in an epidemic situation where the number of patients is increasing, hospitals may be forced to discharge patients earlier than usual due to a lack of equipment such as ventilators, intensive care equipment, and adequate hospital beds, and thus the average hospital length of stay may be reduced. Hence the above dynamics are the most important factors influencing the hospital length of stay of COVID-19 patients in various nations, and they should be considered in the results and interpretations.

5.1.5 Diagnostic performance of targeted parameters in Covid-19 infected patients, in Kakamega, Kenya

There is evidence showing that there are enormous challenges in diagnostic approaches that require rapid and accurate identification of cases of SARS-CoV-2 infections and asymptomatic cases, however, numerous constraints in diagnostics that can rapidly and accurately identify infected persons exist in low resource settings (*Figliozzi et al.,2020*) Approaches that can detect disease progression in order to classify patients for

appropriate care and that can thereby prevent exacerbation of the disease, have been recommended. Diagnostic testing is a major component of outbreak detection and emergency response (*Gillen et al.,* 2020). At the beginning of the coronavirus outbreak globally, there was pressure to expand to an effective response to the testing capacity for the novel corona virus. In some countries there was a need to get clearance before test kits could be shipped in deployed and approved for use (*Kermali et al., 2020).*

In our study as part of the scaling up it was important to use best practices for timely detection of the virus causing the pandemic. The gold standard for SARS-CoV-2 detection is RT-PCR, however, to scale up testing capacity with a good TAT, it was necessary to incorporate use of rapid test kits which could be adopted to increase coverage. All the samples identified as Covid-19 positive by red blood cell counts (RBS) and all those samples identified as positive using Urea test were confirmed using RT-PCR technique (*Ferrari et al.,2020*) (Table 4.10). Only 33.3% of the samples that were purposively identified to be Covid-19 positive using D-dimer were confirmed by RT – PCR while 56.9% of the positive samples according to ESR technique were confirmed to be Covid-19(*Ghahramani et al., 2020*) This showed that the two methods i.e. RBS and Urea were the good performing techniques to use in Covid-19 testing apart from the gold standard method.

5.2 Conclusions

1. Most patients who admitted at Kakamega County referral hospital with asymptomatic COVID 19 were males (185/52.9%) while 165(47.1%) were females.

2. A high prevalence of 84.6% of Covid-19 among patents who attended Kakamega County referral hospital.
3. The Covid-19 main preclinical presentations established were; dry cough (50.29%), pneumonia (25.14%), DIB (21.14%), chest pain/ chest congestion (10.86%).
4. Covid-19 positive patients had lower AST and direct bilirubin levels.
5. A large proportion of the patients (33.33%) spent 4 days or less at the hospital and 31.75% of the patients reported that they were in the hospital for between 5 to nine days.
6. Red blood sugar and Urea were the good performing techniques to use in Covid-19 testing apart from the gold standard method.

5.3 Recommendations

1. Patient characteristics, including, leucocyte count, platelet count, inflammatory markers status, hypokalemia, and blood oxygen saturation in COVID-19 diagnosis should be included.
2. The biochemical parameters needs further evaluation in a larger number of cases and should be used only for risk stratification purposes. Therefore, high-risk patients need strict monitoring round the clock and supportive management timely.
3. To scale up testing capacity with a good TAT, it is necessary to incorporate use of rapid test kits which could be adopted to increase coverage.
4. There is an urgent need for rapid diagnosis of SARS-CoV-2 infected COVID-19 patients even before an immune response can occur and for asymptomatic carriers.

5.3.1 Suggestions for Future Research

1. There is need for careful consideration of the biochemical marker in relation to the other tests when making diagnostic decisions. Biomarkers may help in diagnosis and risk stratification of the disease.
2. Validation studies are necessary to confirm the findings of the studies and gather more robust evidence in different settings, including comparative studies with larger sample sizes and diverse populations.

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APPENDICES

Appendix I: Graduate School approval

KENYATTA UNIVERSITY
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P.O. Box 43844, 00100
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Our Ref: P150/27770/2018

DATE: 31st July, 2023

Director General,
National Commission for Science, Technology
and Innovation
P.O. Box 30623-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MS. LILIAN GATAVI NJUE REG. NO. P150/27770/2018

I write to introduce Ms. Lilian Gatavi Njue who is a Postgraduate Student of this University. She is registered for M.Sc. degree programme in the **Department of Medical Laboratory Science.**

Ms. Njue intends to conduct research for a M.Sc. thesis Proposal entitled, **"Biochemical Markers in Sars-Cov-2 Infected Patients in Kakamega County, Kenya."**

Any assistance given will be highly appreciated.

Yours faithfully,

KENYATTA UNIVERSITY OFFICE OF EXECUTIVE DEAN 31 JUL 2023 GRADUATE SCHOOL P.O. Box 43844-00100 NAIROBI

PROF. ELISHIBA KIMANI
EXECUTIVE DEAN, GRADUATE SCHOOL

RM/ww

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Our Ref: P150/27770/2018

DATE: 31st July, 2023

Director General,
National Commission for Science, Technology
and Innovation
P.O. Box 30623-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MS. LILIAN GATAVI NJUE REG. NO. P150/27770/2018

I write to introduce Ms. Lilian Gatavi Njue who is a Postgraduate Student of this University. She is registered for M.Sc. degree programme in the **Department of Medical Laboratory Science.**

Ms. Njue intends to conduct research for a M.Sc. thesis Proposal entitled, **"Biochemical Markers in Sars-Cov-2 Infected Patients in Kakamega County, Kenya."**

Any assistance given will be highly appreciated.

Yours faithfully,

KENYATTA UNIVERSITY
OFFICE OF EXECUTIVE DEAN
31 JUL 2023
GRADUATE SCHOOL
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PROF. ELISHIBA KIMANI
EXECUTIVE DEAN, GRADUATE SCHOOL

KM/eww

Appendix II: Ethical approval

KENYATTA UNIVERSITY
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Our Ref: **KU/ERC/APPROVAL/VOL.1** Date: 3rd October, 2023

Lilian G.Njue
P.O Box 43844, 00100
Nairobi.

Dear Ms. Njue,

APPLICATION NUMBER: PKU/2789/I1914- BIOCHEMICAL MARKERS IN SARS-COV-2INJECTED PATIENTS IN KAKAMEGA COUNTY, KENYA

This is to inform you that ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** has reviewed and approved your above research proposal. Your application approval number is **PKU/2789/I1914.**The approval period is ***29th/09/2023 to 29th/09/2024.***

This approval is subject to compliance with the following requirements;

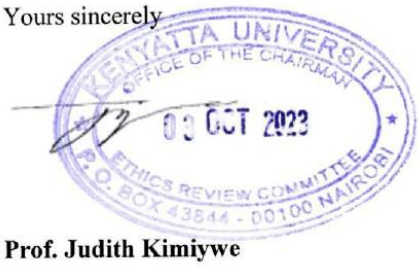
i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE***
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.

vii. Submission of an executive summary report within 90 days upon completion of the study to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE***

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

To serve you better, researchers are kindly requested to access and complete a customer feedback form and sent it back online as you continue with research and upon completion of data collection found on the following website link; ;(https://docs.google.com/forms/d/1ytWefDwvyz5h1oz_VIn0xbxg3uGdlDzMXFWNDsMrRPQ/edit?usp=sharing

Yours sincerely



Prof. Judith Kimiywe

Director: Centre for Research Ethics and Safety

Appendix III: NACOSTI Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: **735056**

Date of Issue: **06/October/2023**

RESEARCH LICENSE

This is to Certify that Ms.. LILIAN GATAVI NJUE of Kenyatta University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kakamega on the topic: Biochemical Markers in Sars-Cov-2 Infected Patients in Kakamega County, Kenya for the period ending : 06/October/2024.

License No: **NACOSTI/P/23/30359**

735056

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Verification QR Code

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See overleaf for conditions