ASSOCIATION OF LIVER BIOCHEMICAL PROFILE AND CLINICAL OUTCOME OF COVID-19 INFECTION IN PATIENTS ADMITTED AT MOUNT KENYA HOSPITAL NYERI, KENYA

BY

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DECLARATION

I declare that the work contained in this thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

I dedicate this research work to my family for their unwavering support throughout the study period, especially my loving husband Joseph M.G. To my dear children for the many play sessions they have understandably forfeited to allow me work on this project, special thanks to them.

ABSTRACT

Coronavirus disease 2019 (covid-19) pandemic is the most recent significant global health crisis after the influenza pandemic of 1918. It has caused consequential global economic decline with loss of over 6 million lives with most of the deaths occurring in the early days of the pandemic when little was known about the virus. The disease is associated with diffuse lung injury causing acute respiratory distress syndrome which is the principal cause of death. Liver injury of variable magnitude has been documented in numerous studies. The aim of this study was to determine the association between liver biochemical profile and clinical outcome of hospitalized covid-19 patients. The specific objectives were; to determine the association between age and clinical outcome of covid-19 infection, to evaluate the association between cholestatic-hepatocellular enzymes and clinical outcome of covid-19 infection, to assess the association between serum albumin levels and clinical outcome of covid-19 infection and to determine the association between serum total bilirubin levels and clinical outcome of covid-19 infection among covid-19 patients admitted at Mount Kenya Hospital, Nyeri, Kenya. This was a retrospective cross-sectional study involving 117 covid-19 patients admitted at Mount Kenya Hospital, Nyeri. A census approach was used. Inclusion criteria was patients with positive covid-19 tested using rqRTPCR with LFTs done on admission. Exclusion criteria was patients with positive covid-19 rqRTPCR whose medical records were incomplete, patients with positive covid-19 tested using rapid antigen test, patients with comorbidities, recent history of alcohol use and pregnant women. Severity of covid-19 was based on at least one of the following: respiratory rate >/ 30 breaths/minute, oxygen saturation < 93%, mechanical ventilation, shock and need for ICU admission. Data on age, liver biochemical parameters and clinical outcome was gathered from medical records. LFT abnormalities were defined as elevation of liver enzymes in reference to Mount Kenya hospital laboratory reference range: ALT (0-42 U/L), AST (0-37 U/L), ALP (40-150 U/L), GGT (8-46U/L), TBIL (0-22 UMOL/L), DBIL (0-6.8 UMOL/L), total protein (66-87 G/L), albumin (37-57 G/L) and graded as mild liver injury (x 1-2 ULN), moderate liver injury (x 3-5 ULN) and severe liver injury (> x 5 ULN). Primary data was entered and cleaned using statistical package for social sciences version 26.0 (SPSS 26.0, 2019). Data was presented as mean ± standard deviation and frequency for normally distributed data and categorical data respectively. Statistical differences for categorical data were compared using chi-square test. Logistic regression analysis was used to assess for association between serum ALP level and clinical outcome (survival or non-survival).Statistical analysis was performed in SPSS (version 26.0, 2019) and $P < 0.05$ was considered statistically significant. The study established a significant association between patient's age, serum ALP and albumin levels and clinical outcome of covid-19.Notably increasing age, high serum ALP levels and hypoalbuminemia were found to associate with clinical outcome. The study concluded that these parameters may be used for risk stratification and prognostication with prompt scaling up of clinical interventions for better health outcomes.

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LIST OF ACRONYMS AND ABBREVIATIONS

ARDS	:	Acute Respiratory Distress Syndrome
ACE-2	:	Angiotensin Converting Enzyme 2
ALP	:	Alkaline Phosphatase
ALT	:	Alanine Transaminase
AST	:	Aspartate Transaminase
BP	:	Blood Pressure
COVID-19	:	Coronavirus Disease-2019
COV-SARS 2	:	Coronavirus Severe Acute Respiratory Syndrome 2
DBIL	:	Direct Bilirubin
GGT	:	Gamma Glutamyl Transferase
LFTs	:	Liver Function Tests
Mrna	:	Messenger Ribonucleic Acid
PR	:	Pulse Rate
RNA	:	Ribonucleic Acid
RTC	:	Replicase Transcriptase Complex
SIRS	:	Systemic Inflammatory Response Syndrome
TMPRSS2	:	Transmembrane Serine Protease 2
TBIL	:	Total Bilirubin
ULN	:	Upper Unit of Normal
WHO	:	World Health Organization

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CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

Coronavirus disease (covid-19) is an ever-changing ‘’Mosaic Disease’’ which affects numerous and a large spectrum of biochemical elements of several organ systems (Waleed et al., 2020). It is caused by the Severe Acute Respiratory Syndrome Coronavirus- 2 (SARS-COV 2). Initially the disease was largely recognized as an exclusive respiratory disorder. However, with time evolving reports have maintained that covid -19 is a multisystem infirmity. Characterized by remarkable extra pulmonary in addition to pulmonary pathology with the involvement of the liver and biliary tract. Angiotensin converting-enzyme 2 (ACE2) receptor, is the main viral entryway and its wide-ranging distribution may delineate why SARS-COV 2 has the capacity to produce an extensive infirmity marked by numerous organs implication inclusive of the heart, kidneys, pancreas, intestine, nervous system, muscular, biliary and liver (Nardo et al., 2021). Patients harboring the SARS-COV -2 will not entirely manifest with regular respiratory symptoms of the infection at the time of hospitalization (AlSamman et al., 2020).

Liver dysfunction has been described as a frequent presentation, although the clinical utility and the effect of this pathology on clinical outcome is uncertain. Abnormal levels of liver parameters are seen more frequently in severe covid-19 infection and are related to poor outcome.Covid-19 is commonly linked to varying levels of deranged liver function tests especially transaminases, which are generally temperate and transient (Garrido & Liberal, 2020).The degree of severity of covid-19 is related to liver damage and can be a predisposing factor to abnormal liver function tests(LFTS). Liver enzyme abnormalities have been inconsistently reported in covid-19 patients (Moon & Barritt, 2021).Few studies have been done in Kenyan counties on covid-19 and the liver, among them is a study done in Nairobi and coastal regions of Kenya that associated high

aspartate transaminase levels with increased risk of death (Ombajo et al., 2022).Despite derangement in liver biochemical parameters being a common phenomenon in covid-19, evidence to support their clinical utility is inconsistent with paucity of information in Nyeri county, Kenya.

Elevation in alanine transaminase (ALT) levels is one of the commonly observed laboratory result in covid-19 infection. Some studies have associated it with severe covid-19 disease and death (Christensen et al., 2020). Similarly, a high level of ALT was associated with 2 times increase in chances of poor covid-19 disease outcome (Sharma et al., 2021, Wu et al., 2020). However, some studies do not show this association. Equally elevation in aspartate transaminase (AST) levels has been observed in severe covid-19 disease. In a study done by Amin (2021), raised AST levels were noted in 18% of patients with non-severe covid-19 and in 56% of those with severe covid-19 disease. High AST levels offer valuable prognostic guidance on covid-19 mortality. In a study done by Sharma et al., (2021) elevated levels of AST in blood were associated with poor covid-19 disease outcomes. There was a 3 time higher risk of poor disease outcome compared to patients with normal AST levels. Similar findings have been noted whereby elevated levels of ALT and AST have been associated with a poor covid -19 disease outcome such as increased mortality (Wu et al., 2020). It is important to note that in severe covid-19 disease high AST levels are common compared to ALT elevation (Kumar-M et al., 2020).

Angiotensin converting-enzyme 2 (ACE2) receptors expression is high in cholangiocytes forming the hypothesis biliary pattern of deranged enzymes is as a result of direct effect of SARS-CoV-2 binding on these receptors. Nevertheless ALP levels have not been shown to be

constantly high in patients with covid-19, which is out of support of this hypothesis. High levels of ALP have been linked to severe covid 19 disease (Kumar et al., 2020). According to Radivojevic et al., (2022) elevated levels of ALP were associated with increased risk of ICU admission and mortality. GGT is a more specific marker for biliary disease compared to ALP (Lala et al., 2021).Some studies have shown rise of GGT levels in a similar frequency to those of aminotransferases however, its prognostic utility in covid-19 is not clear.

Albumin plasma protein accounts for 80% of intravascular osmotic pressure. It plays critical homeostatic role in patients with severe illness through its thiol antioxidant effect and enhances vasomotor tone by counteracting nitric oxide vasodilation action. Albumin also offers buffer effect during metabolic acidosis (Caironi & Gattinoni 2009). Cytokine storm is attributed to cause hypoalbuminemia in covid-19 infection. Xu et al., (2022) reported 82% mortality of covid-19 disease patients with hypoalbuminemia at admission. Hypoalbuminemia is also associated with prolonged hospitalization and it is an early predictor of death independent of other factors such as age, inflammatory markers and comorbidity (Viana Llamas et al., 2021).

Other functions of the liver is to metabolize bilirubin which is an endogenous antioxidant with a bulk of antioxidant effects. In studies done in China, most of covid-19 patients had high levels of bilirubin. It is associated with severe covid-19 and increased chances of ICU admission. (Papadopoulos et al., 2020). In another study on 1,788 covid 19 patients, elevated total bilirubin level was associated with high deaths as out of the group with elevated serum total bilirubin 5.8% of them died compared to 0.6% in the group with normal total bilirubin levels (Liu et al., 2020).From the literature reviewed there exist inconsistencies in liver function parameters associated with poor clinical outcome of covid-19 infection.

1.2 Statement of the Problem

From the literature reviewed, it has been noted that while liver dysfunction has been acknowledged as a frequent phenomenon in covid-19 patients, studies on covid -19 and liver involvement are scarce among the African population and Kenya in particular. The actual spectrum of liver injury in covid-19 is not completely understood. The nature and the characteristics of liver biochemical derangement in covid-19 is still unclear. Serum liver enzymes, bilirubin and albumin levels can be used as prognostic tools for covid-19 infection. However, there are no such studies in Nyeri county, Kenya. The liver is the principal organ of the human body responsible for metabolic and detoxification functions. It synthesizes albumin, alanine transaminase (ALT), aspartate transaminase (AST), bilirubin and other acute phase reactants like C-reactive protein (CRP),transferrin and hepcidin (Gulhar et al., 2022). The role of acute phase reactants is to modulate inflammation. Therefore, injury to the liver may have an effect on the overall presentation and outcome of covid-19 infection. In this light, the pattern and nature of liver involvement in covid-19 needs to be evaluated to highlight its effects and association to the clinical outcome of the disease. Availability of valid and reliable prognostic markers/tests for SARS-CoV-2 is limited. This largely limits accurate and prompt clinical decision-making and thereby delaying efficient and effective patient –centered care which may be associated with poor clinical outcomes. Avid determination of the prognostic value of liver involvement in covid-19 disease is thus imperative.

1.3 Study Justification

The Center for disease Control and Prevention (CDC)documented the earliest case of severe acute respiratory syndrome secondary to coronavirus (SARS-COV) in November 2002 and in 2012 the American Select Agent Registry Program declared SARS-Coronavirus a select agent

(CDC,2013). Covid -19 is a highly infectious disease and has the potential to re-emerge. The disease has caused great suffering to humans across the globe. It has been shown to cause derangement in the functions of the liver. The present study proposed to evaluate the association between liver biochemical profile and outcome of covid -19 infection with the aim to establish laboratory parameters that may be used to evaluate the severity of covid -19 and forecast the likelihood of advancing towards life threatening states namely; Acute Respiratory Distress Syndrome (ARDs), Disseminated Intravascular Coagulation (DIC), Multiple Organ dysfunction (MOD) and death. Also to expand the pool of knowledge that already exists on SARS-COV 2 infection, its manifestation and prognostic indicators.

1.4 Significance of the Study

This study will inform the scientific community on the evidence of clinical utility of liver chemistry parameters in prioritizing response and assigning risk status in SARS-COV 2 infections. It will also provide evidence-based clinical intervention to the patient facilitating efficient, effective and prompt decision making possibly with improved clinical outcomes. It is important to note that pandemics have history of recurrences and as such new knowledge derived from this study may be applied in future by medical practitioners and policymakers. The study will also increase the pool of knowledge to be used in future pandemics as well as form a foundation for further research.

1.5 Objectives of the Study

To determine the association between liver biochemical profile and clinical outcome of covid-19 infection in patients admitted at Mount Kenya hospital Nyeri, Kenya.

1.5.1 Specific Objectives

i. To determine the association between age and clinical outcome of covid-19 infection among covid -19 patients admitted at Mount Kenya hospital Nyeri, Kenya.

ii. To evaluate the association between cholestatic-hepatocellular enzymes and clinical outcome of covid-19 infection in covid-19 patients admitted at Mount Kenya hospital Nyeri, Kenya.

iii. To assess the association between serum albumin levels and clinical outcome of covid-19 infection in covid-19 patients admitted at Mount Kenya hospital Nyeri, Kenya.

iv. To determine the association between serum total bilirubin levels and clinical outcome of covid-19 infection in covid-19 patients admitted at Mount Kenya hospital Nyeri, Kenya.

1.6 Hypothesis

Null hypothesis: There is no association between liver biochemical profile and the clinical outcome of covid -19 infection, in patients admitted at Mount Kenya hospital Nyeri, Kenya.

Alternative hypothesis: There is association between liver biochemical profile and the clinical outcome of covid -19 infection, in patients admitted at Mount Kenya hospital Nyeri, Kenya.

CHAPTER TWO
LITERATURE REVIEW

2.1 SARS-COV-2 and Pathophysiology of COVID-19

In December 2019, in the capital of Wuhan, Hubei province of China, cases of pneumonia of unidentified etiology emerged. Genetic mapping showed that it was a new corona virus, the seventh one of the *Coronaviridae* recognized to infect humans, and was named 2019-novel coronavirus(nCoV).World health organization (WHO) would later rename it covid-19.International committee on taxonomy of viruses (ICTV) renamed it SARS-COV-2 (Sun et al., 2020).

SARS-CoV-2 is similar to Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV), belongs to the B lineage of beta-CoVs (Li *et al*., 2020). During viral replication mutations are generated due to low proofreading ability of their viral ribonucleic acid(RNA)-dependent RNA polymerase (viral-RdRP).Mutations in SARS-COV-2 have led to its continuous evolvement to different groups (Yi et al., 2020). Genetic sequence of the covid-19 revealed genetic identity of >80% to SARS-CoV and 50% to MERS-CoV of which both have their origin in bats and can infect animals and humans (Rothan & Byrareddy, 2020).According to Aleem et al., (2021), since the first detection of the covid -19 virus and its declaration as a global pandemic by WHO in March 2020,there has been several emerging variants of SARS-CoV-2 with WHO distinguishing them into either variants of concern (VOCs) or variants of interest (VOIs) as follows:

Table 2.1: Variants of SARS COV-2 virus

	Strain & lineage	Country first detected	Date first detected	VOCs or VOIs
1	Zeta (P.2)	Brazil	April, 2020	VOI
2	Epsilon(B.1.427; B.1.429)	US	June, 2020	VOI
3	Beta (B.1.351)	South Africa	Oct, 2020	VOC
4	Eta (B.1.525)	New York	Nov, 2020	VOI
5	Iota (B.1.526)	New York	Nov 2020	VOI
6	Alpha B.1.1.7	UK	Dec 2020	VOC
7	Gamma (P.1)	Brazil	Dec 2020	VOC
8	Delta (B.1.617.2)	India	Dec 2020	VOC
9	Kappa (B.1.617.1)	India	Dec 2020	VOI
10	Theta (P.3)	Philippine& Japan	Feb 2021	VOI
11	Lambda (C.37)	Peru	June 2021	VOI

Key: *UK-united kingdom; US-united states of America; VOCs-variants of concern; VOIs-variants of interest* (Aleem et al., 2021).

SARS-COV-2 adheres on a membrane carboxypeptidase known as angiotensin II converting enzyme (ACE-2), which acts as a receptor for particular proteins expressed by this virus, to get into the cells (Beyerstedt et al., 2021). The ubiquitous occurrence of ACE-2, which is the principal viral entry-way receptor, elucidates SARS- COV- 2 capacity to generate a widespread pathology manifested by multiple organ inclusion affecting lungs, liver, bile ducts, intestines, heart, kidney, pancreas, muscular and nervous system. This may explain the occurrence of liver dysfunction in covid events. On that account covid-19 may be evaluated as a widespread infectious and inflammatory condition (Waleed et al., 2020).

Spike (S) protein priming by the transmembrane serine protease-2 (TMPRSS2) of the host occurs the cleavage allows for fusion. SARS-COV-2 internalization occurs via endocytosis

causing the release of viral genetic material by the endosome. The viral RNA produces two different polyproteins namely PP1a and PP1ab.Processing of these proteins provide the elementary units of the viral replicase-transcriptase complex (RTC) (Krichel *et al*., 2020).The complete viral genome replicates in RTC-containing vesicles. At the same time a collection of particular sub-genomic mRNA (Messenger – Ribonucleic Acid) is generated forth to manufacture SARS-COV-2 structural and accessory proteins (Liu et al., 2014).

These proteins convene at the endoplasmic reticulum (ER) –Golgi intermediate compartment for configuration of nucleocaspid and viral envelope, consequently resulting in the freeing of mature virions (Nardo et al., 2021).The viral-binding domain on attaching to the target cell produces significantly higher amounts of angiotensin II and can bring about localized vasoconstriction (song et al., 2020).Clinical presentation of covid-19 is heterogeneous from asymptomatic to severe lung infection. The tissue locale of ACE-2 is associated with how the affliction impacts various tissues and organ systems (Li et al., 2020).

Once the SARS-COV-2 virus enters into the host cell various inflammatory cytokines are released by the host cell. These include interleukins (1, 6, 8, and 12), tumor necrosis factor – alpha, interferons (gamma and beta), chemokine (C-X-C motif) ligand 1(CXCL-10) as well as monocyte chemo attractant protein (MCP-1) and macrophage inflammatory protein -1 alpha (Parasher &Anant., 2020). This high and uncontrolled production of proinflammatory mediators is referred to as cytokine storm and it happens systemically making covid-19 a multisystem disease (Montazersaheb et al., 2022). In the lungs, the cytokine storm attracts other inflammatory cells such as neutrophils, CD8 cytotoxic T cells and CD4 helper T cells, increasing

inflammatory injury to the lungs with subsequent exudation in the alveolar and impaired gaseous exchange capacity (Parasher, 2021)

SARS-COV-2 is associated with a decrease in lymphocytes cell count. This is occasioned by the virus attaching to the ACE-2 receptors on the lymphocytes and subsequent entry into the cell stimulating an inflammatory process with resultant death of the lymphocytes. It has been established that the lymphocytes in the oral mucosa, digestive system and lungs express ACE-2 receptors. Thrombocytopenia may be secondary to destruction of hematopoietic progenitor cells due to cytokine storm (Violetis et al., 2020)

SARS-COV-2 is as well associated with thrombosis. The virus causes vascular endothelial dysfunction dysregulating the innate antithrombotic mechanisms which comprises of molecules such as tissue factor pathway inhibitor (TFPI), heparin sulfate and endothelial cell protein C receptor (EPCR). These molecules work to ward off activation of platelets which would eventually lead to coagulation (Conway et al., 2022). Thus the disease is associated with thrombosis in the deep venous system, thrombosis and embolism in the pulmonary and systemic arteries, venous thromboembolism, cerebral vascular accidents (ischemic stroke) and coronary artery disease leading to myocardial ischemia and infarction (Jain., 2020).

The extra pulmonary manifestations of covid-19 is as a result of direct viral toxicity, impairment of vascular endothelial cells, viral induced disequilibrium of the immune system and response, as well as imbalance of the renin-angiotensin-aldosterone system (RAAS) (Gupta et al., 2020). In the heart the virus causes thrombosis in the coronary arteries eventually leading to ischemia and infarction. Equally, myocarditis may arise as a result of direct viral invasion leading to arrhythmias as well as heart failure. In the kidneys, the disease is associated with acute kidney

failure. This is because of the viral entry into the organ through ACE-2 receptors leading to direct viral toxicity. Cytokine storm as well as hypoxemia also contribute to the virus-associated kidney damage (Jain., 2020).

Pancreatic involvement in covid-19 disease is due to direct viral entry through the ACE2 receptors that are significantly expressed in islet cells of the pancreas, leading to cytopathic injury. Through this, the islets cells destruction eventually lead to acute diabetes mellitus. Expressed ACE-2 receptors on the gastric mucosa lymphocytes form a direct entry point for the virus to the gastrointestinal (GI) system. However, gastrointestinal epithelium may as well be damaged by the inflammatory response. These leads to dysregulated intestinal secretion, stimulation of the GI enteric nervous system and onset of symptoms such as diarrhea (Patel et al., 2020)

Diagnosis of covid-19 is based on epidemiological history, clinical manifestations and radiological tests. Laboratory parameters coupled with demographic data of patients could permit categorization in the early stages of the disease, to help identify critically-ill individuals thus enhancing their clinical care by scaling their therapeutic approaches (Wu et al., 2020).

2.2 Effects of covid-19 on the liver

Hepatic injury in covid-19 in contrast with pulmonary and myocardial damage has been controversially deliberated. Effect of covid-19 on the liver may be congruous with general disease severity thus may aid as a prognostic marker of Acute Respiratory Distress Syndrome (ARDS) (Velavan & Meyer, 2020).According to Fan et al., (2020) the climax of liver enzyme rise occurs on the 7^{th} -12^{th} day post infection, on average at day 10.Additionally, studies have shown that liver dysfunction is frequent in SARS-COV-2 patients with non-severe respiratory

failure (Afra et al., 2020)**.** The amplitude of hepatic damage in SARS-CoV-2 infection may scale from direct infection, indirect implication by systemic inflammatory processes, hypoxic states, clinical-intervention related factors for example medications, ventilation and amplification of underlying liver pathology (Nardo et al., 2021).

The possible pathomechanisms involved in liver injury in covid-19 is wide-ranging including direct cytotoxicity by active replication of the virus in hepatocytes, immune-associated liver injury resulting from massive systemic inflammatory response syndrome (SIRS), changes caused by inadequate oxygen supply to tissues due to respiratory dysfunction, vascular system changes occasioned by coagulation dysfunction, endothelial inflammation, cardiac congestion due to right-sided heart failure, medication-induced liver injury and worsening of pre-existing liver diseases (Moreira et al., 2021). Interplay between ACE-2 receptors and SARS COV-2 result in cytotoxic effects that result in hepatocyte damage which involves cell membrane impairment, mitochondrial swelling and dilatation of the reticulum. The ability of the virus to proliferate in the hepatocytes fuels the cellular dysfunction (Metawea et al., 2021).Direct infection of the liver by SARS-COV-2 or medication-induced injury is demonstrated in liver biopsies showing moderate microvascular steatosis, mild lobular and portal activity (Parohan et al., 2020). Massive systemic immune responses (SIRS) associated with raised levels of plasma cytokines including IL1, IL6, INF, and TNF may contribute to liver injury (Metawea et al., 2021). These cytokines enhance recruitment of more inflammatory cells such as neutrophils and CD4 T- cells, as well as the complement system that lead to liver injury (Cai et al., 2021). Release of viral-induced cytotoxic T-cells occasions hepatocellular-immune mediated damage. Alterations in the gut vascular barrier and gut micro biota has been ascribed to hepatic destruction in covid-19 (Dhar & Mohanty, 2020).

Hypoxia and cardiac failure is related to acute hepatitis in patients with critical disease, with the utilization of extreme levels of positive end expiratory pressure (PEEP) causing increased right atrial pressure leading to decreased venous return causing liver congestion (Metawea et al., 2021). The liver is the major metabolic and purifying organ and antiviral drugs safety profile and therapeutic potency can be disrupted by deranged hepatic function even of moderate degree (Nardo et al., 2021).

2.3 Association between age and clinical outcome of covid-19

Occurrence of covid-19 in various populations and heterogeneity in susceptibility may be attributed to non-identical molecular expression of ACE-2, restricting the ingress of SARS-CoV-2 into the cells (Yi et al., 2020). According to Wu et al., (2020) lower expression of ACE-2 in African-descent populations has been reported with attributes to higher susceptibility to systemic hypertension and premature end organ damage. The principal factors reported to greatly impact the probability of dying from covid-19 is male, age, presence of comorbidities such as hypertension, diabetes, chronic respiratory diseases, cancer and cardiovascular disorders. A number of studies have documented higher fatalities among male, probably due to increased prevalence of the disease among the male sex as opposed to correlation with the male sex (Gagliardi et al., 2020) . Among various patient characteristics linked to poor prognosis in covid-19 age is the principal one. It is not related to the presence of pre-existing conditions (H M Henkens et al., 2021).A multicenter study that included hospitals in Europe, South America, North America, Asia and South Africa, found increasing age to be a principal risk factor of poor outcomes in covid-19 (Spearman et al., 2021).Another multicenter study done in Kenya (Nairobi and coastal regions) highlighted older age, comorbidities and high levels of serum aspartate

transaminases as crucial elements in influencing risk of poor outcomes in covid-19 (Ombajo et al., 2022).

2.4 Association of cholestatic-hepatocellular liver enzymes and clinical outcome of covid-19

Derangement of liver biochemical enzymes ALT,AST,ALP and GGT levels has been reported to characterize liver dysfunction in covid-19.ALT and AST levels are raised mainly in hepatocyte damage or injury thus they are referred to as hepatocellular enzymes. ALP and GGT are elevated in bile ducts involvement commonly resulting in cholestasis thus is referred to as cholestatic enzymes.

2.4.1 Alanine Aminotransferase in covid-19

Alanine aminotransferase (ALT) was formerly known as serum glutamate-pyruvate transaminase (SGPT). It was for the first time described in 1950's by Karmen *et al.* Serum ALT and aspartate aminotransferase (AST) and their ratio (AST/ALT)are measured in blood as biomarkers for liver health(Metón et al., 2015) . Unlike AST , ALT is predominantly formed and stored in the cytosol of the hepatocytes therefore making it more specific indicator of liver inflammation (Agbafor et al., 2017).Although it is also present in clinically negligible amounts in red blood cells and other organs such as the pancreas, kidneys ,heart and muscle cells with the normal blood levels ranging from 5 to 35 IU/L.ALT amount in blood is commensurable to the magnitude of liver damage. ALT levels can rise up to 50x the normal following severe damage of tissue especially of the liver or heart (Huang et al., 2006,Wang et al., 2016)**.**ALT is a 496 amino acids protein, which is coded in chromosome 8 long arm and has a half-life of 47 ± 10 hours (Liu et al., 2014). It is homodimeric in structure composed of two 50 kilo Daltons subunits and is involved in metabolism of amino acids and gluconeogenesis. It catalyzes amino group transfer from alanine to alpha-ketoglutarate in the alanine cycle, forming pyruvate and L-glutamate (Metón et al.,

2015).Its laboratory measurement is readily available and is a relatively inexpensive test used to check for liver disease and general health of the body. Several factors affect ALT activity and plasma levels such as viral hepatitis, alcohol use and various medications (Liu et al., 2014).Other factors that affect ALT include muscular diseases, metabolic syndrome that is associated with mild to moderate ALT elevation, demographic factors such as ethnicity, age and gender, laboratory method of analysis as well as diurnal variations (Liu et al., 2014).

Elevated level of alanine transaminase (ALT) is one of the most commonly identified laboratory result seen in patients suffering from covid -19 infection. It is correlated with severe covid -19 disease and mortality (Christensen et al., 2020). In covid-19, ALT and AST levels usually rise one to two times the upper normal limit which is incongruous with the postulated causes such as covid -19 direct viral hepatotoxicity, hypoxia and ischemia. This is inconsistent with hypoxic or ischemic liver injury (Sharma et al., 2021) which is associated with massive rise of ALT and AST up to 20-fold upper limit normal (ULN).There could be other unknown mechanisms involved in liver injury in covid-19

2.4.2 Aspartate Aminotransferase in covid-19

The enzyme was formerly known as serum glutamic oxaloacetic transaminase (SGOT).It is present mainly in the liver but also in other organs just like ALT (Huang *et al*., 2006).It has a half-life of 17 hours with less specificity for liver inflammation compared to ALT (Metón *et al*., 2015).Its gene is located in chromosome 16 and it maintains nicotinamide adenine dinucleotide /reduce nicotinamide adenide dinucleotide ratio in cells by involving in malate aspartate shuttle (Ndrepepa, 2021). Chen *et al.,* in a cohort of 799 patients observed liver function as a critical predictor for covid-19 patient mortality with non-survivors having much higher levels of AST, ALT, lactate dehydrogenase, creatinine ,creatinine kinase ,cardiac troponin I,N-terminal pro-b

natriuretic peptide and D-dimer (Ponti *et al.*, 2020) . Literature review has showed that 46% of covid -19 admitted patients had high serum levels of AST while 35% had elevated serum levels of ALT on admission and had a more severe disease. Elevation of AST and ALT was more common in American patients compared to Chinese patients. A study done in Kenya found a rise in ALT and AST levels in 48% and 51% of patients respectively. The presence of preexisting liver dysfunction in covid patients with high levels of LFTs on admission has not been fully evaluated ,it is unlikely to be responsible for all LFTs abnormalities noted in covid -19 patients on admission (Bertolini *et al.*, 2020). Most of the studies done on covid -19 and liver disease had only about 3-8% of patients, elevated AST levels were noted in 18% of patients with non-severe covid -19 disease compared to 56% of patients with severe covid -19 disease. While elevated ALT was observed in 20% of non-severe covid -19 patients in contrast to 28% of those with severe covid-19 disease (Amin, 2021). According to (Izcovich *et al.*, 2020),elevated blood AST levels provide invaluable prognostic information on mortality.

2.4.3 Alkaline phosphatase in covid-19

This enzyme was first discovered in 1923 by Dr. Robert Robinson (Brichacek & Brown, 2019).It is found on the outer surface of the plasma membrane aiding in catalyzing hydrolysis of phosphate groups from various substrates in an alkaline environment (Rader, 2017).This phosphate is used for bone and dental mineralization (Liedtke et al., 2020).In the liver elevated levels of ALP signifies cholestasis Brichacek & Brown (2019) as would happen with inflammation secondary to viral binding of SARS COV-2 on the ACE-2 receptors in the hepatic and biliary cells(Fierro, 2020). ALP (30-120 IU/L) belongs to a family of zinc metalloenzymes and has high concentration levels in the bile cannaliculi microvilli. Elevation of ALP and bilirubin disproportionately to ALT and AST points to a cholestatic pattern of liver injury while

the opposite denotes a hepatocellular disease (Lala et al., 2021). AST and ALT levels evaluate hepatocyte injury while ALP and GGT levels are markers of bile duct injury or cholestasis. Bilirubin levels indicate hepatic clearance ability. Cholestasis secondary to covid-19 (ALP ≥3x ULN) affects 1% of covid-19 patients with severe illness at admission and is linked to a poor prognosis(Kulkarni et al., 2020)

Serum elevations of ALP were found not to be significantly higher in the severe group of covid -19 patients however AST elevations was found to be more than ALT in the group with severe covid -19 disease (Kumar-M *et al*., 2020).There are high levels of ACE-2 receptors expression in cholangiocytes promoting hypothesis that biliary involvement is secondary to direct effect of the SARS-COV-2 binding on cholangiocytes ACE-2 receptors. However, elevation of ALP has not been shown to be consistently high in covid -19 patients ,out of support of this hypothesis (Cha *et al.,* 2020).

2.4.4 Gamma-Glutamyl Transferase in covid-19

Gamma-Glutamyl Transferase (GGT) is an enzyme found in gallbladder, liver, spleen, pancreas and kidneys and weighs 68,000 Dalton. It is essential in metabolism of the antioxidant glutathione, and also helps in drug metabolism in the liver. It also has potential to exert a pro-oxidant role (Ali *et al*., 2017). It is not present in bone thus it is more specific for biliary disease compared to ALP (Lala *et al*., 2021).Elevation of GGT was found to be higher than that of ALP, but similar in frequency to elevations in aminotransferases. It is not clear whether these high GGT levels are secondary to oxidative stress or chronic inflammation since it is a surrogate marker for the same, or whether its secondary to biliary injury by the SARS-COV-2 virus (Kumar-M *et al*., 2020**)**.Studies done in Wuhan China showed initial elevations of AST/ALT that was later followed by elevation of GGT,ALP and bilirubin levels in coronavirus disease (Zhao

et al., 2021).This suggests that inflammatory processes within the liver may be the primary cause of AST elevation later on, followed by cholestasis with impaired bilirubin clearance.

2.5 Association of serum albumin and clinical outcome of covid-19

The cause of hypoalbuminemia in SARS-CoV-2 infection has been attributed to cytokine storm (Xu *et al*., 2021) .Albumin has been generally documented to be low in covid-19 patients .Some studies have indicated that 77% of patients with severe covid-19 disease had hypoalbuminemia and 82% of those that had succumbed to the disease had hypoalbuminaemia at admission (Xu et al., 2021)

Length of hospitalization was indicated in some studies to be prolonged in hypoalbuminemic covid-19 patients. According to Viana-Llamas *et al.*, (2021) hypoalbuminemia was an early forecaster of inpatient death in covid-19 infection independent of age, comorbidity and markers of inflammation. In their cohort study involving 609 patients, hypoalbuminemia on admission was rampant in covid-19 non-survivors (65.6%) versus covid-19 survivors (38%).According to Hariyanto *et al*., (2021),fifteen studies(n=4744) showed that patients with hypoalbuminemia had severe disease demonstrating that levels of serum albumin provided reliable distinction between severe and non-severe covid-19 infection.

2.6 Serum total bilirubin and clinical outcome of covid -19

Bilirubin is the end product of the heme degradation process in mammals. Bilirubin that is conjugated with glucoronic acid in the liver is known as conjugated or direct bilirubin while the unconjugated or indirect bilirubin is the one not conjugated with glucoronic acid (Valášková & Muchová, 2016).It is the most potent endogenous antioxidant (Ali *et al*., 2017).At physiological levels ,bilirubin exerts antioxidant effects such as scavenging reactive oxygen species (ROS) and

repressing the action of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. However bilirubin at elevated levels is cytotoxic (Maruhashi *et al*., 2019).Some studies link high serum bilirubin levels with development of ischemic strokes with poor functional outcome (Song et al., 2022).The normal ranges for total bilirubin is 0.3-1.0 mg/dl (5.1-17 μmol/L),indirect bilirubin 0.2-0.8 mg/dl (3.4-12.0 μmol/L) and direct bilirubin 0.1-0.3 mg/dl(1.7-5.1 μmol/L) (Medical Encyclopedia: Bilirubin, 2005).According to several clinical studies, the most frequent extra pulmonary manifestations of covid -19 is liver injury. Elevated bilirubin is defined as bilirubin above 1mg/dl which was found to be elevated in a significant proportion of the Chinese patients (Papadopoulos *et al*., 2020).Elevated total bilirubin, as well as ALT and AST was found among the main liver outcomes in severe covid -19 disease patients, in contrast to non-severe patients , in China and Singapore (Ghahramani *et al*., 2020). A study by Ding *et al* 2021 showed admission bilirubin levels to forecast in-patient mortality in covid-19.

CHAPTER THREE
METHODOLOGY

3.1 Study Area

This study was conducted at Mount Kenya Hospital, an annex of Nyeri county referral hospital. The county has a total population of 759,164(Kenya population census, 2019) on an area of 2361km^2.The hospital has a bed-capacity of 40 and was the main covid-19 disease isolation and treatment center in the county. According to the county department of health, covid-19 positivity rate was 25% in March 2021 which was higher than the national rate of 17%. All confirmed covid-19 patients in the county who required admission were referred to this facility, during which only covid-19 patients were admitted; therefore, the hospital exclusively admitted only confirmed covid-19 patients in the year 2021 thus making it a good choice for the current study.

3.2 Study Design

This was a retrospective cross-sectional study where medical records including laboratory results of patients admitted with positive covid -19 test for the months of April-September 2021 were reviewed.

3.3 Study Population

Nyeri county has a total population of 759,164 on an area of 2361km^2.The positivity rate of covid-19 in the county in the year 2021 was 25%. The study population was confirmed covid-19 patients admitted at Mount Kenya Hospital Nyeri, between April and September of 2021. A sum aggregate of 460 confirmed covid-19 patients were admitted at Mount Kenya Hospital Nyeri, in the year 2021. Highest number of covid-19 admissions were in the months of April – September 2021.The sum number of patients admitted with confirmed covid -19 infection in the period 1st April – 30th September 2021 was 306 patients, this was taken as the study population. Diagnosis of covid-19 was founded on the Ministry of Health (Kenya) guidelines, 2021.

3.4 Sample Size Calculation and Sampling Technique

Due to the inconsistency in manual record keeping, confounding variables and underlying bias that may have led to significant exclusion of the selected study population, a simple census technique was applied where all the 306 covid-19 patient files were used as the target population. For example, only patients without comorbidities and with positive polymerase chain reaction test were included in this study to reduce bias and ensure consistency in the current study findings. This therefore significantly reduced the final sample size for the research.

3.5 Selection criteria

3.5.1 Inclusion criteria

1. Patients with positive covid -19 tested with real-time quantitative reverse transcription polymerase chain reaction test (rqRTPCR).
2. Patients with LFTs done at admission.

3.5.2 Exclusion criteria

1. Patients with positive covid-19 rqRTPCR whose medical records were incomplete
2. Patients with positive covid-19 tested using antigen rapid test
3. Patients with comorbidities
4. Recent history of alcohol use (last 4 weeks)
5. Pregnant women

The clinical outcome of interest was survival or non-survival (death) of the patient.

After subjecting the 306 medical records through the selection criteria, only 117 files met the focused inclusion criteria and thus were used as the study sample for the current research.

3.6 Data Collection Procedure

Data was collected from patient files. Manual retrieval from the Mount Kenya Hospital inpatient registry of medical files of patients admitted in the month of April-September of 2021 was done. The medical records were evaluated and those meeting the inclusion criteria were picked. The data from each patient file was entered and saved into the researcher's data collection form,

electronic excel sheet. The data excel sheet was secured using a password. Data entered in the excel sheet included:

Table 3.1: Data captured on electronic excel sheet

Independent variables	Dependent/outcome variable
Age Sex Blood pressure(Bp) Pulse rate (PR) Temperature Oxygen saturation (Spo2) Respiratory rate(RR) LFTs -Serum aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma glutamyl transferase, total bilirubin, direct bilirubin, total protein and albumin levels Duration of symptoms Hospitalization stay	Clinical outcome (survival or non-survival)

Other data gathered from the participants' medical records was the patient's level of consciousness, features of shock and need for intensive care unit (ICU) admission. Patient's name and other identifiers were kept anonymous to ensure confidentiality. The medical records were arranged back into their respective shelves.

3.7 Classification of Liver Function Tests Abnormalities

Liver function test abnormalities was defined as the elevation of the following parameters in serum referring to Mount Kenya Hospital Nyeri laboratory reference range standards: ALT (0-42 U/L), AST (0-37 U/L), ALP (40-150 U/L), GGT (8-46U/L),TBIL (0-22 UMOL/L), DBIL (0-6.8 UMOL/L), total protein(66-87 G/L) and albumin (37-57 G/L). There is still no consensus on classification of liver injury in covid-19 patients. However, in the context of the current study classification of liver injury was as follows: patients with raised liver enzymes >5× the upper limit normal (ULN) were categorized as severe liver injury; patients with raised liver enzymes 3–

5x ULN were categorized as moderate liver injury while those with raised liver enzymes 1–2 ULN were categorized as mild liver injury.

3.8 Data Analysis

Following data collection, the data was checked for completeness and uniformity. Missing entries were identified and rectified as much as possible. Data was entered into statistical package for social sciences software, SPSS (version 26.0). Descriptive analysis for continuous variables such as age of the patient in years were done using means ± standard deviation (SD) while categorical data such as classification of liver injury using serum levels of ALT, AST, ALP, GGT, albumin and total bilirubin were presented as frequency and the chi-square test was employed to test for associations. Logistic regression analysis was used to assess for association between age, liver function parameters and clinical outcome (survival or non-survival). All statistical analyses were conducted in SPSS (version 26.0) and $P< 0.05$ was considered statistically significant.

3.9 Ethical Approval

The study was submitted to school of graduate studies for academic approval. Ethical approval was sought and granted by university of eastern Africa, Baraton (UEAB/ISERC/20/04/2023) and National Commission for science, technology and innovation (NACOsti) (Ref. no NACOSTI/P/23/25707). Request for waiver of informed consent was made to the Maseno university Ethical review board. As the study is of retrospective nature and the data analyzed was anonymous clinical data of the patients. Permission to access and collect data from the medical records was sought and granted by Nyeri County Director of Health and Mount Kenya Hospital, Nyeri.

CHAPTER FOUR

RESULTS

4.1 Demographic and clinical characteristics of the study population

The study included 117 patients who met the study's inclusion criteria, of the total participants, 64(54.7%) were males while 53(45.3%) were females with a mean age of 56.76 (range 18-98) (Table 4.1)

Table 4.1: Clinical characteristics of covid-19 patients admitted at Mount Kenya Hospital Nyeri, Kenya

		Frequency	Percent	Cumulative Percent
Sex	Male	64	**54.7**	54.7
	Female	53	**45.3**	100.0
	Total	117	100.0	
Outcome	Survived	88	**75.2**	74.3
	Deceased	29	**24.5**	100.0
	Total	117	100.0	
ICU	No ICU admission required	5	38.5	38.5
	ICU admission required	8	61.5	100.0
	Total	13	100.0	
GCS	3	1	.9	.9
	15	105	89.7	100.0
	Total	117	100.0	
COVID Severity	Severe	91	77.8	77.8
	Non-Severe	26	22.2	100.0
	Total	117	100.0	
BP STATUS	Normal	52	44.4	44.4
	Abnormal	65	55.6	100.0
	Total	117	100.0	
PR STATUS	Normal	89	76.1	76.1
	Abnormal	28	23.9	100.0
	Total	117	100.0	

Key: *ICU-Intensive care Unit; GCS-Glasgow coma scale; BP-Blood pressure; PR-Pulse rate*

Those who survived the disease were 75.2% (88) while 24.5% (29) were deceased. Cases defined as severe were 91 (77.8%) while non-severe cases were 26 (22.2%). Participants with

normal Bp (130/85mmHg) (International Society of hypertension, 2021) measurements at admission were 44.4% while 55.6% had abnormal Bp measurements. Only 2 out of 65(3.08%) of subjects had Bp measurements less than 130/85 mmHg. At admission 76.1% of the patients had normal (60-100 bpm) pulse rate (PR) and 23.9% had abnormal (increased) PR. The mean hospitalization duration was 9.74±9.66 days (Table 4.1)

The liver enzyme level means were as follows: ALT 50.55±47.75 U/L, AST 57.74±79.77 U/L, ALP 104.51±72.68 U/L, GGT 70.23±63.43 U/L while total bilirubin and albumin means were 12.94±9.60 UMOL/L and 32.89±10.94 G/L respectively (Table 4.2)

Table 4.2: Age, symptom duration, hospital stay and liver function parameter means

	n	Minimum	Maximum	Mean	Std. Deviation
AGE	117	18.00	98.00	56.76	18.41
SYMPTOM DURATION (DAYS)	117	1.00	21.00	6.98	4.28
HOSPITAL STAY (DAYS)	117	1.00	74.00	9.74	9.66
AST (U/L)	116	6.30	834.26	57.74	79.77
ALT (U/L)	116	2.86	367.00	50.55	47.75
ALP (U/L)	116	5.77	385.00	104.51	72.68
GGT (U/L)	110	12.82	368.20	70.23	63.43
TBIL (UMOL/L)	116	0.00	69.90	12.94	9.60
DBIL (UMOL/L)	117	0.00	23.10	4.76	3.35
TP (G/L)	117	5.10	95.32	63.41	19.48
ALB (G/L)	116	2.59	51.94	32.89	10.94

Key: *AST-aspartate transaminase; ALT-alanine transaminase; ALP-alkaline phosphatase; GGT-gamma glutamyl transferase; TBIL- total bilirubin; DBIL- direct bilirubin; TP-total protein; ALB-albumin.*

4.2 Clinical characteristics based on the clinical outcome of covid-19

The mean age of survivors was 51.5±16.48 SD and non-survivors was 72.17±15.98 SD. The mean symptom duration (days) defined as (time between onset of symptoms and presentation to the hospital) was 7.61 ±4.43 SD in survivors and 5.28 ±3.21 in non-survivors (Table 4.3)

Table 4.3: Clinical characteristics based on the clinical outcome of covid-19

OUTCOME		n	Minimum	Maximum	Mean	Std. Deviation
Survived	AGE	88	18.00	92.00	**51.50**	16.48
	SYMPTOM DURATION (DAYS)	84	1.00	21.00	**7.61**	4.43
	HOSPITAL STAY (DAYS)	84	1.00	29.00	9.37	6.13
	AST (U/L)	83	6.30	834.26	58.36	91.04
	ALT (U/L)	83	3.78	367.00	52.45	52.63
	ALP (U/L)	84	27.16	362.00	94.82	65.04
	GGT (U/L)	81	12.82	368.20	70.77	68.75
	TBIL (UMOL/L)	83	0.00	69.90	13.33	10.35
	DBIL (UMOL/L)	84	0.00	23.10	4.74	3.43
	TP (G/L)	84	5.60	86.06	62.83	21.22
	ALB (G/L)	83	2.80	51.94	33.08	11.92
Deceased	AGE	29	39.00	98.00	**72.17**	15.98
	SYM DURATION (DAYS)	29	1.00	14.00	**5.28**	3.21
	HOSPITAL STAY (DAYS)	29	1.00	74.00	10.76	16.43
	AST (U/L)	29	8.69	202.00	56.95	42.22
	ALT (U/L)	29	2.86	110.00	40.63	23.61
	ALP (U/L)	28	5.77	385.00	137.30	88.30
	GGT (U/L)	25	16.89	195.82	68.46	46.90
	TBIL (UMOL/L)	29	3.16	37.77	12.06	7.85
	DBIL (UMOL/L)	29	0.01	13.00	4.83	3.25
	TP (G/L)	29	5.10	95.32	63.75	14.91
	ALB (G/L)	29	2.59	47.00	31.79	8.36

Key: *AST-aspartate aminotransferase; ALT-alanine aminotransferase; ALP-alkaline phosphatase; GGT- gamma glutamyl transferase; TBIL- total bilirubin; DBIL- direct bilirubin; TP-total protein; ALB-albumin*

4.3 Association between age and clinical outcome

Chi-square test for age and clinical outcome was carried out. Age was found to significantly predict the covid-19 outcome *(P*=0.000), indicating that the clinical outcome non-survival was more likely as age increased. Chi-square tests indicated that the likelihood of survival or death is not uniformly distributed across different age groups. Younger individuals, particularly those below 18 and in the 19-39 age range, exhibit higher percentages of survival, reaching 100% in

the youngest category. Conversely, older age groups, notably those above 75, show a higher rate of non-survival (Table 4.4)

Table 4.4: Association of age and clinical outcome of covid-19 infection

		OUTCOME		Chi-Square	*P*-value
LEVEL		SURVIVORS	NON-SURVIVORS		
Age	Below 18	1 (100.0%)	0 (0.0%)	31.171	0.000
	Young adults (19-39 years)	20 (95.2%)	1 (4.8%)		
	Middle aged adults (40-59 yrs)	37 (88.1%)	5 (11.9%)		
	young-old adults (60-74 yrs)	22 (75.9%)	7 (24.1%)		
	old-old adults (75 years)	8 (33.3%)	16 (66.7%)		

4.4 Association between hepatocellular-cholestatic enzymes and clinical outcome of covid-19

For hepatocellular enzymes, normal AST levels were observed in 80.4% of survivors and 19.6% of non survivors while severe liver injury based on AST levels was seen in 50% of survivors and 50% of non-survivors (Table 5) Normal ALT serum levels were noted in 73.8% of survivors and 26.2% of non-survivors. Moderate liver injury based on ALT was noted in 92.3% of survivors and 7.7% of non-survivors. Serum levels of AST (P=0.483) and ALT (P=0.408) showed no statistical significance in predicting covid-19 clinical outcome.

Table 4 5: Association of hepatocellular enzymes and clinical outcome of covid-19 infection

		OUTCOME		Chi-Square	*P*-value
LEVEL		SURVIVORS	NON-SURVIVORS		
AST	Normal	37 (80.4%)	9 (19.6%)	2.458	0.483
	Mild	36 (75.0%)	12 (25.0%)		
	Moderate	13 (65.0%)	7 (35.0%)		
	Severe	1 (50.0%)	1 (50.0%)		
ALT	Normal	48 (73.8%)	17 (26.2%)	2.898	0.408
	Mild	26 (70.3%)	11 (29.7%)		
	Moderate	12 (92.3%)	1 (7.7%)		
	Severe	1 (100%)	0 (0.0%)		

Key: *AST-aspartate aminotransferase; ALT-alanine aminotransferase*

Of the total participants, 80.4% of patients who survived and 19.6% of patients who were deceased had normal serum AST levels at admission while 50% of patients who survived and 50% of patients who were deceased had severely elevated AST levels at admission (Figure 4.1)

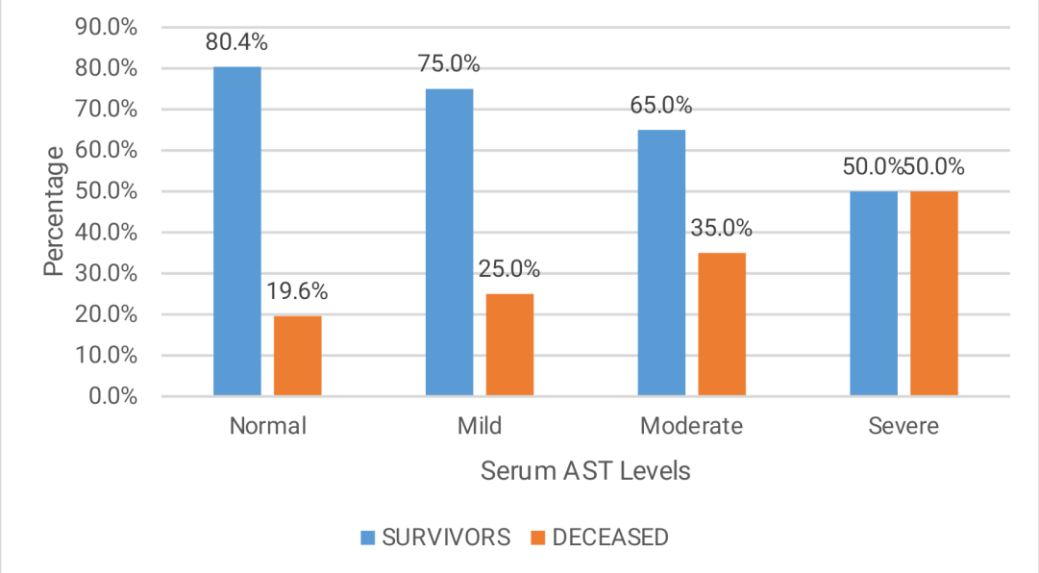


Figure 4.1: The proportion of serum AST levels in survivors vs. deceased patients

Normal ALT levels were observed in 73.8% of survivors and 26.2% of non-survivors at admission. Moderately elevated ALT levels were seen in 92.3% of survivors and 7.7% of non-survivors at admission (Figure 4.2)

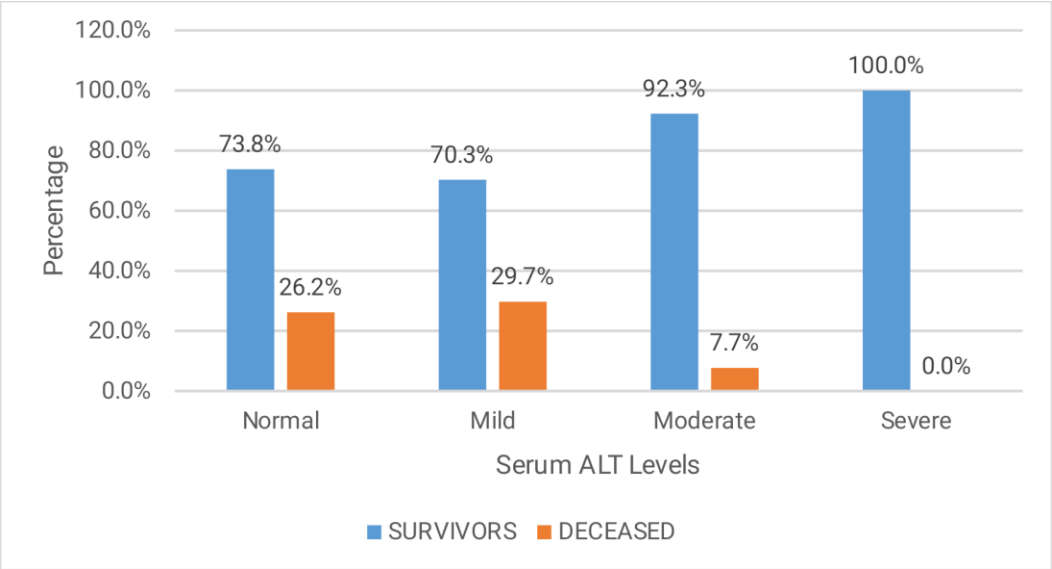


Figure 4.2: The proportion of serum ALT levels in survivors vs. deceased patients

For cholestatic enzymes, a chi-square test was performed to evaluate association between ALP and clinical outcome and GGT and clinical outcome. ALP (P=0.017) demonstrated statistical significance in forecasting covid-19 clinical outcome and GGT (P=0.452) indicated no statistical significance in predicting covid-19 clinical outcome at admission (Table 4.6).

Table 4.6: Association of cholestatic enzymes with clinical outcome of covid-19 infection

		OUTCOME		Chi-Square	P-value
		SURVIVORS	NON-SURVIVORS		
ALP	Normal	73 (79.3%)	19 (20.7%)	3.538	0.017
	Mild	7 (63.6%)	4 (36.4%)		
	Moderate	5 (55.6%)	4 (44.4%)		
GGT	Normal	43 (81.1%)	10 (18.9%)	2.63	0.452
	Mild	24 (70.6%)	10 (29.4%)		
	Moderate	14 (73.7%)	5 (26.3%)		
	Severe	4 (100.0%)	0 (0.0%)		

Key: *ALP-alkaline phosphatase; GGT-gamma glutamyl transferase*

Of the total participants, 79.3% of patients who survived and 20.7% of patients who were deceased had normal serum ALP at admission while 55.6% of patients who survived and 44.4% of patients who were deceased had moderately elevated serum ALP levels at admission (Figure 4.3)

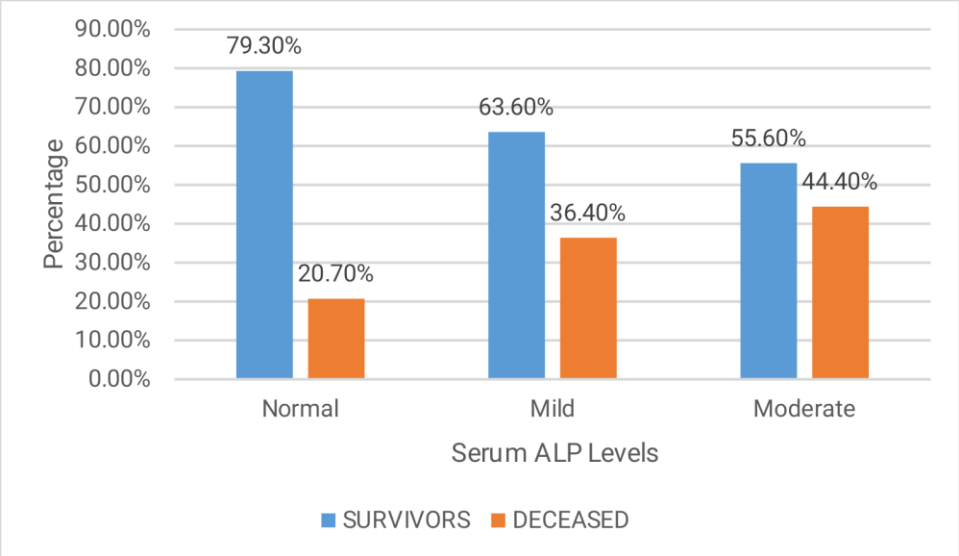


Figure 4.3: The proportion of serum ALP levels in survivors vs. deceased patients

Normal serum GGT levels were observed in 81.1% of survivors and 18.9% of non-survivors at admission. Moderately elevated serum GGT levels were seen in 73.7% of survivors and 26.3% of non-survivors at admission (Figure 4.4)

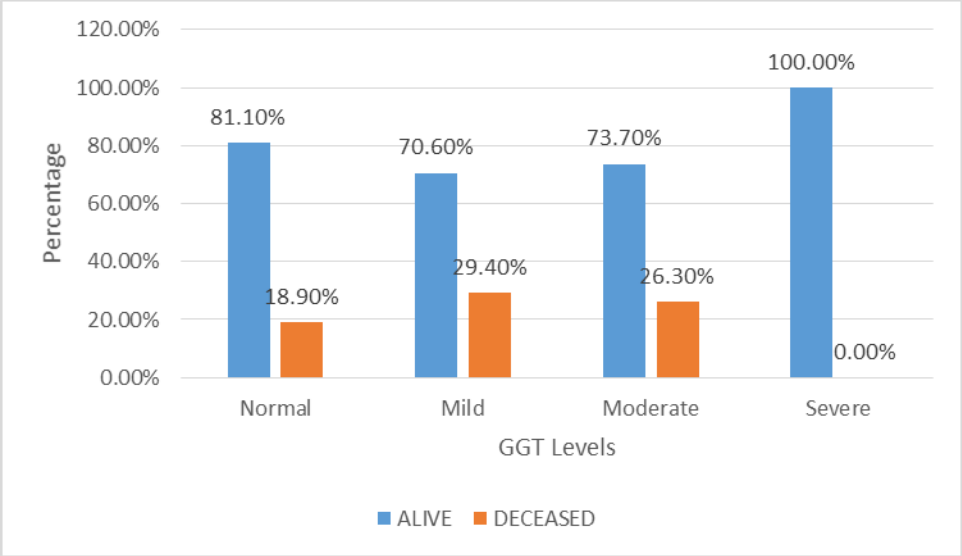


Figure 4.4: The proportion of serum GGT levels in survivors vs. deceased patients

On logistic regression analysis the model with ALP as the predictor variable showed statistical significance,(*P*=0.010) in forecasting the clinical outcome of covid-19 infection. The odds ratio for ALP coefficient was 1.007 with 95% CI [1.002, 1.013] suggesting that patients with high serum ALP levels were more likely to have non-survival outcome compared to those with normal levels (Table 4.7)

Table 4.7: Logistic Regression of ALP and clinical outcome

	B	S.E (β)	Wald (χ^2)	*p*	*O.R.*	*95% C.I. OR*
Constant	-1.956	.399	24.012	0.000	0.141	
ALP	.007	.003	6.563	**0.010**	**1.007**	[1.002, 1.013]
		Dependent Variable:R^2 =0.085				

Key*: B-coefficient estimates; S.E (β)- standard error of coefficient estimates;* χ^2-Chi-square; P-p value; O.R-odds Ratio; *ALP-Alkaline Phosphatase*

4.5 Association between serum albumin levels and clinical outcome of covid-19

A chi-square test was carried out to evaluate the association of serum albumin levels with clinical outcome (*P=0*.019) was statistically significant (Table 4.8)

Table 4.8: Association of serum albumin levels with clinical outcome of covid-19

		OUTCOME		Chi-Square	*P*-value
	LEVELS	SURVIVORS	NON-SURVIVORS		
ALBUMIN	Normal	42 (87.5%)	6 (12.5%)	7.941	**0.019**
	Mild low	28 (62.2%)	17 (37.8%)		
	Very low	11 (73.3%)	4 (26.7%)		

Normal serum albumin levels were seen in 87.5% of survivors and 12.5% of non-survivors. Mild hypoalbuminaemia amongst survivors was at 62.2% and 37.8% in non-survivors.

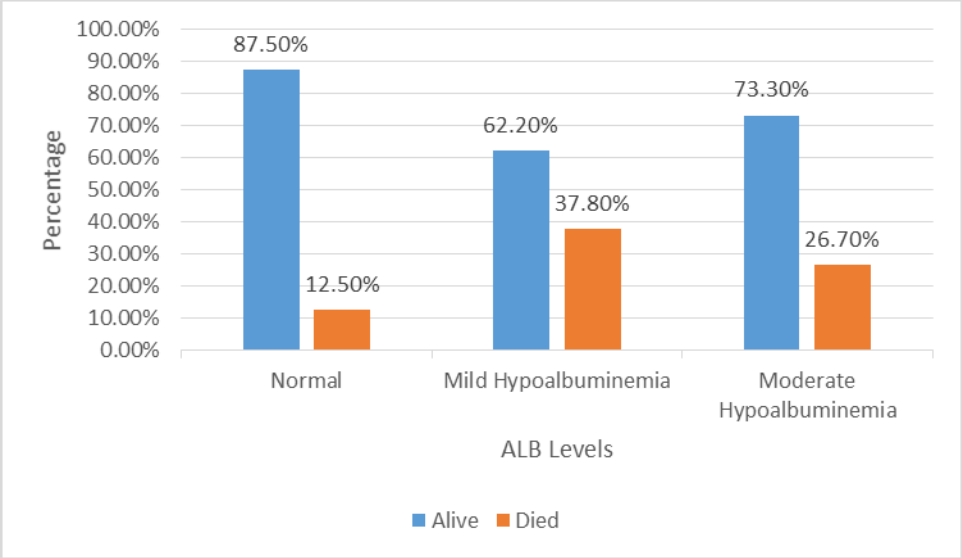


Figure 4.5: The proportion of albumin levels (normal and hypoalbuminaemia) in survivors vs. deceased patients

4.6 Association between serum total bilirubin levels and clinical outcome of covid-19

Chi-square test was carried out, serum total bilirubin and clinical outcome showed no significant statistical association *P*=0.390 (Table 4.9)

Table 4.9: Association of serum total bilirubin with clinical outcome

		OUTCOME		Chi-Square	P-value
		SURVIVORS	NON-SURVVORS		
TBIL	Normal	81 (75.7%)	26 (24.3%)	1.885	0.390
	Mild	4 (57.1%)	3 (42.9%)		
	Moderate	2 (100.0%)	0 (0.0%)		

Key: *TBIL- total bilirubin*

Of the total participants 75.7% of survivors had normal serum total bilirubin levels compared to 24.3% of non-survivors at admission (Figure 4.6)

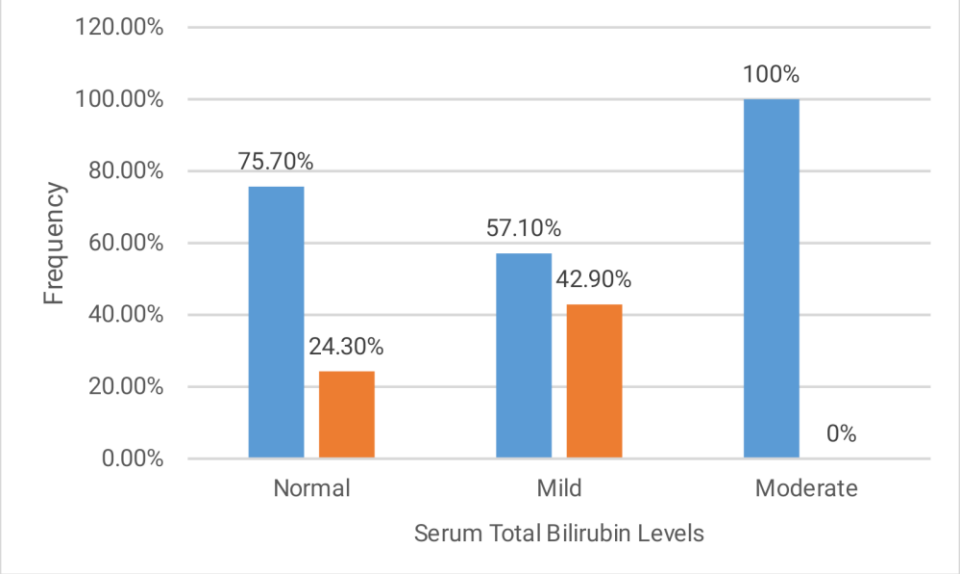


Figure 4.6: The proportion of serum total bilirubin levels in survivors vs. non-survivors

4.7 Overall multivariate logistic regression

On multivariate logistic regression analysis model age as the predictor variable showed statistical significance, (P=0.002) in forecasting the clinical outcome of covid-19 infection. The odds ratio for age coefficient was 1.007 with 95% CI [1.002, 1.013] suggesting that older patients were more likely to have non-survival outcome compared to younger ones (Table 4.7)

Table 4.10: Overall Multivariate Logistic Regression

	β	S.E (β)	Wald (χ^2)	*p*	*O.R.*
AGE	.096	.031	9.802	**.002**	1.100
GENDER(1=Male, 2=Female)	-.524	.847	.383	.536	.592
SYM DURATION (DAYS)	-.148	.157	.894	.344	.862
DURATION STAY (DAYS)	-.062	.055	1.287	.257	.940
BP STATUS(1)	.234	.823	.081	.776	1.263
PR	.029	.028	1.015	.314	1.029
TEMP	-.857	.669	1.639	.200	.424
spo2	-.034	.026	1.715	.190	.966
GCS	-1.479	1.024	2.087	.149	.228
COVID SEVERITY(1=Severe, 2=Non-severe)	1.504	1.256	1.434	.231	4.500
AST	.000	.009	.001	.980	1.000
ALT	-.017	.015	1.326	.249	.983
ALP	.006	.006	.829	.363	1.006
GGT	.009	.007	1.767	.184	1.009
TBIL	-.008	.061	.016	.899	.992
DBIL	.248	.188	1.751	.186	1.282
TP	.034	.046	.556	.456	1.035
ALB	-.041	.082	.249	.618	.960
Constant	44.514	32.955	1.825	.177	2.15E+19

R^2 = 0.635: *BP-blood pressure; PR-pulse rate; Temp-body temperature;spo2-saturation of oxygen; GCS- Glasgow coma scale; AST-aspartate aminotransferase; ALT-alanine aminotransferase; ALP- alkaline phosphatase; GGT-gamma glutamyl transaminase; TBIL-total bilirubin; DBIL- direct bilirubin; ALB-albumin*

CHAPTER FIVE

DISCUSSION

5.1 Association between age and clinical outcome of covid-19 patients admitted at Mount Kenya Hospital Nyeri, Kenya

In the current study age was significantly associated with covid-19 clinical outcome (non-survival) (*P*=0.000). Advancement in age is associated with poor lung capacity and atrophy of the muscles involved in respiration. These affects the integrity of the airway biological defense barrier affecting ability of the airway to clear antigens thus portending poor outcome in this age group. Equally, low lung capacity leads to hypoxemic liver injury. With advanced age there is also likelihood of underlying comorbidities (Zhang et al., 2022).This finding was in agreement with Gagliardi et al. (2020) where older persons were nearly twice more likely to die compared to the younger ones. According to a study by(Starke, 2021) age was associated with increased risk of poor covid-19 disease outcome that included recurrent hospitalization and death. Similarly according to a study by Henkens et al., (2022), age was found to be the major factor determining mortality in the hospital and this was not significantly affected by presence of comorbidities that preexisted. This concurs with this study in that likelihood of non-survival was high in the older age group with mean age 72.17 ± 15.98 years. Studies have documented that increased age beginning 40 years was associated with higher likelihood of death.

5.2 Association between hepatocellular-cholestatic liver enzymes and clinical outcome of covid-19 patients admitted at Mount Kenya Hospital Nyeri, Kenya

In the present study liver enzymes derangement was common in both survivors and non-survivors. Liver involvement in covid-19 is a common phenomenon with contribution to the disease outcome. A similar observation was reported on covid-19 patients by Li et al., (2022)**.**In the current evaluation there was no statistically significant association between AST (P=0.483)

and ALT (P=0.408) levels with the clinical outcome of covid-19 disease. These liver enzymes (AST and ALT) were noted not to be significant in predicting covid-19 clinical outcome. AST and ALT levels are markers of hepatocellular injury. Association of hepatocellular enzymes (AST and ALT) with survival or non-survival in covid-19 may be a comorbidity-based feature. It is hypothesized that this feature is fueled by heightened general inflammation in comorbid patients. Since the current study focused on non-comorbid patients thus no association between hepatocellular enzymes and clinical outcome was demonstrated. Our study findings were similar to those of with who noted that liver dysfunction does not influence survival and the elevations may be explained by underlying other diseases.

However in several other studies, it was found that AST and ALT were associated with severe disease and mortality (Christensen et al 2020)**.** Similar findings were documented by Ibrahim et al., (2022) in a study population of covid-19 patients with severe disease, 77% of them had elevated AST while 49% had elevated ALT. Patients presenting with severe covid-19 disease had high levels of AST (P=0.015) and ALP (P=0.03). According to a study done by Oh et al., (2021) moderate to severe elevation of AST was associated with high mortality (P<0.001) in comparison to the normal AST population. The study population included patients with comorbidities. It is important to note that severity and mortality of covid-19 is affected by other factors such as underlying comorbidities. In this study patients with comorbidities were excluded and perhaps this is why patients with deranged AST and ALT levels did not have statistically significant associated non-survival.

Similarly, in a study done by (Pozzobon et al., 2021) high levels of serum ALT were associated with mortality in covid-19 patients. Patients who at admission presented with ALT levels 2x

ULN and above had statistically significant mortality rate (P<0.001) compared to patients with ALT levels <2x ULN.In another study, patients with elevated ALT levels were found, in comparison with normal group, to have an increased total 7 day non-survival rate (Salık et al., 2021)

The postulated causes of ALT and AST elevation are covid-19 direct viral toxicity, hepatic hypoxia, ischemia and immune response. Sharma et al 2021 also noted inconsistencies in transaminases rise in covid-19 being 1-2 times ULN while in hepatic ischemia/hypoxia is commonly massive up to 20-fold ULN. Variability in liver enzymes pattern in covid-19 has been documented in previous studies. The elevations might have been due to cytotoxic effects of SARS-CoV-2 on the liver since only 3.08% of the patients had blood pressure <130/85mmhg thus the elevations may not be due to ischemia, and equally neither due to hypoxia as the enzyme levels were not massively elevated.

With cholestatic enzymes (ALP and GGT), ALP (*P*= 0.010) (8.5% (Nagelkerke R2) of the variance of covid-19 outcome was associated with clinical outcome of covid-19 infection. High concentration of ACE-2 receptors in cholangiocytes compared to hepatocytes may underscore that bile ducts are the principle viral attack point, and not the liver. This variability in ACE-2 receptors concentration may promote bile ducts injury with resultant cholestasis. This is in agreement with Kulkarni et al., 2020 who highlighted cholestasis due to covid-19 was associated with severe disease and is linked to a poor prognosis. In a study done by Krishnan et al., (2022) elevated levels of ALP were associated with all-cause mortality in covid-19 patients. However, GGT association with covid-19 outcome was not statistically significant (*P*=0.17). A study on elevated GGT levels in covid-19 patients was associated (P=0.004) with severe disease and

longer in-hospital stay. However, the study population included patients with comorbidities (Liu et al., 2021). Similarly, the study found elevated GGT to be associated with mortality. However those who succumbed were significantly (*P*= 0.001) older than those who survived. In this study patients with comorbidities were excluded perhaps reducing the magnitude of the relationship between elevated GGT and non-survival.

5.3 Association between serum albumin and clinical outcome of covid-19 patients admitted at Mount Kenya Hospital Nyeri, Kenya

In the current study low serum albumin levels was statistically significantly associated with clinical outcome (non-survival) of covid-19 disease ($\chi 2$ 7.94, *P*=0.019). Albumin plays a pivotal role in homeostasis maintenance in critical disease states. Hypoalbuminemia on the other hand, is a negative acute phase reactant implicated to promote marked inflammatory processes with associated poor prognosis in infectious diseases. Hypoalbuminemia fosters adverse disease events including acute respiratory distress syndrome. This concurs with Vianna-Llamas et al., (2021) who noted hypoalbuminaemia as an early predictor of inpatient death in covid-19 infection independent of age, comorbidity and markers of inflammation. Similarly , according to Huang et al., (2020) in his study on 299 adult patients ,160 males and 139 females found that hypoalbuminaemia was an independent predictor of covid-19 outcome (P<0.001).Patients demonstrated an inverse relationship between albumin and white blood cells (P=0.01).Similar findings were reported by Xu et al., (2021)

The cause of hypoalbuminaemia in covid-19 infection has been linked to cytokine storm Xu et al., (2021).Severe inflammatory response as seen in covid-19 causes a rise in vascular permeability and capillary leakage, this results in albumin redistribution toward the extravascular space. According to Soetedjo et al., (2021) inflammation and hypoxia also reduce albumin half-

life and as well as direct injury to epithelial-endothelial barrier causing extravasation of albumin. Further, being a protein that regulates homeostasis through various ways such as scavenging for free radicals, controlling of platelet function and maintaining the osmotic pressure then its low levels associates with poor covid-19 disease outcomes. This agrees with this study in that hypoalbuminaemia was associated with poor disease outcome- non-survival.

Notably, albumin has been shown to have the ability to down regulate ACE-2 receptors which critically modulates covid-19 infection; thus low albumin levels may result in up regulation of ACE-2 receptors with increased covid-19 infectivity**,** Xu et al 2021. This up regulation of ACE receptors may explain poor covid-19 outcome of patients with hypoalbuminaemia in this study as it results in promoting entry of the virus into the cells through binding to the receptors.

5.4 Association of Serum total bilirubin and clinical outcome of covid-19 patients admitted at Mount Kenya Hospital Nyeri, Kenya

In this study, serum total bilirubin showed no statistically significant (P=0.390) association with clinical outcome of covid-19 infection. Bilirubin within normal levels exerts antioxidant properties, with the ability to trap and break peroxyl radicals. In in vitro experiments, the antioxidant potency of bilirubin is optimized by presence of normal oxygen levels. Therefore, serum bilirubin levels may not correlate with poor prognosis but with the extent of oxidative-stress in the beginning of the disease process. This observation concurs with Elmunzer et al., (2021) in their retrospective study on various digestive manifestations of covid-19 that involved 1992 inpatients they found that even though gastrointestinal and hepatic anomalies including elevated total bilirubin are common they are usually mild and were not associated with worse clinical outcomes.

According to a study on bilirubin and ischemic stroke by Pineda et al., (2008) high bilirubin level was related to the severity of oxidative-stress related disorders including liver diseases and cerebral vascular accidents but it was not independently linked to the discharge outcome. Bilirubin offers therapeutic advantages because it possesses antioxidant properties. According to Ali et al., (2017) bilirubin is the most powerful endogenous antioxidant and cushions against cell membrane lipid peroxidation. Pineda et al., (2008) highlighted high bilirubin levels in these conditions as an indicator of initial degree of oxidative-stress that was linked to disease severity but not to discharge outcome.

This finding fails to concur with Russo et al., (2022) who reported a higher serum bilirubin level at hospital admission independently associated with the mortality risk during hospitalization. Liu et al., (2020) in a multicenter retrospective study reported high direct bilirubin levels in covid-19 patients in absence of pre-existing comorbidities was linked to severe disease and mortality. In some studies direct bilirubin was observed to be a better predictor of disease outcome compared to total bilirubin levels. The mechanistic explanation needs further evaluation. According to Maruhashi et al., (2019) bilirubin at elevated levels is cytotoxic and causes deoxyribonucleic acid (DNA) damage even in non-neuronal cells, this may elucidate the association of elevated amounts of bilirubin with severe disease and non-survival.

5.5 Summary of Key findings

Liver biochemical profile parameters were commonly deranged in covid-19 patients. Age is a predictor of covid-19 clinical-outcome (non-survival). Older persons were nearly twice more likely to die compared to the younger ones. Even on a multivariate linear logistic regression, the significance level of age to clinical outcome was at per with other patient characteristics as GCS and Spo2 levels. Among the liver enzymes serum ALP levels was a strong predictor of mortality

in in-patient covid-19 patients. Liver enzymes AST, ALT and GGT were observed not to confer association with clinical outcome of covid-19.Serum total bilirubin levels were not predictive of covid-19 clinical outcome. Serum albumin levels specifically hypoalbuminemia forecasted poor prognosis and non-survival in covid-19 disease. Variability in the association of liver biochemical markers and clinical outcome of covid-19 infection in different studies has been demonstrated with a number of factors including comorbidity being hypothesized as possible attributes.

5.6 Strengths of the study

The study results highlight statistical significance of association without suggesting cause and effect relationship between the exposure and outcome.

5.7 Limitations of the study

This was a single-center study. Longitudinal cross sectional study would have allowed for evaluation of possible changes in liver biochemical profile of participants at different study points.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATION

6.1 Conclusion

i. Age has a predictive value in covid-19 clinical outcome with older patients nearly twice more likely to succumb than younger ones.

ii. Cholestatic enzyme ALP level in serum confer an association with covid-19 clinical outcome with those with high levels of ALP being more likely to succumb compared to those who had low levels

iii. Hypoalbuminaemia is a predictive factor in prognosis of covid-19 infection and is related to clinical outcome (non-survival)

iv. Serum bilirubin levels have no predictive value in forecasting covid-19 in-hospital clinical outcome (survival or non-survival)

6.2 Recommendations

From the study findings the following recommendations have been made:

i. Age be used as a reliable predictive patient characteristic to inform likely disease outcome of covid-19 infection to help in patient risk stratification and prioritizing response.

ii. Serum ALP levels be utilized as the main liver enzyme element in forecasting covid-19 infection clinical outcome in non-comorbid subjects. Patients with high levels of serum ALP should receive prompt and higher-level clinical therapy interventions to caution from advancing to more life-threatening afflictions and to improve clinical outcome

iii. Hypoalbuminemia at admission should be utilized as a predictive factor of poor disease outcome and thus prompt and escalated measures of clinical intervention should be employed to avert poor clinical outcome of patients

iv. Future studies need to evaluate the role of serum GGT levels in predicting covid-19 clinical outcome and the mechanistic explanation me in of differences in clinical outcome parameters that predict disease outcome comorbid vs. non-comorbid patients

v. Medical practitioners need to appreciate variability in liver biochemical markers and their association to covid-19 disease outcome, to ensure their reliability in prognosticating covid-19 outcome.

REFERENCES

Afra, H. S., Amiri-Dashatan, N., Ghorbani, F., Maleki, I., & Rezaei-Tavirani, M. (2020). Positive association between severity of COVID-19 infection and liver damage: a systematic review and meta-analysis. *Gastroenterology and Hepatology From Bed to Bench*, *13*(4), 292. /pmc/articles/PMC7682972/

Agbafor, K. N., Nwaka, A. C., Dasofunjo, K., Asuk, & Ugwu, M. N. (2017). Idosr Journal of Scientific Research 2(2) 97-107, 2017. Serum Activity of Alanine Aminotransferase and Aspartate Aminotransferase in Albino Rats Administered Aqueous Extract of Fresh Leaves Pterocarpus Santalinoids. *Idosr Journal of Scientific Research*, *2*(2), 97–107. www.idosr.orgwww.idosr.org

Aleem, A., Akbar Samad, A. B., & Vaqar, S. (2023). Emerging Variants of SARS-CoV-2 and Novel Therapeutics Against Coronavirus (COVID-19). In *StatPearls*. StatPearls Publishing.

Ali, S. J., Bazzaz, A. A., Arif, A. I., Ali, S. J., Bazzaz, A. A., & Arif, A. I. (2017). Activity of the Enzyme Gamma-Glutamyl Transferase (GGT) as a Prognostic Tool for Heart Failures. *Advances in Bioscience and Biotechnology*, *8*(9), 324–341. https://doi.org/10.4236/ABB.2017.89024

AlSamman, M., Caggiula, A., Ganguli, S., Misak, M., & Pourmand, A. (2020). Non-respiratory presentations of COVID-19, a clinical review. *The American Journal of Emergency Medicine*, *38*(11), 2444. https://doi.org/10.1016/J.AJEM.2020.09.054

Amin, M. (2021). COVID-19 and the liver: overview. *European Journal of Gastroenterology & Hepatology*, *33*, 309–311. https://doi.org/10.1097/MEG.0000000000001808

AR, B., AE, A., W, T., JL, H., GJ, N., SJ, G., MC, B., G, D., AA, V., AD, O., PH, van der V., DJ, M., & H, van G. (2020). Angiotensin-converting enzyme 2 (ACE2), SARS-CoV-2 and the pathophysiology of coronavirus disease 2019 (COVID-19). *The Journal of Pathology*, *251*(3), 228–248. https://doi.org/10.1002/PATH.5471

Bertolini, A., Peppel, I. P. van de, Bodewes, F. A. J. A., Moshage, H., Fantin, A., Farinati, F., Fiorotto, R., Jonker, J. W., Strazzabosco, M., Verkade, H. J., & Peserico, G. (2020). Abnormal liver function tests in COVID- 19 patients: relevance and potential pathogenesis. *Hepatology (Baltimore, Md.)*, *72*(5), 1864–1872. https://doi.org/10.1002/HEP.31480

Beyerstedt, S., Casaro, E. B., & Rangel, É. B. (2021). COVID-19: angiotensin-converting enzyme 2 (ACE2) expression and tissue susceptibility to SARS-CoV-2 infection. *European*

Journal of Clinical Microbiology and Infectious Diseases, *40*(5), 905–919. https://doi.org/10.1007/s10096-020-04138-6

Brichacek, A. L., & Brown, C. M. (2019). Alkaline Phosphatase: A Potential Biomarker for Stroke and Implications for Treatment. *Metabolic Brain Disease*, *34*(1), 3. https://doi.org/10.1007/S11011-018-0322-3

Caironi, P., & Gattinoni, L. (2009). The clinical use of albumin: the point of view of a specialist in intensive care. *Blood Transfusion*, *7*(4), 259. https://doi.org/10.2450/2009.0002-09

Cha, M. H., Regueiro, M., & Sandhu, D. S. (2020). Gastrointestinal and hepatic manifestations of COVID-19: A comprehensive review. *World Journal of Gastroenterology*, *26*(19), 2323. https://doi.org/10.3748/WJG.V26.I19.2323

Christensen, B., Favaloro, E. J., Lippi, G., & Cott, E. M. Van. (2020). Maintaining Hemostasis and Preventing Thrombosis in COVID-19—Part I: Hematology Laboratory Abnormalities in Patients with Coronavirus Disease 2019 (COVID-19). *Seminars in Thrombosis and Hemostasis*, *46*(7), 845. https://doi.org/10.1055/S-0040-1715458

Conway, E. M., Mackman, N., Warren, R. Q., Wolberg, A. S., Mosnier, L. O., Campbell, R. A., Gralinski, L. E., Rondina, M. T., van de Veerdonk, F. L., Hoffmeister, K. M., Griffin, J. H., Nugent, D., Moon, K., & Morrissey, J. H. (2022). Understanding COVID-19-associated coagulopathy. *Nature Reviews Immunology 2022 22:10*, *22*(10), 639–649. https://doi.org/10.1038/s41577-022-00762-9

Dhar, D., & Mohanty, A. (2020). Gut microbiota and Covid-19- possible link and implications. *Virus Research*, *285*. https://doi.org/10.1016/J.VIRUSRES.2020.198018

Elmunzer, B. J., Spitzer, R. L., Foster, L. D., Merchant, A. A., Howard, E. F., Patel, V. A., West, M. K., Qayed, E., Nustas, R., Zakaria, A., Piper, M. S., Taylor, J. R., Jaza, L., Forbes, N., Chau, M., Lara, L. F., Papachristou, G. I., Volk, M. L., Hilson, L. G., … Durkalski, V. (2021). Digestive Manifestations in Patients Hospitalized With Coronavirus Disease 2019. *Clinical Gastroenterology and Hepatology*, *19*(7), 1355. https://doi.org/10.1016/J.CGH.2020.09.041

Fierro, N. A. (2020). COVID-19 and the liver: What do we know after six months of the pandemic? *Annals of Hepatology*, *19*(6), 590. https://doi.org/10.1016/J.AOHEP.2020.09.001

Gagliardi, M. C., Tieri, P., Ortona, E., & Ruggieri, A. (2020). ACE2 expression and sex disparity in COVID-19. *Cell Death Discovery*, *6*(1), 1234567890. https://doi.org/10.1038/S41420-020-0276-1

Garrido, I., Liberal, R., & G. M.-A. pharmacology, & 2020, undefined. (2020). COVID- 19 and liver disease—what we know on 1st May 2020. *Wiley Online Library*, *52*(2), 267–275. https://doi.org/10.1111/apt.15813

Ghahramani, S., Tabrizi, R., Lankarani, K. B., Kashani, S. M. A., Rezaei, S., Zeidi, N., Akbari, M., Heydari, S. T., Akbari, H., Nowrouzi-Sohrabi, P., & Ahmadizar, F. (2020). Laboratory features of severe vs. non-severe COVID-19 patients in Asian populations: a systematic review and meta-analysis. *European Journal of Medical Research*, *25*(1). https://doi.org/10.1186/S40001-020-00432-3

Gulhar, R., Ashraf, M. A., & Jialal, I. (2023). Physiology, Acute Phase Reactants. *StatPearls*. https://www.ncbi.nlm.nih.gov/books/NBK519570/

Gupta, A., Madhavan, M. V., Sehgal, K., Nair, N., Mahajan, S., Sehrawat, T. S., Bikdeli, B., Ahluwalia, N., Ausiello, J. C., Wan, E. Y., Freedberg, D. E., Kirtane, A. J., Parikh, S. A., Maurer, M. S., Nordvig, A. S., Accili, D., Bathon, J. M., Mohan, S., Bauer, K. A., … Landry, D. W. (2020). Extrapulmonary manifestations of COVID-19. *Nature Medicine 2020 26:7*, *26*(7), 1017–1032. https://doi.org/10.1038/s41591-020-0968-3

H M Henkens, M. T., Raafs, A. G., J Verdonschot, J. A., Linschoten, M., van Smeden, M., Wang, P., M van der Hooft, B. H., Tieleman, R., F Janssen, M. L., A ter Bekke, R. M., Hazebroek, M. R., C van der Horst, I. C., Asselbergs, F. W., H Magdelijns, F. J., & B Heymans, S. R. (2021). Age is the main determinant of COVID-19 related in-hospital mortality with minimal impact of pre-existing comorbidities, a retrospective cohort study on behalf of the CAPACITY-COVID collaborative consortium. *BMC Geriatrics*, *22*, 184.https://doi.org/10.1186/s12877-021-02673-1

Huang, X. J., Choi, Y. K., Im, H. S., Yarimaga, O., Yoon, E., & Kim, H. S. (2006). Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. *Sensors*, *6*(7), 756–782. https://doi.org/10.3390/s6070756

Izcovich, A., Ragusa, M. A., Tortosa, F., Marzio, M. A. L., Agnoletti, C., Bengolea, A., Ceirano, A., Espinosa, F., Saavedra, E., Sanguine, V., Tassara, A., Cid, C., Catalano, H. N., Agarwal, A., Foroutan, F., & Rada, G. (2020). Prognostic factors for severity and mortality in patients

infected with COVID-19: A systematic review. *PLoS ONE*, *15*(11). https://doi.org/10.1371/JOURNAL.PONE.0241955

Jain, U. (2020). Effect of COVID-19 on the Organs. *Cureus*, *12*(8). https://doi.org/10.7759/CUREUS.9540 Montazersaheb, S., Hosseiniyan Khatibi, S. M., Hejazi, M. S., Tarhriz, V., Farjami, A., Ghasemian Sorbeni, F., Farahzadi, R., & Ghasemnejad, T. (2022). COVID-19 infection: an overview on cytokine storm and related interventions. *Virology Journal 2022 19:1*, *19*(1), 1–15. https://doi.org/10.1186/S12985-022- 01814-1

Kopel, J., Perisetti, A., Roghani, A., Aziz, M., Gajendran, M., & Goyal, H. (2020). Racial and Gender-Based Differences in COVID-19. *Frontiers in Public Health*, *8*, 418. https://doi.org/10.3389/FPUBH.2020.00418

Krichel, B., Falke, S., Hilgenfeld, R., Redecke, L., & Uetrecht, C. (2020). Processing of the SARS-CoV pp1a/ab nsp7–10 region. *Biochemical Journal*, *477*(5), 1009–1019. https://doi.org/10.1042/BCJ20200029

Krishnan, A., Prichett, L., Liu, Y., Ting, P. S., Alqahtani, S. A., Kim, A. K., Ma, M., Hamilton, J. P., Woreta, T. A., & Chen, P. H. (2022). Risk of Severe Illness and Risk Factors of Outcomes of COVID-19 in Hospitalized Patients with Chronic Liver Disease in a Major U. S. Hospital Network. *Canadian journal of gastroenterology & hepatology*, *2022*, 8407990. https://doi.org/10.1155/2022/8407990

Kulkarni, S., Jenner, B. L., & Wilkinson, I. (2020). COVID-19 and hypertension. In *JRAAS - Journal of the Renin-Angiotensin-Aldosterone System* (Vol. 21, Issue 2). SAGE Publications Ltd. https://doi.org/10.1177/1470320320927851

Kumar-M, P., Mishra, S., Jha, D. K., Shukla, J., Choudhury, A., Mohindra, R., Mandavdhare, H. S., Dutta, U., & Sharma, V. (2020). Coronavirus disease (COVID-19) and the liver: a comprehensive systematic review and meta-analysis. *Hepatology International*, *14*(5), 1. https://doi.org/10.1007/S12072-020-10071-9

Lala, V., Goyal, A., Bansal, P., & Minter, D. A. (2021). Liver Function Tests. *StatPearls*. https://www.ncbi.nlm.nih.gov/books/NBK482489/

Li, H., Liu, S.-M., Yu, X.-H., Tang, S.-L., & Tang, C.-K. (2020). Coronavirus disease 2019 (COVID-19): current status and future perspectives. *International Journal of Antimicrobial Agents*, *55*(5), 105951. https://doi.org/10.1016/J.IJANTIMICAG.2020.105951

Liedtke, D., Hofmann, C., Jakob, F., Klopocki, E., & Graser, S. (2020). Tissue-Nonspecific

Alkaline Phosphatase—A Gatekeeper of Physiological Conditions in Health and a Modulator of Biological Environments in Disease. *Biomolecules*, *10*(12), 1–28. https://doi.org/10.3390/BIOM10121648

Liu, D. X., Fung, T. S., Chong, K. K. L., Shukla, A., & Hilgenfeld, R. (2014). Accessory proteins of SARS-CoV and other coronaviruses. *Antiviral Research*, *109*(1), 97–109. https://doi.org/10.1016/J.ANTIVIRAL.2014.06.013

Liu, W., Yang, C., Liao, Y.-G., Wan, F., Lin, L., Huang, X., Zhang, B.-H., Yuan, Y., Zhang, P., Zhang, X.-J., She, Z.-G., Wang, L., & Li, H. (2022). Risk factors for COVID-19 progression and mortality in hospitalized patients without pre-existing comorbidities. *Journal of Infection and Public Health*, *15*, 13–20. https://doi.org/10.1016/j.jiph.2021.11.012

Liu, Y.-C., Kuo, R.-L., & Shih, S.-R. (2020). COVID-19: The first documented coronavirus pandemic in history. *Biomedical Journal*, *43*(4), 328. https://doi.org/10.1016/J.BJ.2020.04.007

Liu, Z., Que, S., Xu, J., & Peng, T. (2014). Alanine aminotransferase-old biomarker and new concept: A review. *International Journal of Medical Sciences*, *11*(9), 925–935. https://doi.org/10.7150/ijms.8951

Maruhashi, T., Kihara, Y., & Higashi, Y. (2019). Bilirubin and Endothelial Function. *Journal of Atherosclerosis and Thrombosis*, *26*(8), 688. https://doi.org/10.5551/JAT.RV17035

Medical Encyclopedia: Bilirubin. (2005). http://www.nlm.nih.gov/medlineplus/ency/article/003479.htm

Metawea, M. I., Yousif, W. I., & Moheb, I. (2021). COVID 19 and liver: An A–Z literature review. *Digestive and Liver Disease*, *53*(2), 146. https://doi.org/10.1016/J.DLD.2020.09.010

Metón, I., Salgado, M. C., Anemaet, I. G., González, J. D., Fernández, F., & Baanante, I. V. (2015). Alanine aminotransferase: A target to improve utilisation of dietary nutrients in aquaculture. *Recent Advances in Pharmaceutical Sciences V*, *37/661*(2), 133–148. https://www.trnres.com/ebook/uploads/munozvcontent/T_1444299727Munozv 9.pdf

Moon, A. M., & Barritt, A. S. (2021). Elevated Liver Enzymes in Patients with COVID-19: Look, but Not Too Hard. *Digestive Diseases and Sciences*, *66*(6), 1767–1769. https://doi.org/10.1007/s10620-020-06585-9

Moreira, J. L. de S., Barbosa, S. M. B., & Júnior, J. G. (2021). Pathophysiology and molecular mechanisms of liver injury in severe forms of COVID-19: An integrative review. *Clinics and Research in Hepatology and Gastroenterology*, *45*(6), 101752. https://doi.org/10.1016/J.CLINRE.2021.101752

Nardo, A. D., Schneeweiss- Gleixner, M., Bakail, M., Dixon, E. D., Lax, S. F., & Trauner, M. (2021). Pathophysiological mechanisms of liver injury in COVID- 19. *Liver International*, *41*(1), 20–32. https://doi.org/10.1111/LIV.14730

Ndrepepa, G. (2021). Aspartate aminotransferase and cardiovascular disease—a narrative review. *Journal of Laboratory and Precision Medicine*, *6*(8), 6–6. https://doi.org/10.21037/jlpm-20-93

Ombajo, L. A., Mutono, N., Sudi, P., Mutua, M., Sood, M., Loo, A. M., Juma, P., Odhiambo, J., Shah, R., Wangai, F., Maritim, M., Anzala, O., Amoth, P., Kamuri, E., Munyu, W., & Thumbi, S. M. (2022). Epidemiological and clinical characteristics of patients hospitalised with COVID-19 in Kenya: A multicentre cohort study. *BMJ Open*, *12*(5). https://doi.org/10.1136/BMJOPEN-2021-049949

Papadopoulos, N., Vasileiadi, S., & Deutsch, M. (2020). COVID-19 and liver injury: where do we stand? *Annals of Gastroenterology*, *33*(5), 459. https://doi.org/10.20524/AOG.2020.0522

Parasher, A. (2021). COVID-19: Current understanding of its Pathophysiology, Clinical presentation and Treatment. *Postgraduate Medical Journal*, *97*(1147), 312–320. https://doi.org/10.1136/POSTGRADMEDJ-2020-138577

Parohan, M., Yaghoubi, S., & Seraj, A. (2020). Liver injury is associated with severe Coronavirus disease 2019 (COVID- 19) infection: a systematic review and meta- analysis of retrospective studies. *Hepatology Research*, *50*(8), 924–935. https://doi.org/10.1111/HEPR.13510

Patel, K. P., Patel, P. A., Vunnam, R. R., Hewlett, A. T., Jain, R., Jing, R., & Vunnam, S. R. (2020). Gastrointestinal, hepatobiliary, and pancreatic manifestations of COVID-19. *Journal of Clinical Virology*, *128*, 104386. https://doi.org/10.1016/J.JCV.2020.104386

Pineda, S., Bang, O. Y., Saver, J. L., Starkman, S., Yun, S. W., Liebeskind, D. S., Kim, D., Ali, L. K., Shah, S. H., & Ovbiagele, B. (2008). Association of Serum Bilirubin with Ischemic Stroke Outcomes. *Journal of Stroke and Cerebrovascular Diseases : The Official Journal of*

National Stroke Association, *17*(3), 147. https://doi.org/10.1016/J.JSTROKECEREBROVASDIS.2008.01.009

Ponti, G., Maccaferri, M., Ruini, C., Tomasi, A., & Ozben, T. (2020). Biomarkers associated with COVID-19 disease progression. *Critical Reviews in Clinical Laboratory Sciences*, 1. https://doi.org/10.1080/10408363.2020.1770685

Pozzobon, F. M., Perazzo, H., Bozza, F. A., Rodrigues, R. S., de Mello Perez, R., & Chindamo, M. C. (2021). Liver injury predicts overall mortality in severe COVID-19: a prospective multicenter study in Brazil. *Hepatology International*, *15*(2), 493. https://doi.org/10.1007/S12072-021-10141-6

Rader, B. A. (2017). Alkaline Phosphatase, an Unconventional Immune Protein. *Frontiers in Immunology*, *8*(AUG). https://doi.org/10.3389/FIMMU.2017.00897

Radivojevic, A., Abu Jad, A. A., Ravanavena, A., Ravindra, C., Igweonu-Nwakile, E. O., Ali, S., Paul, S., Yakkali, S., Teresa Selvin, S., Thomas, S., Bikeyeva, V., Abdullah, A., & Balani, P. (2022). A Systematic Review of SARS-CoV-2-Associated Hepatic Dysfunction and the Impact on the Clinical Outcome of COVID-19. *Cureus*, *14*(7), e26852. https://doi.org/10.7759/cureus.26852

Rothan, H. A., & Byrareddy, S. N. (2020). The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *Journal of Autoimmunity*, *109*, 102433. https://doi.org/10.1016/J.JAUT.2020.102433

Salık, F., Uzundere, O., Bıçak, M., Akelma, H., Akgündüz, M., Korhan, Z., Kandemir, D., & Kaçar, C. K. (2021). Liver function as a predictor of mortality in COVID-19: A retrospective study. *Annals of Hepatology*, *26*, 100553. https://doi.org/10.1016/J.AOHEP.2021.100553

Sharma, P., Kumar, A., Anikhindi, S., Bansal, N., Singla, V., Shivam, K., & Arora, A. (2021). Effect of COVID-19 on Pre-existing Liver disease: What Hepatologist Should Know? *Journal of Clinical and Experimental Hepatology*, *11*(4), 484. https://doi.org/10.1016/J.JCEH.2020.12.006

Song, Y., Zhang, X., Li, C., Xu, S., Zhou, B., & Wu, X. (2022). Is Bilirubin Associated with the Severity of Ischemic Stroke? A Dose Response Meta-Analysis. *Journal of Clinical Medicine*, *11*(12), 3262. https://doi.org/10.3390/JCM11123262/S1

Soetedjo, N. M., Iryaningrum, M. R., Damara, F. A., Permadhi, I., Sutanto, L. B., Hartono, H., & Rasyid, H. (2021). *Prognostic properties of hypoalbuminemia in COVID-19 patients: A systematic review and diagnostic meta-analysis*. https://doi.org/10.1016/j.clnesp.2021.07.003

Spearman, C. W., Aghemo, A., Valenti, L., & Sonderup, M. W. (2021). COVID-19 and the liver: A 2021 update. *Liver International*, *41*(9), 1988–1998. https://doi.org/10.1111/LIV.14984

Starke, R. R. (2021). The isolated effect of age on the risk of COVID-19 severe outcomes: a systematic review with meta-analysis. *BMJ Global Health*, *6*, 6434. https://doi.org/10.1136/bmjgh-2021-006434

Sun, J., He, W.-T., Wang, L., Lai, A., Ji, X., Zhai, X., Li, G., Suchard, M. A., Tian, J., Zhou, J., Veit, M., & Su, S. (2020). COVID-19: Epidemiology, Evolution, and Cross-Disciplinary Perspectives. *Trends in Molecular Medicine*, *26*(5), 483. https://doi.org/10.1016/J.MOLMED.2020.02.008

Valášková, P., & Muchová, L. (2016). Metabolism of bilirubin and its biological properties. *Klinicka Biochemie a Metabolismus*, *24*(4), 198–202.

Velavan, T. P., & Meyer, C. G. (2020). Mild versus severe COVID-19: Laboratory markers. *International Journal of Infectious Diseases*, *95*, 304–307. https://doi.org/10.1016/j.ijid.2020.04.061

Viana-Llamas, M. C., Arroyo-Espliguero, R., Silva-Obregón, J. A., Uribe-Heredia, G., Núñez-Gil, I., García-Magallón, B., Torán-Martínez, C. G., Castillo-Sandoval, A., Díaz-Caraballo, E., Rodríguez-Guinea, I., & Domínguez-López, J. (2021). Hypoalbuminemia on admission in COVID-19 infection: An early predictor of mortality and adverse events. A retrospective observational study. *Medicina Clinica*, *156*(9), 428–436. https://doi.org/10.1016/j.medcli.2020.12.018

Violetis, O. A., Chasouraki, A. M., Giannou, A. M., & Baraboutis, I. G. (2020). COVID-19 Infection and Haematological Involvement: a Review of Epidemiology, Pathophysiology and Prognosis of Full Blood Count Findings. *Sn Comprehensive Clinical Medicine*, *2*(8), 1089. https://doi.org/10.1007/S42399-020-00380-3

Waleed, M. S., Sadiq, W., & Azmat, M. (2020). Understanding the Mosaic of COVID-19: A Review of the Ongoing Crisis. *Cureus*, *12*(3). https://doi.org/10.7759/CUREUS.7366

Wang, H. L., Chu, C. H., Tsai, S. J., & Yang, R. J. (2016). Aspartate aminotransferase and alanine aminotransferase detection on paper-based analytical devices with inkjet printer-sprayed reagents. *Micromachines*, *7*(1), 1–10. https://doi.org/10.3390/mi7010009

Wu, J., Song, S., Cao, H.-C., & Li, L.-J. (2020). Liver diseases in COVID-19: Etiology, treatment and prognosis. *World Journal of Gastroenterology*, *26*(19), 2286.

https://doi.org/10.3748/WJG.V26.I19.2286

Xu, Y., Yang, H., Wang, J., Li, X., Xue, C., Niu, C., & Liao, P. (2021). Serum albumin levels are a predictor of covid-19 patient prognosis: Evidence from a single cohort in chongqing, china. *International Journal of General Medicine*, *14*, 2785–2797. https://doi.org/10.2147/IJGM.S312521

Y, Z., Z, Z., Y, W., Y, Z., Y, M., & W, Z. (2020). Single-Cell RNA Expression Profiling of ACE2, the Receptor of SARS-CoV-2. *American Journal of Respiratory and Critical Care Medicine*, *202*(5), 756–759. https://doi.org/10.1164/RCCM.202001-0179LE

Zhao, X., Lei, Z., Gao, F., Xie, Q., Jang, K., & Gong, J. (2021). The impact of coronavirus disease 2019 (COVID-19) on liver injury in China: A systematic review and meta-analysis. *Medicine*, *100*(4). https://doi.org/10.1097/MD.0000000000024369

APPENDICES

TITLE: ASSOCIATION OF LIVER BIOCHEMICAL PROFILE AND CLINICAL OUTCOME OF COVID-19 INFECTION IN PATIENTS ADMITTED AT MOUNT KENYA HOSPITAL NYERI, KENYA

Appendix 1: Data Collection Form

AGE	
GENDER	☐Male ☐Female
DURATION OF SYMPTOMS	
CLINICAL OUTCOME:	☐Survived ☐Died
DATE OF DISCHARGE	
DATE OF DEATH	
DURATION OF HOSPITAL STAY	

BMI(KG/M2)	BP(MMHG)	PR (B/M)
RR(B/M)	SPO2 (%)	TEMPS(.C)

OBTUNDATION (GCS)	
SIGNS OF SHOCK	☐Hypotension ☐Tachypnea ☐Tachycardia ☐GCS
NEED FOR ICU ADMISSION	☐Yes ☐No

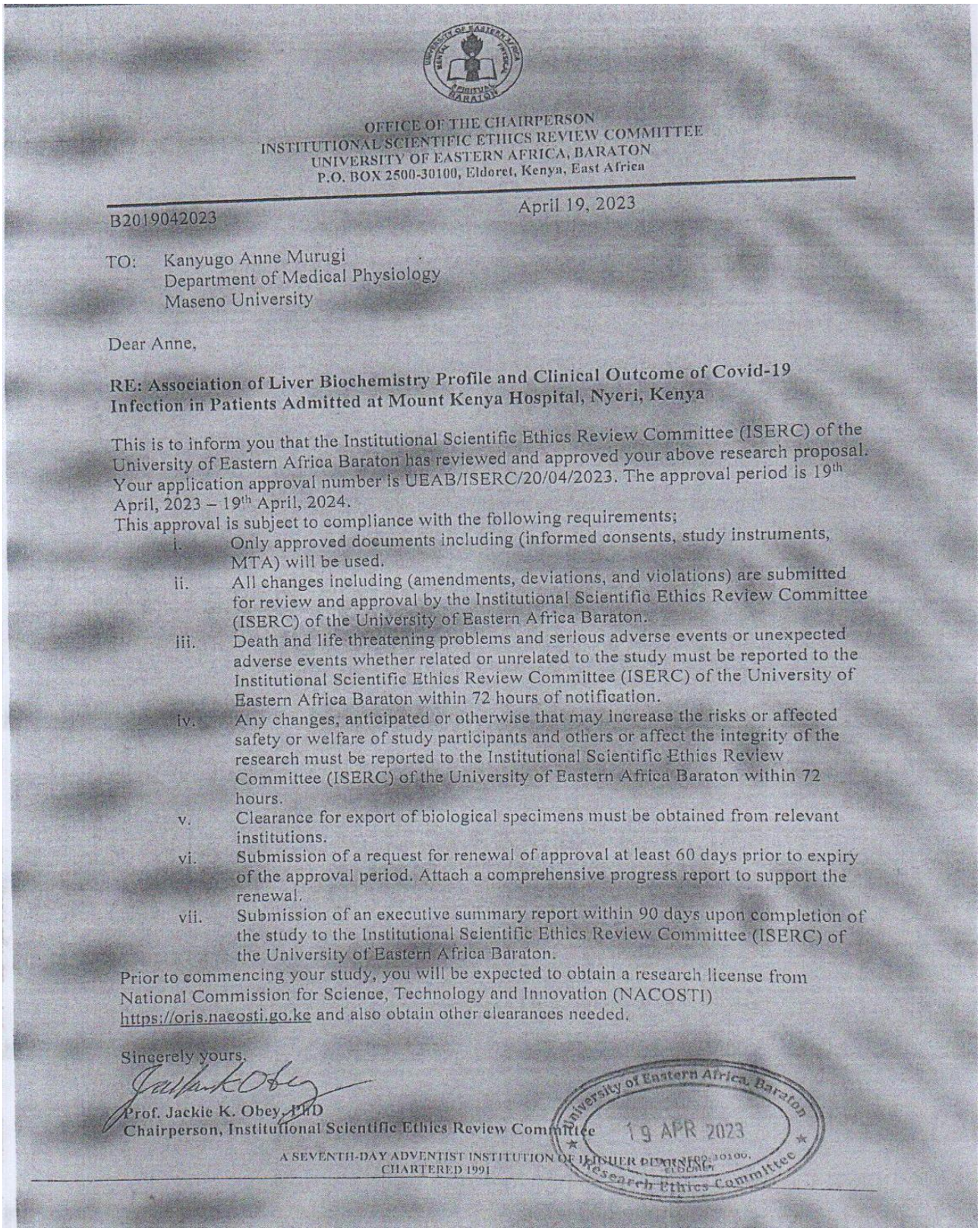
Appendix 2: Liver Biochemical Profile

PARAMETER	VALUE	LOW	NORMAL	ELEVATED		
				MILD 1-2xULN	MODERATE 2-5 ULN	SEVERE >5 ULN
AST (0-37 U/L)						
ALT(0-42 U/L)						
ALP (40-150 U/L)						
GGT (8-46U/L)						
TBIL (0-22 UMOL/L)						
DBIL (0-6.8 UMOL/L)						
TP (66-87 G/L)						
ALB (37-57 G/L)						

Appendix 3: Map of Kenya

Appendix 4: Map of Nyeri County

Appendix 5: Ethical Approval



OFFICE OF THE CHAIRPERSON
INSTITUTIONAL SCIENTIFIC ETHICS REVIEW COMMITTEE
UNIVERSITY OF EASTERN AFRICA, BARATON
P.O. BOX 2500-30100, Eldoret, Kenya, East Africa

B2019042023

April 19, 2023

TO: Kanyugo Anne Murugi
Department of Medical Physiology
Maseno University

Dear Anne,

RE: Association of Liver Biochemistry Profile and Clinical Outcome of Covid-19 Infection in Patients Admitted at Mount Kenya Hospital, Nyeri, Kenya

This is to inform you that the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton has reviewed and approved your above research proposal. Your application approval number is UEAB/ISERC/20/04/2023. The approval period is 19th April, 2023 – 19th April, 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 the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton.
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 the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton 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 the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton 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 the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton.

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

Sincerely yours,

Prof. Jackie K. Obey, PhD
Chairperson, Institutional Scientific Ethics Review Committee

University of Eastern Africa, Baraton
19 APR 2023
Research Ethics Committee

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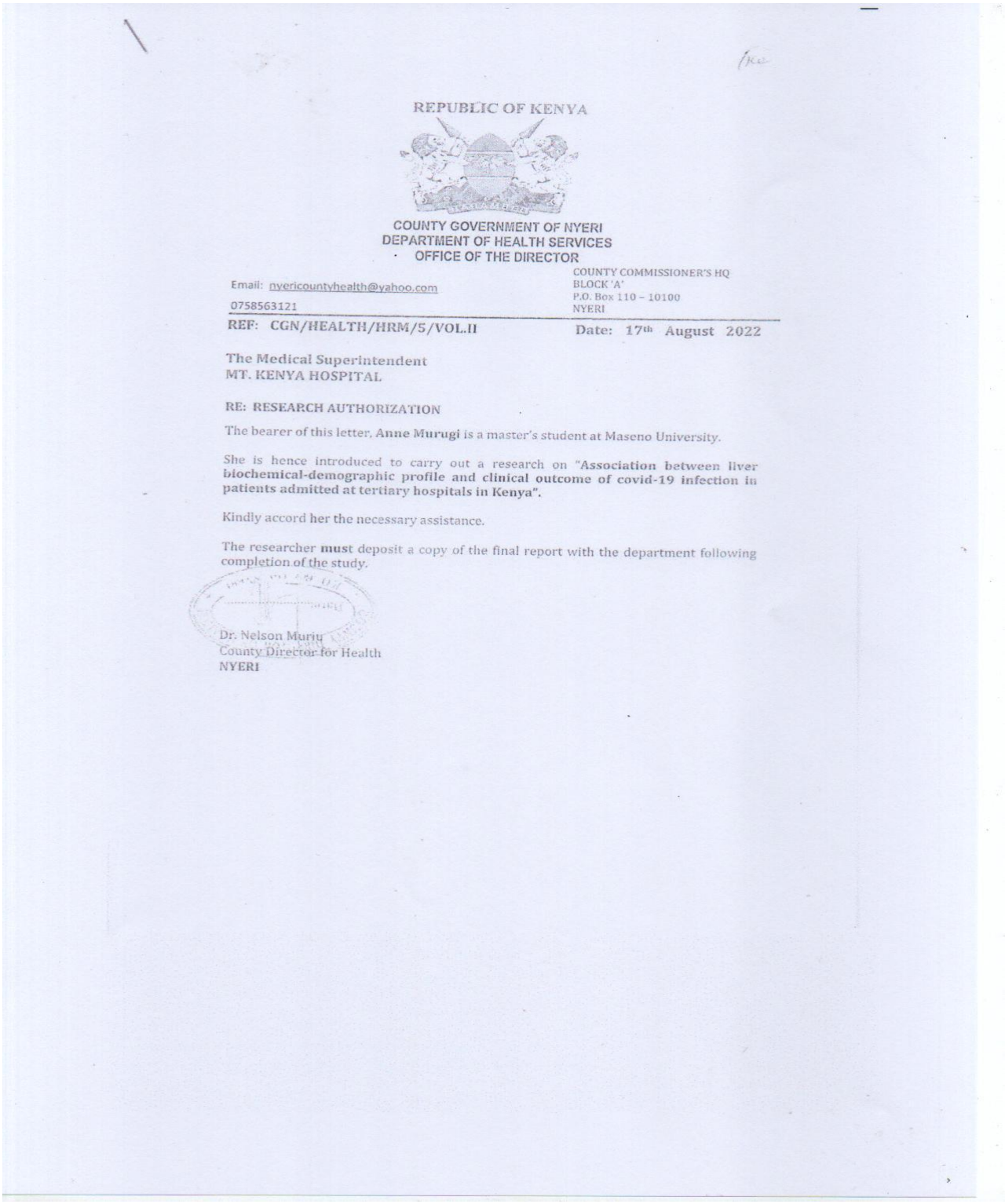
Appendix 6: Nyeri County Authorization Letter



REPUBLIC OF KENYA

COUNTY GOVERNMENT OF NYERI
DEPARTMENT OF HEALTH SERVICES
OFFICE OF THE DIRECTOR

Email: nyericountyhealth@yahoo.com
0758563121

COUNTY COMMISSIONER'S HQ
BLOCK 'A'
P.O. Box 110 – 10100
NYERI

REF: CGN/HEALTH/HRM/5/VOL.II Date: 17th August 2022

The Medical Superintendent
MT. KENYA HOSPITAL

RE: RESEARCH AUTHORIZATION

The bearer of this letter, **Anne Murugi** is a master's student at Maseno University.

She is hence introduced to carry out a research on **"Association between liver biochemical-demographic profile and clinical outcome of covid-19 infection in patients admitted at tertiary hospitals in Kenya".**

Kindly accord her the necessary assistance.

The researcher **must** deposit a copy of the final report with the department following completion of the study.

Dr. Nelson Muriu
County Director for Health
NYERI

Appendix 7: Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **179456**

Date of Issue: **01/May/2023**

RESEARCH LICENSE

This is to Certify that Ms.. Anne Murugi Kanyugo of Maseno University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Nyeri on the topic: ASSOCIATION OF LIVER BIOCHEMICAL PROFILE AND CLINICAL OUTCOME OF COVID-19 INFECTION IN PATIENTS ADMITTED AT MOUNT KENYA HOSPITAL NYERI, KENYA for the period ending : 01/May/2024.

License No: **NACOSTI/P/23/25707**

179456

Applicant Identification Number

Director General
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