HEPATOCELLULAR INJURY AND METABOLIC DYSREGULATION OF SELECTED BIOMARKERS IN HIV-1-HCV CO-INFECTED INDIVIDUALS USING HEROIN IN MOMBASA COUNTY KENYA

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A thesis submitted to the School of Public Health Biomedical Science and Technology in partial fulfilment for the requirements of the award of the Master of Science degree in Medical Laboratory Sciences (clinical chemistry) of Masinde Muliro University of Science and Technology.

AUGUST, 2024

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DEDICATION

This study is dedicated to my wife Anne Miraho, my mother Daisy Kayeli, my children, my sister, brothers and my late father.

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Abstract

Background and study aim: Hepatocellular injury and metabolic dysregulation are significant complications in individuals co-infected with human immune deficiency virus (HIV-1) and hepatitis C virus (HCV), particularly those who use injection heroin. However, this is less recognized. The combined effect of viral pathogenesis, drug intoxication, compromised bone mineralization and systemic inflammation contribute to accelerated liver damage and metabolic abnormalities including osteoporosis, but effective biomarker –based tools for early detection and monitoring remain limited. The current study aimed to characterize and quantify selected biomarkers of hepatocellular injury and metabolic dysregulation in HIV-1 and HCV co-infected injection heroin users, with the intent of developing a laboratory based diagnostic and monitoring algorithm for early detection and disease progression assessment.

Materials and Methods: This case control retrospective study was conducted targeting injection heroin users stratified into HIV-1 and HCV co-infected, mono infected and un-infected groups. A total of 289 samples from persons aged between18 to 65 years were analyzed for liver enzymes (ALP), metabolic indicators (Lipid profile, Calcium and Vitamin D_3), and virological parameters (HIV RNA, HCV RNA and CD4 count). Statistical analyses included group comparisons and correlation modeling to evaluate biomarker association with disease severity.

Results: The co-infected group exhibited significantly elevated liver enzymes compared to mono infected and uninfected participants ($P < 0.05$). Strong correlations were observed between viral load, biomarker alterations and duration of heroin use. The combined biomarker profile demonstrated potential to distinguish early and advanced hepatic and metabolic impairment.

Conclusion: HIV-1 and HCV co-infection in injecting heroin persons is associated with compounded liver injury and metabolic dysregulation that can be effectively assessed through selected biomarkers. These findings support the development of a biomarker –based diagnostic and monitoring algorithm to facilitate early detection and guide targeted clinical interventions in this high-risk population to avert a rise in mortality rate.

Key words: HIV-1-HCV co-infection, Injection heroin use, Hepatocellular injury, metabolic dysregulation, Biomarkers, Liver function, Diagnostic algorithm, Lipid profile.

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ABBREVIATIONS AND ACRONYMS

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

ALP: Alkaline phosphatase

AIDS: Acquired Immune Deficiency Syndrome

CVD: Cardiovascular disease

CD4: Cluster of differentiation 4

EDTA: Ethylene Diamine Tetra acetic Acid

Facs: Florescence activated cell sorter

GB: Giga bytes

HCV: Hepatitis C virus

HIV-1: Human immunodeficiency virus type one.

IFN-β: Interferon beta

IR: Insulin resistance

KENPHIA: Kenya Population Based HIV Impact Assessment

LDL-C: Low density lipoprotein cholesterol

NAFLD : Nonalcoholic fatty liver disease

NACADA: National Commission against Drug Abuse

NACC : National Aids Control Council

NASCOP: National Aids and Sexually transmitted disease Control pro gram.

PAMP: Pathogen associated molecular pattern

PWID: People who inject drugs

PWIH: People who inject heroin

PT: Prothrombin time

RNA: Ribonucleic acid

RAM: Random Access Memory

ROM: Read Only Memory

SGPT: Serum glutamic pyruvic transaminase

Tg: Triglycerides

UPR: Unfolded protein response

USA: United States of America

UNAIDS: Joint United Nations Program on HIV/AIDS

UNODC: United Nations Office on Drug and Crime

Vit.D_3: Vitamin D_3

WHO: World health organization

OPERATIONALIZATION OF TERMS

Study population: Those who inject illicit drugs and are HIV- HCV infected in Mombasa County and those who inject drugs and are HIV-HCV

Sample size: The total number of injection and non -injection substance users that will be examined during the study.

Key populations: Those at risk of contracting HIV and HCV through injection drug use in Mombasa County.

Injection drug users: Individuals with needle scars in there ante cubital region of their hand and reporting to have used illicit injection substances even once, as per the united nation's office on drug and crime (UNODC) report.

Non Injection Drug Users: Individuals who have never injected drugs but have at least used one non injection listed in the UNODC registry.

People Who Inject Drugs: Persons who use injectable heroin

People who inject heroin: Persons who use injectable heroin

Underlying pathology: Other comorbidities/complications that arise as a result of HIV and HCV infection.

Support therapy: Nutritional and psychosocial counselling

Algorithm: Documented procedure for carrying out a test

ART naïve HIV-1: HIV positive persons who are not on treatment

LP-(a): Lipoprotein (a)

CHAPTER ONE
INTRODUCTION

1.0 Overview

This chapter discusses the background of the study, the statement of the problem, purpose of the study, objectives of the study and the research questions. The researcher also discussed the significance and justification for the study, the scope, the limitations, and the theoretical framework of the study.

1.1 Background

Chronic hepatitis C infection and Acquired Immunodeficiency Syndrome together are immunosuppressive diseases caused by hepatitis C virus (HCV) and human immune virus (HIV) respectively (Artenie A, 2025). This is complicated by the use of illicit psychoactive substances, which is of increased public health concern globally (WHO, 2022). Worldwide, approximately 11.2 million people inject drugs. Out of this number, approximately 1.4 million were diagnosed with HIV-1, half of them were living with hepatitis C virus and 1.2 million were living with both (UNODC, 2021). On the other hand, the use of heroin in Africa is gradually rising amongst persons of 18 years and above, mostly in informal settlements of urban centers (Sema Baltazaret et al,, 2020).This paints a similar picture in East Africa, with Kenya registering a rise in cases of drug use, having approximately 37.1 % of subjects confessing to have used an injectable illicit drug in their life time (NACADA, 2022). However, majority of illicit substance users' advance from taking non-injectable to injectable drug use (Mburu G et al, 2019), with injectable and non-injectable illicit drug

amounting to worldwide burden of injection drug use and Africa carrying a substantial number of the affected persons (Wantuck et al, 2019). The wide spread of injectable illicit drug use within Africa is as a result of increased availability of illicit injectable drugs with heroin being key, in addition to marijuana and cocaine in informal settlements of the urban centers (Singh et al, 2022); (Monroe-Wise et al, 2024). Just like cases in other African countries, in Kenya, wide spread use of injectable heroin began during the rise in tourism on the Kenyan coast in 1980s, and has slowly infiltrated the inland across the country (Mburu G et al, 2019). In response to high probability of blood borne pathogenic organisms among people who inject heroin (PWIH), the government of Kenya started a needle and syringe program in 2013 and a program for methadone maintenance therapy in 2014 as a calculated strategy to reach out to the county's most vulnerable populations (Kiburi S K et al, 2022). However, poverty and lack of employment are key factors determining the health status of illicit drug users (Budambula et al, 2024). This passively influence personal characteristics of injectable drug users like sharing needles and negotiating for drug exchange with illicit sex or as a mode of evading police arrest (Sema Baltazar C et al, 2024).

Human immunodeficiency virus has remained a major public health challenge globally, due to psychoactive substance use which increases the risk of HIV-1 infection in regard to poor mental judgement ability (Kiburi S K et al, 2022); (Budambula et al, 2024). Approximately 40% of new cases of HIV-1 infections globally are as a result of illicit injectable drug use (UNAIDS, 2023).

At the end of 2015, it was estimated that 36.7 million people were having the human immunodeficiency virus and acquired immunodeficiency syndrome in the world, while at the same period a good number of people injected drugs out of whom approximately 1.7 million had HIV-1. About 70% of this sub population inhabit sub- Saharan Africa (UNAIDS, 2021); (WHO, 2022). Studies carried out in Texas, USA and China documented that injection drug users are more prone to HIV-1 infection as compared to non-injectable illicit drug users (Mahmud et al, 2020) (Global HIV/AIDS response, 2021). In Kenya, approximately 1.5 million people who included 18,327 persons who inject illicit drugs lived with HIV-1 as at the end of 2020. Currently the estimated wide spread of HIV-1 in injectable illicit drug users is at 18.3%, which is projected to be much elevated than in the general population (NACC, 2020). It is eluded that in Kenya, the predictive indicators of HIV-1 in the general public are poverty and unemployment. Mombasa County in Kenya has a prevalence rate of 33% for injectable drug users who are HIV-1 infected (Mburu *et al*, 2019), hence this has led to the elevated cases of HIV-1 and HCV infections (NACADA, 2022).

Opioids are associated with persistent immune stimulation, hence inducing chronic inflammation (Obare et, 2024) and with this, HIV-1 infected people who inject drugs can progressively develop marked inflammation of the hepatocytes, which can be that can be assessed through determination of hepatic functionality. Injectable illicit drug use also causes chronic liver deterioration and severe derangement in hepatic synthetic capabilities (Anna et al, 2020);(Gardner A R et al, 2023). A rise in ALT, GGT and

ALP levels are associated with injection drug use in addicted individuals (Marin RC et al, 2025). While long term use of heroin causes reduction in albumin synthesis in the liver (Eckart et al, 2020), the two events indicate increase in inflammatory mediated hepatic derangement in injection substance users.

Hepatitis C viral infection is a worldwide health problem affecting approximately 71 million individuals (Grebely et al, 2019); (Chemaitell et al, 2019); (WHO, 2022). Chronic HCV disease is noted to be the leading cause of liver fibrosis, liver cirrhosis and hepatic cells carcinoma with progressive liver failure, causing 500 000 preventable death yearly (El-Khoury et al, 2024), what derails health care systems (Mahmud et al, 2020). In 2015 alone, HCV disease caused 21% of all liver cancer deaths worldwide (Akinyemiju et al, 2017); (Forner A et al, 2018). People using injectable illicit drugs are key to HCV elimination efforts, because they constitute the group with the highest HCV spread across many countries and often associated with risk factors for HCV transmission (Khoury, 2024). Widespread anti-HCV antibodies vary among PWIH, with an approximation of 52% globally amounting to an equivalent of 8.2 million PWID who give positive results with anti HCV sera.

Sub-Saharan Africa is inhabited by a large number of people who live with HCV (Akiyama et al, 2019), although little information is in public domain about PWIH and the degree of prevalence of HCV among them in the entire region. People who inject drugs in the lower part of Africa are among those populations with the highest risk index for HCV infection and HIV-

1 infection, and comprise a substantial proportion of HCV and HIV-1 recorded transmissions in the region (Sonderup et al, 2017). Majority of HCV infected persons are noted to suffer from extra-hepatic symptoms associated with fatigue, joint disorders, depression, insulin resistance, hyperglycemia, nephropathy and lymphoproliferative disorders which cause about 15% of HCV patients to be hospitalized per year (Mazzaro C et al, 2021) (Redwan E M et al, 2022); (Cartwright et al, 2024), hence the need to closely monitor defined chemistry parameters in this patients.

Estimate of anti-HCV antibody dominance among PWIH in sub-Saharan Africa are limited in both quantity and quality, and the available data suggest that this prevalence is similar to that in other settings worldwide (Boateng et al, 2019); (Sema Baltazaret et al,, 2020). In Kenya, reports indicate that between 22% and 70% of PWIH are positive of HCV (Akiyama et al, 2019).

Globally, it is indicated that four to five million HIV-1 infected individuals have episodes of chronic hepatitis C virus infection (WHO, 2022), becoming an outstanding cause of death in HIV infected individuals mainly in economically stable countries (Singh et al, 2022); (Khoury, 2024). Monitoring of HCV-HIV-1 co-infection is paramount among PWIH because HIV infection can lead to reduction in spontaneous HCV clearance, higher HCV viral load and increased rate of HCV disease progression (Vergara- Samur H et al, 2023); (Gardner A R et al, 2023), hence complicating the pathogenicity of the two conditions, calling for the need to evaluate lipid and liver enzymes closely. In the resource limited settings, like in Kenya, data on widespread of HCV infection in

HIV-1 infected individuals (18% to 32%), and its effect on long term clinical successes are limited (Akiyama et al, 2019); (Boateng et al, 2019).

As customarily viral load analysis is used in monitoring disease prognosis and treatment in HCV and HIV-1 infections (NASCOP, 2021), some biomarkers are not to some extend specific to HCV and HIV-1 infection as a result of injection drug use. There is also scarce documented evidence in regard to clinical chemistry biomarkers in injection heroin users in Mombasa County, Kenya. Looking at this, there is need to closely monitor liver and lipids biomarkers in people who inject drugs to boost their clinical management. The evaluation of structural hepatic biomarkers, functional hepatic biomarkers, lipid biosynthesis, calcium and 25-hydroxyvitamin D_3 gave a clear picture of the prognosis of HIV-1 and HCV co-infection in people who inject drugs that helped to structure a proper regimen for disease management, without the use of the most expensive viral load monitoring and liver biopsies to assess the extent of liver damage.

This study notably looked at the clinical chemistry laboratory biomarkers in HIV-1- HCV co-infected people who use injectable heroin and HIV-1-HCV negative people who use injectable heroin, in order to map up an alternative disease diagnosis, treatment and prognosis monitoring algorithm in addition to the routine viral load testing.

1.2 Problem statement

There is an increase in prevalence of injecting drug use in Mombasa and other Kenya's Indian Ocean coastal towns. This is because of the strategic location of the county as part of the Indian Ocean coast of Kenya, as a major seaport and tourist attraction and abuse drug smuggling transit point,

as well as commercial center. Its location on the Indian Ocean coast favors easy access to abuse substances because of their easy access from the Far East, which is famous as a source for the substances of abuse. Persons who are infected concurrently with both HIV-1-HCV and who inject illicit drugs have been noted to develop various conditions such as liver disease from hepatocyte injury, which leads to disturbance of lipid metabolism, leading to cardiovascular complications (Matsumori et al, 2022). Further, some of the people suffer disturbance of calcium metabolism leading to bone diseases, from resultant abnormalities in the body's calcium and vitamin D_3 homeostasis. However, treatment and monitoring of prognosis of HIV-1-HCV concurrent infection has not included the use of clinical chemistry of lipids, liver function, calcium and Vit. D_3 biomarkers owing to lack of sufficient data on these clinical chemistry parameters, hence the need for less -invasive procedures to help determine disease severity.

Many families at the coastal region are affected as a result of increase in numbers of young population of persons who use injectable illicit drugs and are co infected with HIV-1 and HCV, making them non-productive economically in terms of income generation and expenses for clinical management of the conditions (Mburu G et al, 2019).This calls for proper measures to be instituted to control these conditions. The rate at which young people at the coast and other parts of Kenya are becoming mentally derailed due to illicit injectable drug use is on the rise, a vice that need timely intervention. The number of young people who drop out of school along the coastal line due to increased use of injectable illicit drugs is high, majority of whom get infected with HIV-1 and HCV leading to increase in

prevalence of these two conditions (Fraser H et al, 2021). There is no structured algorithm for management and prognosis monitoring of HIV-1-HCV concurrent infection in persons who use injectable illicit drugs, a gap this study has provided information that will address.

1.3 Objectives

1.3.1 Broad objective

To evaluate hepatocellular injury and metabolic dysregulation of selected biomarkers in HIV-1-HCV co-infected injecting heroin users.

1.3.2 Specific objectives

i. To determine the influence of demographic characteristics on injection heroin use.
ii. To determine changes in the concentration of liver function biomarkers in injection heroin users
iii. To measure serum concentration of metabolic biomarkers in persons who use injectable heroin and have HIV and HCV infection.
iv. To establish the correlation of liver biomarkers with clinical outcomes in injection heroin users.

1.4 Research questions

i) What is the association between age, gender and injection heroin use?
ii) Are the serum concentration of liver function biomarkers affected by injection heroin use?
iii) Are serum levels of metabolic biomarkers altered in injection heroin use and HIV/HCV co-infection?
iv) How are liver function biomarkers correlated with BMI, CD4 and HIV infection?

1.5 Justifications of the study

Hepatitis C virus is known to be the outstanding cause of progressive liver inflammation with fibrosis, leading to liver cirrhosis, hepatocarsinoma, liver dysfunction and death (Forner A et al, 2018). Studies indicate that individuals with chronic hepatitis C infection also show increased degree of mortality from circulatory system disease and hepatic failure (Mazzaro C et al, 2021). This calls for the need to determine the serum levels of vitamin D3 in HIV-1-HCV co-infected injection heroin users as stipulated in specific objective (i) and to measure the serum levels of calcium, triglycerides, low density lipoprotein together with high density lipoprotein in HIV-1-HCV co-infected injection heroin users as indicated in specific objective (ii).Derangement of metabolic processes in the liver also called for the establishment of serum albumin and alkaline phosphatase levels as stipulated in specific objective (iii) together with the association of vitamin D3,calcium, anthropometric alongside sociodemographic parameters in HIV-1-HCV co-infected injection heroin users (Younossi et al, 2020).

A high degree of clinical suspicion coupled with early recognition, timely establishment of the diagnosis, clearly targeted treatment with proper management of the underlying pathology are important in the overall management of persons suffering from HIV-1 associated complications of bone tissue and mineral metabolism (Charoenngam et al, 2020). This calls for the evaluation of serum ionized calcium and 25-hydroxyvitamin D_3 levels in these patients.

Notably, there is limited data regarding the impact of HIV-1- HCV concurrent infection in persons who use injectable illicit drugs and their effect on lipid, liver enzymes, calcium and vitamin D_3 metabolism (Reid *et al*,

2020). This shows the need for development of a well -structured algorithm that will guide in diagnosis and monitoring of the prognosis of this condition.

Data on HIV-1-HCV concurrent infection among PWIH in Kenya are limited although its widespread is remarkably 18 - 32% (Akiyama et al, 2019); (Boateng et al, 2019) which contrasts global prevalence of 82%. Previous studies indicate that HIV-1 speeds up the progression of liver damage due to HCV infection (Gardner A R et al, 2023), yet data on the widespread of HCV infection in HIV-1 seropositive population and its effect on long term clinical outcome are limited (Boateng et al, 2019), hence, this study will help to set up timely diagnostic intervention to avert death.

1.6 Significance of the study

A substantial number of injection drug users with HIV-1-HCV co infection die while undergoing clinical management, owing to lack of a proper algorithm for testing and monitoring. Presently, there is no clear clinical chemistry data that is to be used to guide the management of HIV-1-HCV concurrent infection in PWIH, hence this study has come up with clear evaluation of the clinical chemistry informed data on serum level of ionized calcium and 25-hydroxyvitamin D_3 which will be used to follow up on prognosis of this condition.

There is a rise in cases of liver cirrhosis, most of which advance to hepatic carcinoma as a result of a delay in instituting proper diagnosis. Death rate amongst persons who use injectable illicit drugs co infected with HIV-1 and HCV as a result of untimely clinical intervention has been on the rise

(Abdi M et al, 2023). This is owed to lack of structured algorithm for clinical management. Advances in critical analysis of all actors of calcium homeostasis is crucial in treatment and prevention of hypercalcemia. This brings into consideration the shift from a support therapy, which has not been effective to specific and targeted therapy in patients who have chronic hypercalcemia (Vergara- Samur H et al, 2023). This will help to reduce the mortality rate in people who use injectable illicit drugs with HIV-1-HCV co infection. Access to proper treatment and prevention services for stigmatized PWIH with HIV-1-HCV concurrent infection has been insufficient due to lack of sufficient clinical chemistry data (Sema Baltazar C et al, 2024). Treating HIV-1-HCV co-infection concurrently can lead to drug-to-drug interactions and augment their toxicity.

Therefore, the findings documented in this study have informed the structuring of an algorithm that will improve the management and prognosis of HIV-1-HCV concurrent infection in PWIH. This will provide a reference for clinical management of metabolic derangement related to lipid, calcium and 25-hydroxyvitamin D_3 metabolism. Currently there has been no algorithm that addresses this. The proposed algorithm will be shared to the Ministry of Health, NASCOP and NACADA for verification, adaption and in-cooperation into the national disease management protocals.

1.7 Scope of the study

This case control retrospective study was conducted as part of wider studies on HIV-1 - HCV co-infection amongst PWIH at Bomu community hospital in Mombasa County. The study was carried out from 3rdAugust 2023 to 31stJuly 2024, through which data was collected, analyzed and report written. Data was collected for serum lipid profile, liver function profile,

ionized calcium, 25-hydroxyvitamin D_3, anthropometric parameters, HIV and HCV status for study participants. The study involved HIV-1- HCV co-infected people who inject heroin, and who voluntarily sought or were brought in for rehabilitation services or medication for other comorbidities. The findings of this study is a replica of what is happening in other areas of Kenya and, therefore, similar studies will be carried out for better understanding of illicit drug use and HIV-1/HCV co-infection.

1.8 Limitations

The population for HIV-1 that was used in this study were ARV naïve patients, but the HIV-1 seropositive individuals who failed to disclose personal ARV status were considered to be ARV naïve and hence gave outlier results. Due to stigma associated with and criminalization of people who inject drugs, there was valid data missed out during investigation, that otherwise may have positively contributed to the research work. Some participants enrolled for the study failed to give enough sample for analysis which accounted for missed results for some analytes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview

The prevalence of HCV and HIV-1 has been seen to escalate globally due to injection heroin use. Sub-Sahara Africa is one of the regions that has been heavily affected. Many countries especially those on the African continent are far from realizing the WHO target of having a world free from HCV by the year 2030. Kenya for example faces challenges to realize this goal due to limited resources. This chapter, therefore, addresses the contribution of other scholars on clinical, diagnostic, anthropometric and demographic factors that need to be monitored to help Kenya realize a country free from HCV, HIV and injection drug use as stipulated in the vision 2030.

2.1.1 Hepatitis C Virus infection

Hepatitis C viral particle (HCV) is seen as a small single-stranded positive-sense, membrane-enveloped RNA viral particle that is noted to cause chronic liver disease. There are seven documented major genotypes of HCV, which are known as genotypes one to seven. The genotypes are put into several subtypes with the number of subtypes corresponding to the genotype (WHO, 2022). Looking at the United States of America, an estimated 70% of cases were seen to be caused by genotype 1 and 20% associated with genotype 2. Genotype 1 is also the most common in South America and Europe (Elkassas et al, 2019). In Africa, a resembling picture is replicated which also reflect the situation in East Africa. The situation in Kenya is not different (Hwang J P et al, 2019). The half-life of the viral

particles in the serum is noted to be around 3 hours and may reduce to 45 minutes. In an infected individual, it is estimated that 10^{12} viral particles are produced each day. It has been documented that apart from replicating in the liver the viral genome can also multiply in lymphocytes (Pierantoneli et al, 2019); (Smirnovet, 2023); (Nazeer B et al, 2024).

Autophagy, a key process for breaking down cytoplasmic components, has been shown to modulate interactions between RNA viruses and their host cells. However, this mechanism to oversee the functional role of autophagy in the viral life cycle is still under investigation ((Nazeer B et al, 2024).The HCV brings about the unfolded protein response (UPR), which in turn makes the autophagy pathway active, hence promoting HCV RNA replication in human hepatoma cells ((Matsumori, 2022). Further investigation reveals that the autophagy process running up to completion of autolysosome maturation is required to enhance HCV RNA replication and does this by innate antiviral immunity suppression ((Smirnovet, 2023). This is achieved by silencing the target Gene or activation of unfolded protein response autophagy pathway in regard to activation of the IFN-β mediated by an HCV-derived pathogen-associated molecular pattern (PAMP) ((Matsumori et al, 2022); (Smirnovet, 2023); (Nazeer B et al, 2024).

Hepatitis C virus infection is shown to represent an outstanding public health problem across the world and suppression of HCV morbidity and mortality is currently bringing up challenges owing to several viral factors and host factors. Virus molecular evolution plays a key role in HCV transmission, progression of the active disease and clinical management outcome in regard to its ability to alter biochemical markers in the liver, the

kidney with the involvement of the cardiovascular system ((Younossi et al, 2020).

Hepatitis C virus infection molecular evolution is guided by various mechanisms which include a high mutation rate, genetic changes, recombination coupled with temporal variations and compartmentalization (Wantuck et al, 2019); (Nazeer B et al, 2024). These evolutionary processes continuously rearrange the composition of the HCV intra host population in a scaffolding manner (Hwang J P et al, 2019). Remarkable advances in comprehending the molecular mechanism which controls HCV replication has facilitated the development of huge amounts of direct-acting antiviral agents against HCV and in correction of the biochemical markers associated with its pathological characteristics (Gitto et al, 2017); (Mervem et al, 2018); (Gardner A R et al, 2023); (Marin RC et al, 2025). As a result, high grade sustained viral responses have been attained. The fast evolving field of anti-HCV therapy is expected to broaden its landscape with more potent antivirals ((Gitto et al, 2017), drawing people one step closer to the interferon-free era.

Continued sharing of drug injection items by PWIH is an outstanding cause of disease burden and a key contributor to blood-borne viral particle transmission ((Redwan E M et al, 2022). Looking at the worldwide multistage systematic review to determine the pathological effect of liver malfunction in regard to the widespread of injection illicit drug use in persons aged 18 years and above; socio demographic features of and risk factors for people who inject illicit drugs (PWID), and the widespread of HIV-1 and HCV among PWIH is paramount (Cartwright et al, 2024).

2.1.2 Human Immune Virus-1 infection

HIV-1 is known to cause Acquired Immune Deficiency Syndrome (AIDS). The virus in the human body replicates, increase in number with weakening of the immune system. This causes the body to become more susceptible to a wide range of infections. HIV-1 has a lower degree of pathogenicity, hence called a lent virus (Hessou et al, 2019). Currently only HIV-1 and HIV-2 have been identified, noted to be transmitted through similar means and result in clinically similar condition. HIV-2 has a longer window and incubation period. HIV-1 is markedly variable, comprising several varying strains that are put into two groups i.e. group M with approximately 10 subtypes of viruses in which most recognized subtypes are A to J (UNAIDS, 2021). Group 0 is a common category that includes a variety of relatively distinct viruses.

Major difference in groups is in their genetic composition, with sub types prone to different modes of transmission, a factor that needs further investigation. Somebody can become infected by way of transfusion of contaminated blood and blood products, or by sharing needles used for general injection and administering injectable illicit drugs (WHO, 2021).

2.1.3 HIV-1-HCV co-infection

Hepatitis C viral particles (HCV) and Human immune deficiency viral particles type one (HIV-1) have come out as major viral infections as documented through the past two decades. Their co infection rises complications with a significant impact in regard to morbidity and mortality linked to liver abnormalities, cardiovascular complications with renal dysfunction (Forner A et al, 2018) ;(Wantuck et al, 2019); (Nazeer et al, 2023). In

regard to the past decades, it has been indicated that HIV-1-HCV co-infected persons fail to respond well to HCV therapy, complicating the pathology associated with liver cirrhosis, with marked liver fibrosis (Anna et al, 2020). Even as timely treatment interventions have extended the survival period for HIV-1 infected individuals, liver dysfunction has become the leading cause of non-AIDS related untimely deaths in this group (WHO, 2023). The World Health Assembly adopted the first "Global Health Sector Strategy on Viral Hepatitis, 2016-2021" in the year 2016, which pointed out the important role of universal health coverage setting goals that align with those of the Sustainable Development Goals. This goal is set to eliminate viral hepatitis as a public health problem, being encapsulated in the regional and worldwide targets to reduce new cases of viral hepatitis infections by 90%. This will reduce mortality due to viral hepatitis by 65% by 2030 (WHO, 2022). The strategy outlines the steps to be taken by countries and WHO Secretariat to realize the achievement of these goals (WHO, 2017). It is noted that widespread of HCV infection in other WHO regions differ from 0.5% to 1.0%. Looking at different countries, hepatitis C virus infection is noted to concentrate in isolated populations. For example, 23% of new HCV cases and 33% of HCV death is attributed to the use of injectable illicit drugs, although these category of people and those in prisons are considered less during national responses (WHO, 2022).

The data on people who use injectable illicit drugs is sparse, although, studies from Kenya and Tanzania show a related high HIV prevalence degree of 36% and 35% respectively, in comparison to the 6.0% and 5.0%

universally (WHO, 2021). Use of Injectable illicit drugs is of great concern across the region compounded by reports of risky sexual behaviors in these individuals (Mburu G et al, 2019). The lack of mortality reduction programs and persistent high risk exposure has been highly implicated in transmission of HIV (Fraser H et al, 2021). Although there is no effective vaccine developed against hepatitis C viral particles, research towards the same is currently being pursued (Gardner A R et al, 2023).

2.2 Epidemiology of Human Immune Virus (HIV)

Globally, 38.4 million (33.9 million – 43.8 million) person were infected with HIV in 2021. 1.5 million (1.1 million – 2.0 million) people became newly infected with HIV, while about 5.9 million people did not know there HIV status by the end of 2021. Approximately 650 000 people died from AIDS related illness in 2021 (UNAIDS, 2023). The risk of acquiring HIV is 35 times higher in PWID than in adults who do not use injectable illicit drugs (UNAIDS, 2023).

Looking at the global trends, there is an overall rise in HIV-1 prevalence rate, sub-Saharan Africa bearing 70% of the worldwide burden of the infection. Approximately 1.5 million new HIV-1 cases occur each day globally of which two in three, accounting for 51%, are from sub- Saharan Africa (UNAIDS, 2023). According to WHO report-2022, African Region is the most affected. In Kenya, the Government tabled preliminary findings of the Kenya Population-based HIV-1 Impact Assessment (KENPHIA) 2018 survey showing that Kenya's HIV-1 prevalence rate stood at 4.9% (UNAIDS, 2021). It is noted that Mombasa County is inhabited by 1,145,259 persons who comprise 52% males and 48% females and HIV-1

prevalence in Mombasa (7.5%) is higher compared to the national prevalence rate of 5.9% (UNAIDS, 2021). The county was noted to contribute 5% and 3% of the total new HIV-1 infections in Kenya comprising children and adults (NACC, 2022).

2.3 Epidemiology of Hepatitis C virus (HCV)

Total worldwide HCV prevalence rate is estimated at 2.5% (177.5 million of HCV infected adults), ranging from 2.9% in Africa and 1.3% in America, with a worldwide viremia rate of 67% (118.9 million of HCV RNA positive cases), ranging from 64.4% in Asia to 74.8% in Australia (UNAIDS, 2021). The widespread of HCV in the general population in Africa ranges between 0.1% and 17.5%, in regard to individual countries. The countries with the highest rate of prevalence include Egypt (17.5%), Cameroon (13.8%) and Burundi (11.3%) (UNAIDS, 2021).

From a previous study, HCV widespread among HIV-1 positive participants was noted to be 50% (66 of 131 participants) in coastal Kenya, 35% (42 of 121) in Nairobi, and 4% (one of 23) in western Kenya.

According to the world health organization report (WHO, 2022), an estimated 290,000 people died from complications associated with Hepatitis C, mostly from cirrhosis and hepatocellular carcinoma.

Risk associated factors for HIV-1- HCV concurrent infection mirrored those observed for HCV mono-infection (Oguya et al, 2021).

2.4 Epidemiology of HIV-1 and HCV co-infection

HIV-1 infection synergizes the prognosis of HCV infection, accelerating the deterioration rate of liver inflammation. Hepatic injury is a marked disorganization of hepatic tissue structures noted as excess deposition of extracellular matrix.

HIV-1- HCV co-infection is noted to affect approximately 2 million persons worldwide. The acceleration of HCV-associated complications, especially hepatic fibrosis, because of HIV-1 co-infection has been properly established, whereas the effect of HCV on HIV-1 progression in PWIH remain not clear and HCV remains the most common co-infection with HIV-1 and has become a major challenge to the survival rate of HIV-1 infected persons (UNAIDS, 2023).

The current facts on the impact of co-infection on the spread and clinical progression of individual infection is pronounced. The focus is on the alterations associated with virology and immunology of metabolic processes that are involved with HIV-1 and HCV pathogenesis in co-infection and to revised disease-modifying effects of antiretroviral therapy in regard to HCV (Gobran S T et al, 2021).

2.5 Etiology of Hepatitis C Virus (HCV)

HCV particles are causative agents of Hepatitis C infection which spreads when blood contaminated with the virus enters the bloodstream of an uninfected individual (Grebely et al, 2019). Transmission can also happen by coming into contact with small quantities of blood through use of injectable illicit drugs, unsafe injection practices, unsafe health care service delivery, transfusion of infected blood and blood products, and sexual practices involving infected persons (Francois et al, 2020). HCV is a virus

transmitted through blood and blood products which is associated with both acute and chronic liver infection ranging in severity from a less severe illness lasting a few weeks to a serious long lasting illness, which can progress to liver cancer (Forner A et al, 2018);(Wantuck et al, 2019). HCV exists worldwide in several distinct genotypes and more than 67 subtypes (Wantuck et al, 2019).

2.6 Etiology of Human Immune Virus (HIV-1)

Acquired immune deficiency virus is a chronic and life-threatening condition whose causative agent is HIV-1. By destroying the immune system, HIV-1 weakens the body's ability to fight disease causing agents (UNAIDS, 2023)

PWIH are also linked to HIV-1 infection in sub-Saharan Africa, with increased reports from Kenya, South Africa, Mauritius and Tanzania (UNAIDS, 2021).

Current research has shown that injection drug use is a key mode of HIV-1 transmission in North Africa and Middle East. (Global HIV/AIDS response, 2021). This is a significant route of transmission in most regions, including such countries like Pakistan, Afghanistan, Iran, Tunisia, Libya, Bahrain, Kuwait, and Oman. In these countries, persons who use injectable illicit drugs account for an estimated 67% of HIV-1 cases in Iran and 90% in Libya where heroin and other illicit drugs are highly utilized (UNAIDS, 2021). This mirrors the current situation in Mombasa.

2.7.1 Pathophysiology of HCV in people who inject heroin.

HCV is a non-cytopathic virus that gains entry into the liver cell where it replicates simultaneously leading to cell necrosis by immune –mediated

cytolysis, steatosis, oxidative stress and insulin resistance. It also causes chronic antigenic stimulation, direct cell transformation through insertional mutagenesis and expression of oncogenic proteins. In acute HCV infection, the host immune system triggers the secretion of interferon alpha, sensitization of natural killer cells followed by the activation of the adaptive immune system. This causes the impairment of these mechanisms with resultant local inflammation and fibro genesis that causes hepatic injury with cirrhosis (Lin W et al, 2022).

Hepatitis C virus infection, apart from being the key cause of serious liver disease, is recognized as a factor associated with high rate of mortality in clinical cardiovascular disease (Matsumori et al, 2022). In isolated cases, new HCV infections are usually not symptomatic with some persons getting abrupt onset of hepatitis which does not cause a life-threatening disease. There is a probability of 30% (15–45%) of individuals infected to spontaneously clear the virus in a period of 6 months of infection without any therapeutic intervention (Smirnov A et al, 2023). 70% (55–85%) of these individuals are likely to develop long term HCV infection, with 15% to 30% progressing to develop liver cirrhosis within 20 years (Wantuck *et al*, 2019.

Many studies have shown elevated cardiac and inflammatory biomarkers in persons with lifelong HCV infection (Mazzaro C et al, 2021). This is used to risk stratify people for cardiac conditions in the general population. These patients have a rise in cardiac and inflammatory biomarkers in comparison to non-infected control population and hence, may play a key role in CVD risk stratification (Younossi et al, 2020). A step by step review of

inflammatory and cardiac biomarkers in persons with HCV infection and their utility as an indicator for CVD in the target population revealed the need for laboratory monitoring of this parameters. From this, it was noted that HCV infection significantly alters blood levels of inflammatory markers, endothelial function, and cardiac dysfunction prior to HCV therapeutic intervention. Some show changes in response to HCV therapy (Younossi et al, 2020). Looking at the current trend, mortality stratification tools for development of CVD in the general population do not put into consideration the increased inflammatory markers that are elevated among HCV infected individuals, a factor that contributes to increased CVD risk.

2.7.2 Pathophysiology HIV-1 in people who inject heroin

When HIV-1 enters the host cells, it binds to a receptor (Tcells CD4) and releases its RNA into the host cell, where it undergoes reverse transcription converting its RNA into a copy of a double stranded DNA. In HIV advanced disease, patients have reduced total cholesterol, LDL-C and HDL-C with high triglycerides levels which become pronounced as the disease progresses (Kenneth R et al, 2022). CD4 T cells are key immune cells that secret cytokines during HIV infection. Th-1 subset of T helper cells produces important cytokines like interferon-γ (IFN-γ) and Interleukin-2 (IL-2), while Th-2 subset of T helper cells secrets IL-4, IL-5, IL-6, and IL-10. Studies show that infection with HIV down regulates Th-1 and upregulates Th-2 cytokines creating an imbalance and impairing the immune function. Immune activation by HIV triggers cytokine changes (eg. IL-4, IL-5, IL-13, and TGF-β) that increases liver inflammation and marked liver fibrosis (Shaviya et al, 2021).

2.7.3 Pathophysiology of HIV-1-HCV co-infection in people who inject heroin

HIV co-infection leads to elevated HCV persistence following acute onset of infection, increased viral load and increased speed of progression to liver fibrosis and advancement to end-stage liver disease in comparison to HCV mono-infection. HIV-1 - HCV concurrent infection has negative effect on the homeostatic activity of $CD4^+$ T cell count and facilitates HIV replication and viral reservoir persistence (Gobran S T et al, 2021).

2.8 Vitamin D3 metabolism in HIV-1-HCV co infected PWIH

The liver and other tissues metabolize vitamin D, from the skin or in the diet, to 25 hydroxyvitamin D, then to hormonal form 1,25 di hydroxyvitamin D (1,25 (OH)2 D), catalyzed by CYP2RI, the most important 25 hydroxylase enzyme. In the liver, vitamin D is hydroxylated at C-25 to produce 25-hydroxyvitamin D3, which is the major form of vitamin D found in circulation (Jaimni et al, 2021). Its presence and levels in serum serves as one of the most reliable biomarker of vitamin D status. They are transported in blood bound to vit. D binding protein and albumin. Vitamin D functions by triggering intestinal calcium and phosphorus absorption, by triggering bone calcium mobilization and by upregulating renal reabsorption of calcium in distal tubule, which require parathyroid hormone (Kanwal et al, 2022).

Vitamin D is known to regulate cell proliferation and differentiation and has immune modulatory activities such as anti-inflammatory and anti-fibrotic properties. Vitamin D deficiency is present in chronic liver disease and may reveal non response to antiviral therapy in chronic hepatitis C

disease, and is associated with serious non-alcoholic fatty liver disease (Visuthrnukul C et al, 2023). They play a major role in activation and regulation of both innate and adaptive immune system and are strong anti-fibrotic agents against liver injury (Pierantoneli et al, 2019).

Normal blood 25-hydroxyvitamin D_3 levels ranges from 20ng/ml to 50 ng/ml. High levels of 25-hydroxyvitamin D_3 of greater than 150ng/ml can cause elevated blood ionized calcium levels, with calcification of arteries and soft tissues (Reid et al, 2020); (Onyango et al, 2024). Low levels of 25-hydroxyvitamin D_3 below 12 ng/ml is indicative of vitamin D deficiency which can result in increased risk of CVD and loss of bone density which can contribute to osteoporosis and bone fractures (Sing'oei et al, 2021).

2.9 Calcium metabolism in HIV-1-HCV co-infected PWIH

Calcium metabolism entails movement and regulation of calcium ions in and out of the body and between body compartments via the gut. This is dependent on the performance of parathyroid hormone (PTH), whose secretion is strictly controlled by ionized serum calcium by negative feedback mechanism, which is achieved by the activation of calcium –sensing receptors (CaSRs) expressed on the surface of the parathyroid cells (Abel O et al, 2024). Calcium is essential for most metabolic processes such as nerve function, muscles contraction and blood clotting (Sema Baltazar et al, 2024).

Calcium is an important secondary messenger involved in the regulation of multiple hepatic activities which include lipid and carbohydrate metabolic processes and bile secretion. Dysregulation of calcium signaling is

an assay- mark for both acute and chronic liver disease (Escobedo-Monge et al, 2024). Chronic liver disease causes hypercalcemia. Unchecked hypercalcemia may lead to renal impairment by alteration of renal tubular function and recurring nephrolithiasis leading to progressive loss of renal function and serious bone pathological conditions and heart damage (Ralph Kettriz et al, 2020).

The pathological changes in bone and mineral metabolism associated with HIV-1 infection may be triggered by direct interaction of HIV-1 with cells of the bone and bone marrow microenvironment, chronic T cell activation, and compromised cytokine production affecting osteoblast and osteoclast functions, dysregulation of calcium homeostasis, parathyroid hormone (PTH) function, vitamin D metabolism, opportunistic or neoplastic diseases, and adverse effects of drugs (Ravaioli F et al,, 2022). To provide up to date health care for HIV-1 infected patients, early diagnosis and adequate treatment of HIV-1 associated disorders of bone and mineral metabolism are required (Fiamenghi VI et al, 2021). Data on histo-morphometric analyses of bone remodeling in individuals with HIV-1 infection are sparse (WHO, 2022). In HIV-1 infection, reduced production and action of 1, 25 $(OH)_2 D_3$ is noted to be the key cause of hypocalcemia, accounting for 48% of cases (WHO, 2022).

Normal blood ionized calcium levels are between 8.5 to 10.5 mg/dl or 2.2 to 2.6 mmol/L. Higher levels of above 10.5 mg/dl are an indication of hyperparathyroidism or excessive consumption of calcium supplements (Escobedo-Monge et al, 2024). Low levels of below 8.5 mg/dl can be as a

result of hypo albuminemia due to cirrhosis, nephrosis, malnutrition, burns, chronic illnesses and sepsis (Abel O et al, 2024).

2.10 Lipids serum levels in HIV-1-HCV co-infected PWIH

Lipids are noted to be a group of naturally occurring molecules that include fats, oils and waxes. They may also include certain vitamins, hormones, and most of the non-protein membrane of cells (Kenneth R et al, 2022). Notably primary lipids of interest in this study were triglycerides, low density lipoprotein (bad cholesterol) and high density lipoprotein (good cholesterol) (Nazeer et al, 2023).

2.10.1 Triglycerides

In normal circumstances, triglyceride levels are below 150mg/dl of blood. High triglycerides levels (200mg/dl to 499mg/dl) contribute to arteriosclerosis, which increases the risk of stroke, heart attack, heart disease and acute pancreatitis (Matsumori et al, 2022). This may also include obesity and metabolic syndrome which is a cluster of conditions that includes accumulation of fat around the waist, high pertension and diabetes mellitus (Saxena et al, 2023). Elevated triglyceride (TG) levels are common in HIV- positive persons for several reasons. First, conditions that traditionally result in elevated TG, such as insulin resistance, diabetes mellitus (DM) and fatty liver are prevalent in the HIV-positive population (Nazeer B et al, 2024). Second, the physiological distress that results from untreated HIV infection may cause lipid perturbations, in particular elevated TG (Mazzaro C et al, 2021). It has been suggested that HIV-positive patients may experience increased post- prandial TG levels ,this being one potential explanation for the increased risk of myocardial infarction (MI) that is seen in HIV-positive persons (Matsumori et al, 2022). Cases of low

Triglycerides levels are observed in low fat diet, hyperthyroidism, malabsorption syndrome and in malnutrition.

2.10.2 Low density lipoprotein cholesterol

Plasma low density lipoprotein (LDL) patterns are also changed by infection and patients with AIDS have increased levels of small dense LDL particles. Low-density lipoprotein receptor (LDLR) is involved in uptake of cholesterol rich LDL particles from the bloodstream (Spera A M et al, 2024). The association of HCV particles with lipoproteins implicates their role in HCV entry; however, the precise role of LDLR in HCV entry still remains controversial (Obare et al, 2024). The effect of HCV infection on LDLR expression and the underlying mechanism(s) involved was investigated. It was demonstrated that HCV stimulates LDLR expression in both HCV-infected Huh7 cells and in liver tissue from chronic hepatitis C patients. Increased LDLR expression resulted in the enhanced uptake of lipoprotein particles by HCV-infected cells (Kanno T et al, 2025). Analysis of LDLR gene promoter identified a pivotal role of sterol-regulatory element binding proteins (SREBPs), in the HCV-mediated stimulation of LDLR transcription (Chongo S et al, 2024). In addition, HCV negatively modulated the expression of pro protein convertase subtilisin /kexin type 9 (PCSK9), a protein that facilitates LDLR degradation.

Looking at patients with HIV mono infection, HIV/HCV co infection, and neither HIV nor HCV infection, it can be observed that in comparison to those with neither infection, HIV/HCV-co infected adults have the lowest LDL-C followed by HIV-mono infected adults. Importantly, it can be

demonstrated that HIV/HCV co-infection appear to attenuate abnormalities in lipoprotein subclass that have a reported association with CVD relative to those with HIV mono-infection. (Kenneth R et al, 2022). Low LDL-C levels of less than 40 milligrams per deciliter of blood can be associated with an increased risk of cancer, and notably malnutrition, anemia, hyperthyroidism and in liver disease. High levels of LDL-C of over 130 mg/dl of blood is an indication of cardiovascular disease (Pascual-Oliver et al, 2024).

2.10.3 High density lipoproteins

Decreases in high density lipoprotein cholesterol (HDL-C) and Apo lipoprotein A-I, and an increase in lipoprotein (a) [L p (a)] are the usual lipidemic disorders during HIV infection, even in those patients with CVD (Lee T H et al, 2025). The decreased HDL-C and increased triglycerides and L p (a) are associated with an increased risk of myocardial infarction. Some cardiovascular events have been reported in HIV positive individuals (Yu N et al, 2025). Elevated HDL-C of greater than 60 mg/dl are good for the body while low levels of less than 40 mg/dl are associated with CVD risk.

2.11 Liver function biomarkers serum levels in HIV-1-HCV co-infected PWIH

These are employed to determine the levels of enzymes and proteins in blood to help diagnose and monitor prognosis of liver disease or liver damage (Abdi M et al, 2023).

2.11.1 Alkaline phosphatase serum levels in HIV-1 & HCV co-infected PWIH

Previous studies suggest that comorbidities and demographic variables have stronger associations with elevated ALP in HCV and HIV-1, hence the need to evaluate it in both conditions (YU N et al, 2025). Lower than normal ALP levels is indicative of hypophosphatemia, deficiency of protein or Wilson' s disease, and may also point to malnutrition due to celiac disease or an insufficient amount of certain vitamins and minerals. Normal range of ALP is typically 44 to 147 IU/L or 0.73 to 2.45μkat/L (microkatal per liter) (Strauss K L et al, 2025). High levels of above 147 IU/L are indicative of liver damage or bone fracture (Sing'oei et al, 2021) (Smirnov A et al, 2023).

2.11.2 Albumin serum levels in HIV-1-HCV co-infected PWIH

Reduced serum albumin levels are a consequence of HIV-1 infection progression among other conditions and heroin has the ability to reduce serum albumin levels (Huang R et al, 2024). Therefore, serum albumin level is an independent key factor for mortality and an important indicator for prognosis, hence improvement of hypo-albuminemia is considered for improvement of prognosis (Rakesh Kumar et al, 2021).

Reduced serum albumin levels are an important indicator for morbidity and mortality and correlate with an increased risk of morbidity and mortality in HCV and HIV infected individuals (Huang et al, 2024). Albumin, produced by the liver, is the major protein that circulates in the blood and is important for maintaining the oncotic pressure in the vascular system (Moman et al, 2019). A decrease in oncotic pressure due to reduced albumin level causes fluid to leak from the interstitial spaces into the peritoneal cavity, with production of ascites. Albumin is also a key protein in the

transportation of various molecules. It has antioxidant and anti-inflammatory properties that protect the body (Huang et al, 2024). It is monitored in the liver function profile as a clinical marker (Tom Were et al, 2021). Normal albumin range is 3.4 to 5.4 g/dl. Levels lower than 3.4 g/dl are indicative of liver dysfunction, kidney disorder or an inflammatory disease. It is also a sign of malnutrition. Higher levels of above 5.4 g/dl is an indication of acute infection, burns, stress from surgery or a heart attack (Eckart et al, 2020).

2.11.2.1 Alanine aminotransferase

The damage to the liver parenchymal cell causes aminotransferases to leak from the damaged cells into blood circulation resulting in elevated serum levels of these enzymes in individuals with HCV and HIV infections (Spach, 2024). ALT levels in normal cases falls between 7 to 35 U/L in women and between 7 to 40 U/L in men. Levels of above 35 U/L in females and 40 U/L in males is indicative of liver damage, and levels lower than 7 U/L serves as an independent predictive marker for increased long term mortality especially in middle aged adults, seen in cases of Vitamin B6 deficiency or chronic kidney disease (Fittipaldi et al, 2024).

2.11.2.2 Gamma glutamyl aminotransferase (GGT)

In hepatitis C virus (HCV)-mono infected patients, the serum GGT level has been linked to the hepatocyte growth factor level, which rises as fibrosis progresses and has been implicated in fibrogenessis (Bryan et al, 2021). Isolated high GGT levels due to enzyme induction are observed in HIV-1/HCV-co infected patients and elevation may be a manifestation of liver mitochondrial toxicity. This enzyme seemed to be useful as an indirect

marker of more advanced liver disease in chronic hepatitis C (Jose-Abrego et al, 2024). GGT normal levels are between 0 to 30 IU/L. Levels above 30 IU/L are indicative of liver damage due to example, hepatitis. Low GGT levels are observed in certain genetic diseases where the flow of bile from the liver is interrupted or suppressed (Yu N et al, 2025).

2.12 Anthropometric indicators

They are noninvasive quantitative measurements of the body which are used to estimate total body fat, regional fat and fat distribution, i.e. measures of relative adiposity or fatness.

2.12.1 Body mass index. (BMI)

Hepatitis C virus is associated with hepatic steatosis. Patients with HCV are likely to be heavier and have hypertriglyceridemia with elevated body mass index. This is a predictor of non-alcoholic fatty liver disease (Lee T H et al, 2025). This calls for the need to evaluate BMI, noted to be a measure that uses height and weight to estimate body fat. This is derived from the mass and height of a person (Saxena et al, 2023).

2.12.2 Waist circumference

Metabolic syndrome is highly prevalent in HCV Genotype 1 infected patients as a result of fatty liver disease (Karaca B et al, 2023). It is associated with hypertension, Insulin resistance, increased abdominal fat and overweight. This calls for the need to evaluate waist circumference in HCV patients (WHO, 2021).

2.12.3 Waist to hip ratio (WHR)

There is close relationship between HCV infection and metabolic syndrome. Close monitoring of weight gain among patients with HCV is beneficial. This clinical conditions can negatively affect disease prognosis, by reducing the incidence of comorbidities and possibly delay the progression of chronic liver disease (Karaca B et al, 2023). This points to the need to evaluate the waist to hip ratio.

2.12.4 Waist to height ratio (WHtR)

Previous studies suggest that HCV modulates the lipid metabolism of host cells to promote its replication, and is also related to increased lipogenesis, reduced secretion and beta oxidation of lipids, a factor that can lead to central obesity (Karaca B et al, 2023), hence the need to evaluate WHtR in HCV patients.

2.13 Heroin in HIV-1-HCV co-infected PWIH

Heroin metabolism occurs in the liver, which is associated with production of enzymes for this purpose that promote two forms of heroin metabolism. Phase one metabolism which involve drug oxidation or hydrolysis and phase two metabolism where heroin undergoes conjugation reaction. Heroin is de acetylated by esterase enzyme to 6-monoacetylmorphine which is the active metabolite. This is then de acetylated again by esterase enzyme through hydrolysis to morphine, which undergoes glucuronidation to form morphine – 6 – glucuronide (M6G). This metabolites are highly lipophilic (Milella M S et al, 2023).

Heroin is associated with increased neurocognitive deficits in HIV positive people on ARVs, reducing quality of life in 15 to 40% of people.

Morphine dysregulates functional processes in monocyte-derived macrophages to increase neuro pathogenesis. Morphine triggers these cells by increasing phagocytosis and increasing the activity of reactive oxygen species (Malik J A et al, 2024). This causes minimal impact of Anti Retro viral therapy during heroin exposure. It also affects macrophage homeostasis, enhancing inflammatory activities. It impacts more cellular processes in HIV-infected monocyte derived macrophages to effectively increase neuro pathogenesis in people with HIV infection (Tarantino G et al, 2024). Heroin causes severe long lasting liver degeneration and derangements in hepatic metabolic functions (Milella M S et al, 2023), complicating the pathological effect of HIV-1-HCV co-infection in PWIH.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Overview

This chapter contains research design, area of the study, target population, sampling techniques, and sample size. It also presents the research instruments of data collection, reliability, validity of the research instruments, procedures of data collection, analysis of data and ethical consideration.

3.1 Study area

The study was undertaken in Mombasa County in Kenya, which measures 219.9 sq. kilometers and an estimated population of 1.2 million people according to the 2019 census. Bomu, being a community hospital and a rehabilitation center for people who inject drugs was an ideal testing site, with daily turnover of 850 patients both in and out patients. The county of Mombasa, with its six sub-counties, has a remarkable number of people who inject illicit drugs of approximately 37 000 persons as per the 2019 Kenya national population sensors, with heroin being the key drug. Injection heroin users from the six counties seek services at Bomu community hospital. The coastal region has readily available illicit drugs from the far -east and the western countries. As it borders the Indian Ocean, the smuggling in of illicit drugs is rampant. Although the most common ailments in this region are malaria and filariasis, a bigger portion of the population are involved with heroin use. This has resulted to an increase in the prevalence of HIV (7.5%) as per the report by the National Aids control council (NACC, 2022) and HCV (66 of 131 participants), (WHO, 2022), raising

the HIV-1-HCV concurrent infection index among the coastal population in both males and females. Given this raise in number of HIV-1-HCV concurrent infection and PWIH, the study was viable.

3.2 Study design

This was a case control retrospective study which involved samples from HIV-1 and HCV positive people who showed evidence of needle scars for injecting drugs, HIV-1 and HCV negative people who showed evidence of needle scars for injecting drugs and confessed to have used injectable heroin, and also showed evidence of pupil constriction (miosis) in their eyes with track marks on their skin, which are cardinal signs seen as a result of high concentration of heroin in circulation. This was a case control retrospective study. This was ideal for this study because hepatocellular injury and metabolic dysregulation are already present outcomes in a high-risk, hard-to-follow population. This design allowed us to efficiently compare co-infected heroin users with and without liver injury, examine multiple exposures (HIV, HCV, heroin duration, biomarker levels), use existing medical records, reduce ethical challenges, and achieve valid results in a resource-limited setting like Mombasa.

Their serum lipid profile, liver function panel, ionized calcium and 25-hydroxyvitamin D_3 results were documented and analyzed.

3.3 Study population

Key population was ART naïve HIV-1 seropositive (evidenced with presence of antibodies against HIV-1) and HCV seropositive (evidenced by presence of antibodies against HCV) people who use injectable heroin, who showed needle scars at sites of injection, and where the main injection drug was heroin, noted with evidence of pupil constriction in their

eyes and track marks on their skin. The target population comprised people who had never screened for HIV-1. The control group was HIV-1 -ve and HCV -ve persons who inject heroin, and had evidence of using illicit injection drugs as evidenced by pupil constriction (miosis) in their eyes and track marks on their skin. Out of the 500 specimens isolated, data of only 289 participants was valid and relevant to the study.

3.3.1 Inclusion criteria

Considered for the study were adults between 18 years to 75 years with evidence of needle scars in their inner elbow, with evidence of pupil constriction in their eyes, track marks on their skin (ClinicalInfo(NIH), 2024), confessed to have injected themselves with an illicit drug even once and were able to provide a written informed consent. Persons of below 18 years of age were not considered because they could not give informed consent as per the Kenyan laws, and those above 75 years undergo physiological decline in metabolic processes which naturally alter biochemical markers. Included as a control population were the HIV-1- HCV negative individuals with evidence or history of illicit injection drug use and were willing to consent to the study.

3.3.2 Exclusion criteria

Individuals excluded from the study were HIV-1-HCV co-infected and uninfected persons who also tested positive of Hepatitis B surface Antigen, and those who were on antiretroviral therapy. Sample results from these individuals could have compromised the study outcome.

Persons who had other underlying liver diseases and those with pre-existing cardiovascular conditions and mycobacterium infections were also excluded from the study. All these have direct and indirect effect on metabolic processes and immune system.

Injection heroin users who were HIV-1 and or HCV positive but on anti-viral therapy did not qualify for the study.

3.4 Study variables

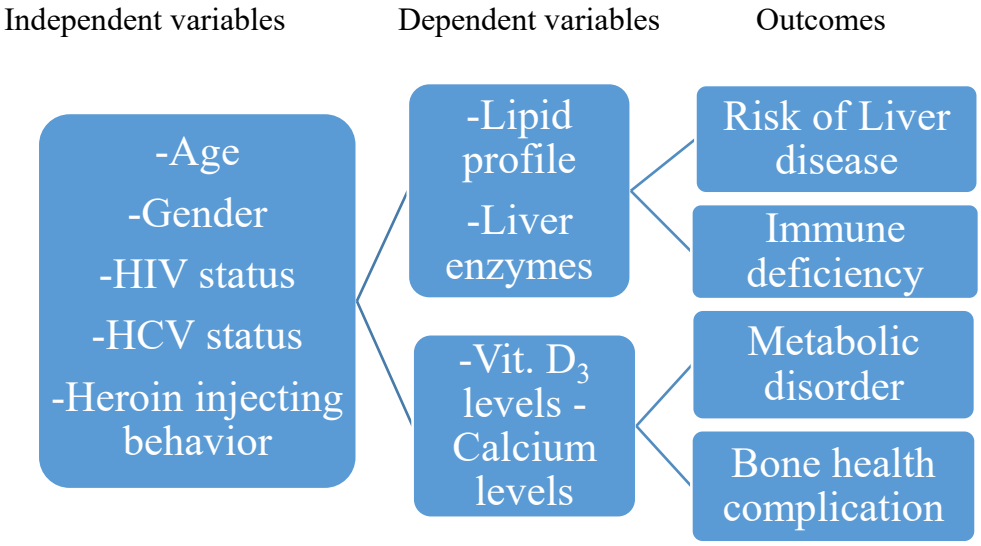
3.4.1 Dependent variables

Dependent variables in this study were serum LDL-C, HDL-C, triglycerides, albumin, alkaline phosphatase, ionized calcium and 25-hydroxyvitamin D_3. Their levels were determined as per the pre-determined reference ranges. Room temperature was also monitored to preserve the integrity of the analytical procedures.

3.4.2 Independent variables

Independent variables of the study included age, gender, HIV-1 and HCV status of the study participants. Males and Females 18years to 75 years who were concurrently infected with HIV-1-HCV and injected illicit drugs were a valid source of data to the researcher. Below 18 years could not give self-consent as per the laws of Kenya and above 75 years have progressive liver decline with physiological derangement of hepatic and lipid markers. Gender parity was key due to differences in result interpretation in regard to variation in reference ranges for various analytes.

Figure 3.4.2 Study variables



3.5 Sampling design

Eligible data on prevalence of illicit injection drug use, HIV antibody and HCV antibody among IHUs was selected. Where multiple estimates were available, they were pooled. Also observed as eligible data was the percentage of IHUs who were females and males 18 years to 75 years with history of engaging in drug injecting risk and whose main drug of injection was heroin. All participants who were recruited were recruited for the study were assigned unique numbers to avoid double registration. County and country estimates in line with previous regional reviews were examined.

3.5.1 Sampling strategy

Injection heroin users who consented to the study were recruited via respondent driven sampling at the make shift outreaches, snowball, needle and syringe program sites. This helped to reduce human bias in selecting persons that were included in the sample. It helped the researcher to have a sample that was highly representative of the population of persons under

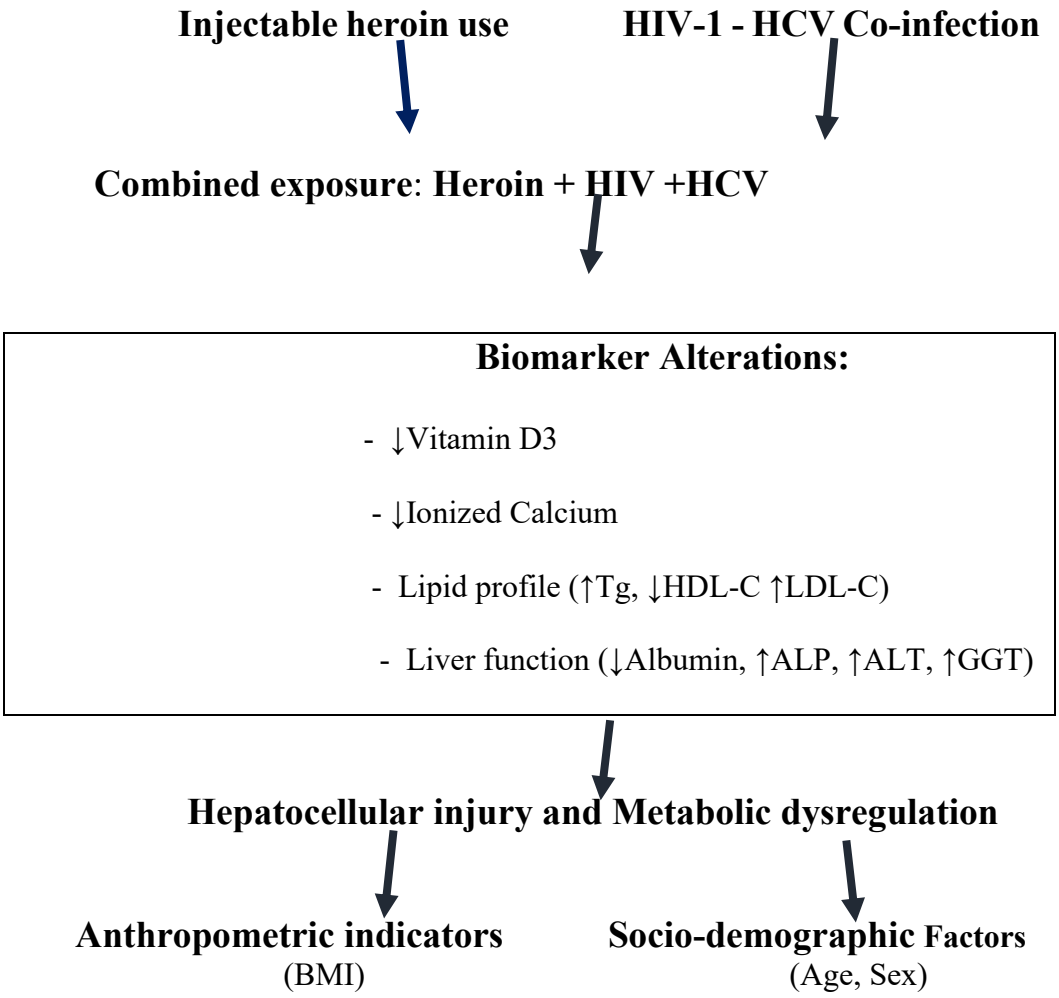
study. This gave allowance for making statistical inferences from the sample to the population to help achieve external validity.

3.5.1 Sampling strategy



3.5.2 Conceptual framework

This illustrated how the combination of viral infections and substance use impacts biomarker levels. It also guided data collection and analysis by showing hypothesized relationships. The framework posited that chronic heroin injection leads to physiological, nutritional and metabolic disturbances that may alter liver function, affect lipid metabolism and impair calcium and vitamin D_3 homeostasis. However, these disturbances manifest through specific biochemical markers and may result in long term health consequences.



3.6 Sample size determination

Sample size was determined with the following Fisher's formula:

$n = z^2 p q / d^{2.}$ (Riley RD et al. 2025).

Where: - n = desired sample size

Z = standard normal deviation set at 1.96 which corresponds to 95% level of confidence

P = the population target estimated to have had particular characteristic (25%).

q = 1-P

d = Degree of accuracy desired in the study. Set at 5% = 0.05.

$n=1.96^2 \times [0.25x (1-0.25)] / 0.05^2 = 289$ respondents.

3.7 Data and information collection

3.7.1 Procedure data

Four milliliters of venous blood was drawn from the brachial vein in the antecubital region of the arm into serum separator tubes (SST) using the standard closed mode system. The samples were allowed to clot for 15 minutes, before being centrifuged at 3000 revolutions/minute to get clear serum. All samples were analyzed within 24 hours after collection and parallel samples stored at negative 80^0 C (-80^0 C) pending analysis. Samples were centrifuged using a swing out head electric centrifuge set at 3000 rpm for 10 minutes. This ensured the collection of clear serum free from traces of cells. Serum was aliquoted into separate 2mls each cryovials for freezing at -80^0 C for a longer period. Serum was used for testing clinical chemistry markers and for HCV and HIV-1 sero testing. Results were verified in comparison with the standard reference ranges. Patients' demographic information, date of sample collection and date for sample analysis were documented on the laboratory requisition form and in the laboratory's information management system.

Screening for HIV and HCV infections were done as per the Kenya National HIV testing algorithm using the immune chromatographic lateral flow techniques. Rapid screening for antibodies against HCV was done using anti HCV rapid test diagnostic kits. Concentration of ALT, AST, ALP, ALB, BIL, HDL-C, LDL-C, Triglycerides, ionized calcium and 25-hydroxyvitamin D_3 were determined using Clinical chemistry auto analyzer.

Collected data was analyzed using SPSS version 20 computer software. Regional reference ranges were used (Sing'oei et al, 2021).

3.7.2 Data collection instruments

Venous blood from study participants was collected in plain BD vacutainer tubes and serum separator tubes (BD, Franklin Lakes, USA), using the standard closed mode system. Reference ranges were based on the locally established ranges.

Automatic pipettes with capacity of measuring 10µl to 200µl and 100µl to 1000µl were used to aliquot serum samples. A swing out head auto stop centrifuge with speed of 3000 rpm was used to separate clot retracted blood samples for clear serum collection, and a refrigerator with ability to attain temperatures of -25 to 8°C was used to store samples pending analysis. Those samples that needed to be stored for longer periods were kept in a -80^{0} C freezer.

A computer installed with Microsoft office and SPSS version 20 software (SPSS Inc. Chicago, Illinois, USA), with a RAM of 4 GB and a ROM of 120 GB was used to compute and store data.

3.8 Methodology

3.8.1. Collection of blood samples

Venous blood samples were collected from consenting individuals by a competent phlebotomist from the median cubital vein at the cubital fossa using a closed system into 4ml EDTA anticoagulant and 4ml plain and clot activator vacutainer TM tubes (BD, Franklin Lakes- USA). Blood samples were collected between 9.00am and 11.00am to obtain precisely compara-

ble values and control for diurnal variations in blood parameters. All laboratory tests were performed on samples that were collected using standard phlebotomy technique and immediately separated to obtain clear serum for storage at -80 ^{o}C to maintain sample integrity.

3.8.2. HCV viral load

HCV viral copies were determined using Real-Time Polymerase Chain Reaction (RT-PCR), by Abbott m2000 molecular analyzer following manufacturer's written protocol (Abbott Molecular Inc., Illinois, USA). RNA particles were extracted from 0.2 ml serum samples using guanidine thiocyanate, washed and taken through the replication process via an RNA-dependent RNA polymerase (NS5B) which synthesizes a negative –single stranded RNA intermediate, which is then used to produce more positive-single stranded genomic RNA, using HCV specific amplifying kit and internal control primers. The new viral genomes are then packaged into virions in the cytoplasm of host cell. Fluorescence intensity of HCV probes were detected and converted into viral load copies/ml of blood by the analyzer.

3.8.3 HIV-1 viral load

HIV-1 viral particle copies were determined by use of fully automated Abbott m2000 molecular analyzer with regard to the manufacturer's written protocol (Abbott Molecular Inc., Illinois -USA). RNA particles were obtained from 0.2 ml serum samples using guanidine thiocyanate, washed and taken through reverse-transcription to obtain cDNA. The cDNA was then amplified with the use of HIV-1 specific amplifying kit and internal

control primers. Fluorescence intensity of the HIV-1 probe was detected and converted by the system's analogue-digital converter into viral load copies/ml of blood by the analyzer.

3.8.4 Clinical chemistry analysis

All samples were analyzed on the Cobas 6000 chemistry auto analyzer at 22^0 C room temperature. Direct clinical chemistry tests were performed for Vitamin D_3 (25-hydroxyvitamin D_3), albumin, ionized calcium and alkaline phosphatase using a clinical chemistry automatic analyzer (Roche COBAS® 6000, Lausanne, Switzerland). The analyzer was calibrated using standard calibrators specific for the tests to be performed. After successful calibration internal quality control samples were run and results verified with the Levi-Jennings chart to ensure they fall within the stipulated reference range. 50µl of clear serum was aliquoted into the sample cup and placed into the sample rack. The rack was then loaded onto the analyzer and results automatically generated for data analysis. Hemolysed and lipemic samples were rejected. The reference values were based on the local laboratory established reference ranges.

3.8.5 CD4 cell count

Data for CD4 cells enumeration was extracted from the archived results. Samples were collected in EDTA anticoagulated vacutainers using the closed mode venipuncture system. 50µl of whole blood was dispensed into trucount tubes and 20µl of fluorescent tagged antibodies added. The mixture is vortexed at moderate speed and incubated at 25^0 C for 15 minutes in a dark place. 450 ml of lysing solution is added, vortexed and incubated

again at 25^0 C for 15 minutes in a dark place. The sample was then analyzed on a Facs caliber flow cytometer automated platform (BD, Franklin Lakes- USA) and results reported as cells/ ml of blood.

3.8.6 HCV antibody testing

Detection of Hepatitis C viral particles (HCV) infections was performed using the single-step HCV-5 panel, immunochromatographic diagnostic tests, respectively (Healthaw Medical limited, Hangzhou, China). Clear serum samples were tested for presence of antibodies against the HIV panel markers and anti-HCV markers. HIV-1 and anti-HCV reactive persons were considered sero-positive for HIV and/or HCV infection.

3.8.7 HIV-1 antibody testing

HIV-1 antibody testing was done using immunochromatographic test kits, 1st Response, Determine™ (Abbott Laboratories, Tokyo, Japan) and Unigold™ (Trinity Biotech Plc, Bray, Ireland). Individuals with reactive results for both 1st Response and Determine were considered HIV infected based on the Kenyan national HIV screening algorithm. Where discordant results were recorded, Unigold was used as a tie breaker to ascertain the HIV status.

3.8.8 Body mass index (BMI)

Anthropometric measurements were documented for each individual at enrolment as per the Centre for Disease Control guidelines. Height (m) was determined to the nearest 0.1 cm using the Health-o-meter PORTROD wall mounted height rod (Health O meter ®, McCook, USA). Individual weight were documented in kilograms (kg) using a

portable digital weight scale (Rich forth Electronics Co., Fuzhou, China). The BMI was calculated using the height and weight measurements from the study participants using the formula:

$$BMI\ (Kg/m^2) = \frac{weight\ (kg)}{Height\ (m^2)}$$

Where BMI < 18.5 was characterized as underweight and above 25.0 as overweight.

Table. 3.8 Laboratory apparatus used to analyze study samples

No.	Item	Manufacturer	specifications
1.	Cobas chemistry analyser	Roche COBAS® 6000, Lausanne, Switzerland	6000, autoanalyzer.
2.	Abbott molecular analyzer	Abbott Abbott Laboratories, Tokyo, Japan	2000SP
3.	Vacutainers	BD, Franklin Lakes- USA	4mls, plain/ clot activator, Red top.
4.	Health-o-meter PORTROD	McCook, USA	wall mounted height rod Health O meter ®
5.	Portable digital weight scale	Rich forth Electronics Co., Fuzhou, China.	Portable, kilograms.
6.	Determine™	Abbott Laboratories, Tokyo, Japan	Immunochromatographic strips with diluent
7.	1st Response	Abbott Laboratories, Tokyo, Japan	Immunochromatographic plate with diluent
8.	Unigold™	Trinity Biotech Plc, Bray, Ireland	Immunochromatographic plate with diluent
9.	Single-step HCV-5 panel	Healthaw Medical limited, Hangzhou, China	Immunochromatographic diagnostic tests
10.	Flow Cytometer	BD, Franklin Lakes- USA	Facs Calibur
11.	Automatic precision pipettes	BD, Franklin Lakes- USA	10µl to 200µl and 100µl to 1000µl
12.	Centrifuge	Roche COBAS® 6000, Lausanne, Switzerland	Swing out head auto stop, 3000 rpm

13.	Computer	SPSS Inc. Chicago, Illinois, USA	Microsoft office and SPSS version 20 software with a RAM of 4 GB and a ROM of 120 GB

3.8.9 Reliability

The accuracy and precision of the automatic pipettes, centrifuge, refrigerator and the clinical auto analyzer calibration and verification reports from accredited field service engineers from internationally approved, registered companies were counterchecked before performance of procedures.

3.9 Quality assurance

Condition of reagents and control materials at reception and before sample analysis were verified by counterchecking the expiry dates and storage temperatures. External quality control data was verified to ascertain the conformance of the testing laboratory to the international and national acceptable standards. Internal quality control was done on the HIV-1 and HCV immunochromatographic testing kits and on clinical chemistry analyzer reagents before use on study samples. Evidence of good clinical laboratory practice was confirmed by counterchecking the testing laboratory's quality indicators performance before sample analysis.

3.9.1 Data analysis

Data was collected, presented and statistical analysis carried out using IBM1 SPSS 1 statistical software version 20 (SPSS Inc. Chicago, USA). Continuous data (age and analytical results) was summarized as medians

and interquartile ranges and if it was not normally distributed, was compared across study groups using a non –parametric ANOVA (Kruskal Wallis) test and Dunn's post hoc test for multiple comparison. Differences in the distribution of biochemistry parameters was compared with the use of Chi square test. Categorical data (i.e Gender) was presented in tables as frequencies and percentages. Spearman's rank correlation test was used to determine the association of clinical chemistry measurements within the study group. In this study, a two sided probability value of ≤ 0.05 was taken to be statistically significant.

3.9.2 Logistical and ethical considerations

To carry out this study, ethical approval was obtained from the Masinde Muliro University of Science and Technology ethical review committee, (MMUST DPS, IERC) protocol MMU/COR:403012 Vol 6 (01), and from the National Commission for Science, Technology and Innovation (NACOSTI), License No: NACOSTI/P/24/32609.

Written informed consent was obtained from the ministry of public health and sanitation (Ref:ADM.3/5/37/121) Mombasa County, to access archived samples for re-analysis, patients' demographic and anthropometric data.

Confidentiality was taken to be a key element to be considered throughout the course of the study for all participants' retrieved samples, which were part of a major study carried out earlier. HIV, HCV, clinical chemistry and viral load results were stored and analyzed by a password controlled computer. The results were only accessed by the researcher and two data entry clerks assisting the researcher.

All samples were given a unique code which only the researcher could use to identify the study respondent.

CHAPTER FOUR
RESULTS PRESENTATION

4.0 Overview

This chapter presents the results from analysis done on archived samples from HIV-HCV infected injection heroin users seeking services in sponsored medical camps and at Bomu community hospital. Generated results and retrieved anthropometric data was presented, interpreted and discussed in this chapter.

4.1 Demographic and clinical information of study participants

The study population comprised 289 participants above 18 years of age, who included both male and female. The population involved persons who injected heroin and were both HIV-HCV –ve (n=128), HCV-ve- HIV +ve (n=102), HCV +ve HIV –ve (n=37) and HCV+ naïve HIV +ve (n=22). The sample size of 289 was divided into four groups based on infection status

after the target number was attained. Table 4.1 shows a summary of demographic and clinical information of the study individuals.

4.1.1 Gender distribution

Females were very few across groups (10.1% in HIV-ve/HCV-ve, 2.7% in HIV-ve/HCV +ve, 18.2% in HIV +ve/HCV +ve). Male were predominant (upto ~ 90% in some groups). The proportion of male and female study individuals were significantly different across the groups of study (***P*= 0.0001**).

4.1.2 Age distribution

The median ages of both male and female participants were notably different (*P*=0.001).Seronegative and HIV monoinfected IHUs were younger (34.9 and 34.4), while HCV monoinfected (39.3 years) and co-infected participants (40.3 years) were older (Table 4.1).

4.1.3 CD4 count

Marked differences were observed in CD4 cell counts across the groups (Kruskal- Wallis test, P<0.001). The highest median CD4 counts were found among uninfected IHUs (1200 cells/µL, range 600-1800), while the lowest were recorded in HIV/HCV co-infected individuals (320 cells/µL, range 60 - 580). These results highlight the strong immunosuppressive effect of HIV-1 infection, further exacerbated in co-infected individuals.

4.1.4 Viral load patterns

HIV RNA across groups with HIV +ve participants were comparable (median 2.2 $\log_{10}$ copies/ml of serum, range 1.6-2.8) among HIV-1 monoinfected and 3.0$\log_{10}$ copies/ ml of serum, range 1.8-4.2 among co-infected, with *P*=0.569) Table 4.1. HCV RNA median 3.9 $\log_{10}$, range 2.2-

5.7 were recorded in HCV monoinfected and 3.7 $\log_{10,}$ range 2.5 – 4.8 in co-infected, with P=0.475.

4.1.5 Duration of heroin injection

A striking difference emerged in duration of injection (P=0.0001). Most HCV monoinfected (89.1%) and co-infected (77.3%) participants had injected heroin for >1 year. In contrast, most HIV-1 monoinfected (15.6%) and uninfected IHUs (2.3%) had injected for <1 year.

4.1.6 Frequency of heroin injection

Frequency of injection differed significantly (P=0.004). High frequency injection (>1 per day) was most common in HCV monoinfected (75.6%) and co-infected (82.0%) groups, compared to lower proportions in HIV monoinfected (27.4%) and uninfected (14.0%).

Table 4. 1 Demographic and characterized clinical information of study individuals with evidence of injection scars.

Characteristics	HCV[-]/HIV[-] IHUs n = 128	HCV[-]/HIV[+] IHUs n = 102	HCV[+]/HIV[-] IHUs n = 37	HCV[+]/HIV[+] IHUs n = 22	P
Gender:					
Female n %	13 (10.1)	48 (47.0)	1 (2.7)	4 (18.2)	**0.0001**
Male n %	115(89.0)	54 (53.0)	36 (97.3)	18 (81.8)	
Age(median, range)	34.9(18.7-51.1)	34.4(21.9-47.0)	39.3(23.1-55.5)	40.3(23.4-57.2)	**<0.001**
CD4 cell count (cells/ ml blood)	1200(600-1800)	650(250-800)	800(200-1400)	320(60-580)	**<0.001**
Log_{10} HIV-RNA copies/ml (Median, range)	-	2.2(1.6-2.8)	-	3.9(2.2-5.7)	0.569
Log10 HCV RNA copies/ml (Median, range)	-	-	3.0(1.8- 4.2)	3.7 (2.5 - 4.8)	0.475
Duration of injection n%					
>1 year	19 (14.8)	20 (19.6)	33 (89.1)	17 (77.3)	

≤ 1 year	3 (2.3)	16 (15.6)	29 (78.3)	19 (86.4)	**0.0001**
Frequency of injection n %					
> 1	18 (14.0)	28 (27.4)	28 (75.6)	18(82.0)	
≤1	4 (3.1)	9 (8.8)	26 (70.2)	16 (72.7)	**0.004**

Data is presented as number and proportions (%) of subjects, and median (range) for age and viral load. Viral load was determined as viral copies/ml of plasma. Statistical evaluations were conducted by use of Fisher's for 2-by-2 cross-tabulations. HIV, human immunodeficiency virus. HCV, Hepatitis C virus, IHUs, injectable Heroin users. Figures in bold indicate significant *P*-values.

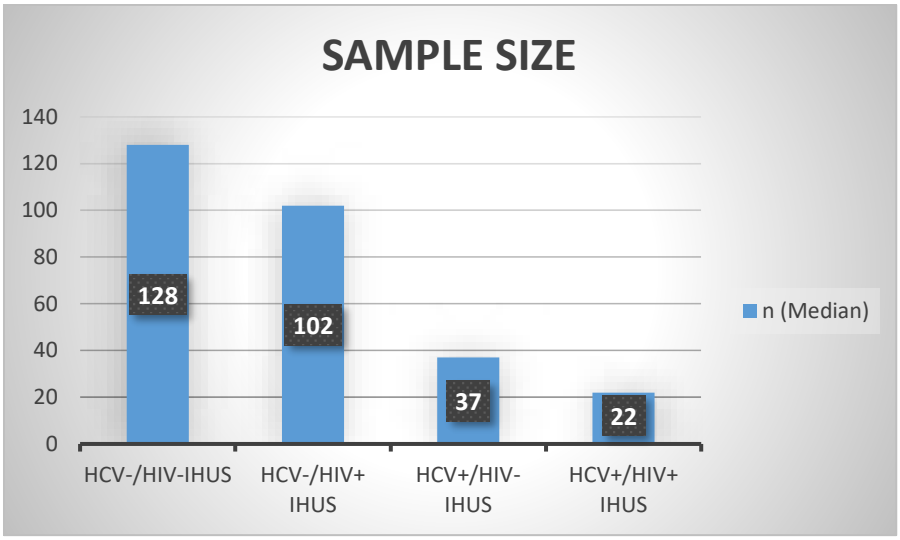


Figure 4.1 (a) Sample size of study participants

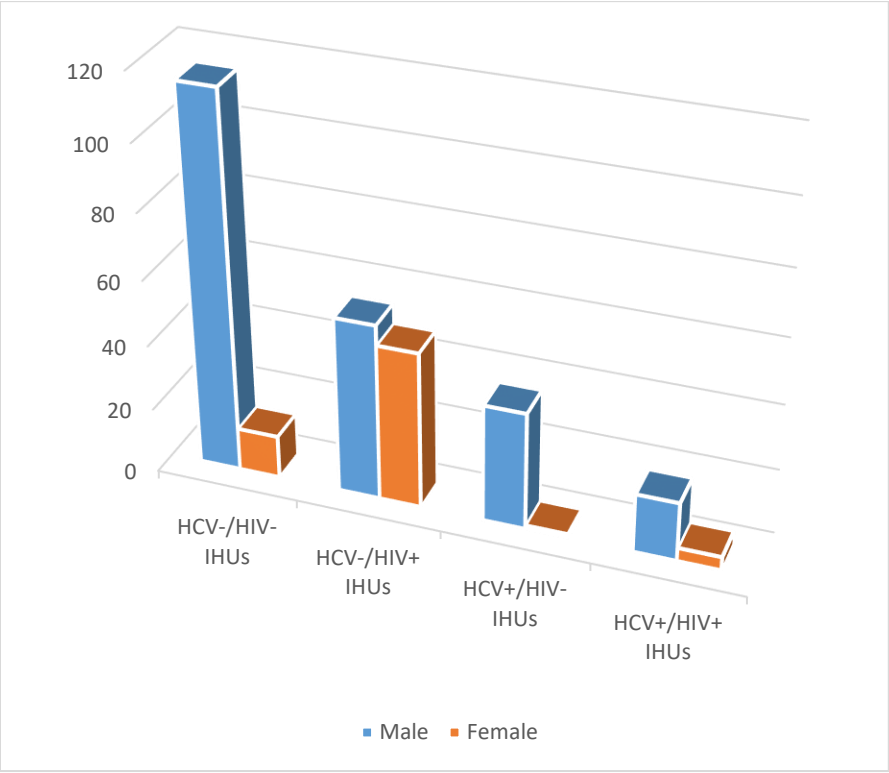


Figure 4.1(b) Gender (n) of study participants

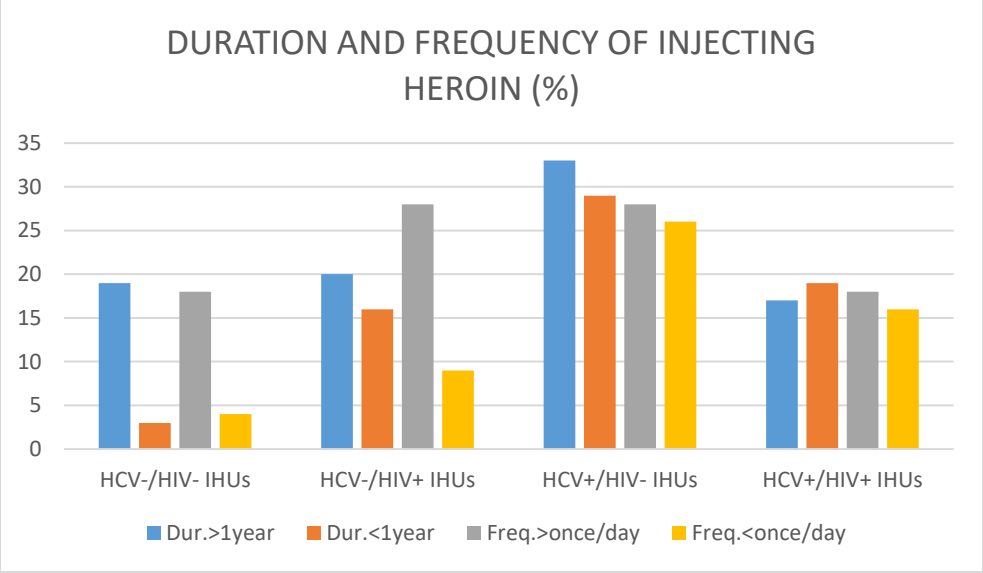


Figure 4.1 (c) Duration and Frequency of injecting heroin

4.2.0 Liver function parameters

4.2.1 Albumin levels

Median albumin was highest in HCV monoinfected IHUs (4.3g/dl, range 2.7 – 5.9) and lowest in HIV/HCV co-infected participants (2.7 g/dl, range 1.8 – 3.6). HIV monoinfected individuals also demonstrated markedly reduced albumin levels (3.2g/dl, range 2.8 – 3.6). The differences across groups were statistically significant (P= <0.0001).

4.2.2 Alanine aminotransferase (ALT)

Alanine aminotransferase (ALT) levels progressively increased from uninfected IHUs (23.4 IU/L, range 9.4 – 37.4) through HIV monoinfected (26.0 IU/L, range 15.6 – 36.4) and HCV monoinfected (29.4 IU/L, range 14.8 – 43.9) individuals, reaching the peak in the co-infected group (32.0 IU/L, range 18.7 – 45.3). This stepwise elevation was significant (P= 0.003), consistent with hepatocellular injury.

4.2.3 Alkaline Phosphatase (ALP)

Alkaline phosphatase (ALP) was lowest in uninfected IHUs (79.4 IU/L, range 43.1 – 115.6) and increased steadily across HIV mono infected (99.2 IU/L, range 68.1 – 130.2), HCV mono infected (99.0 IU/L, range 64.1 – 133.9), and co-infected (102.5 IU/L, range 72.2 – 132.8) groups. This increase was also significant (P <0.0001).

4.2.4 Gamma Glutamyl aminopeptidase (GGT)

Uninfected IHUs had the lowest GGT (28.7 IU/L, range 9.4 – 48.0). Levels were elevated in HIV mono infected (48.1 IU/L, range 22.6 – 73.5), HCV mono infected (56.4 IU/L, range 28.2 – 84.7), and co-infected (51.0 IU/L,

range 26.4 – 75.6) participants. Differences were highly significant (P<

Parameter	HIV-1 [-] HCV[-] n = 128	HIV-1 [+] HCV[n = 102	HIV-1 [-] HCV[+ n = 37	HIV-1 [+] HCV[n = 22	P
Liver function parameters					
Albumin, g/dl	4.0 (3.1-4.9)	3.2 (2.8-3.6)	4.3 (2.7-5.9)	2.7 (1.8-3.6)	**<0.0001**
ALT, IU/L	23.4 (9.4-37.4)	26.0 (15.6-36.4)	29.4 (14.8-43.9)	32 (18.7-45.3)	**0.003**
GGT, IU/L	28.7 (9.4-48.0)	48.1 (22.6-73.5)	56.4 (28.2-84.7)	51.0 (26.4-75.6)	**<0.0001**
ALP, IU/L	79.4 (43.1-115.6)	99.2 (68.1-130.2	99.0 (64.1-133.9)	102.5 (72.2-132.	**<0.0001**

0.0001).

TABLE 4.2: LIVER FUNCTION PARAMETERS OF STUDY PARTICIPANTS

Data shown are median, and interquartile range (IQR) for continuous variables of study subjects. HIV-1 [-]; Human immunodeficiency virus type 1 negative. HIV-1 [+]; HIV-1 positive. ALT; Alanine aminotransferase. GGT; Gamma glutamyl transpeptidase. ALP; Alkaline phosphatase. IU/L; international units per liter. Data

analysis was conducted using Kruskal wallis test. Thereafter, Bonferroni post-hoc test was used for comparisons between study groups. P values in bold are significant.

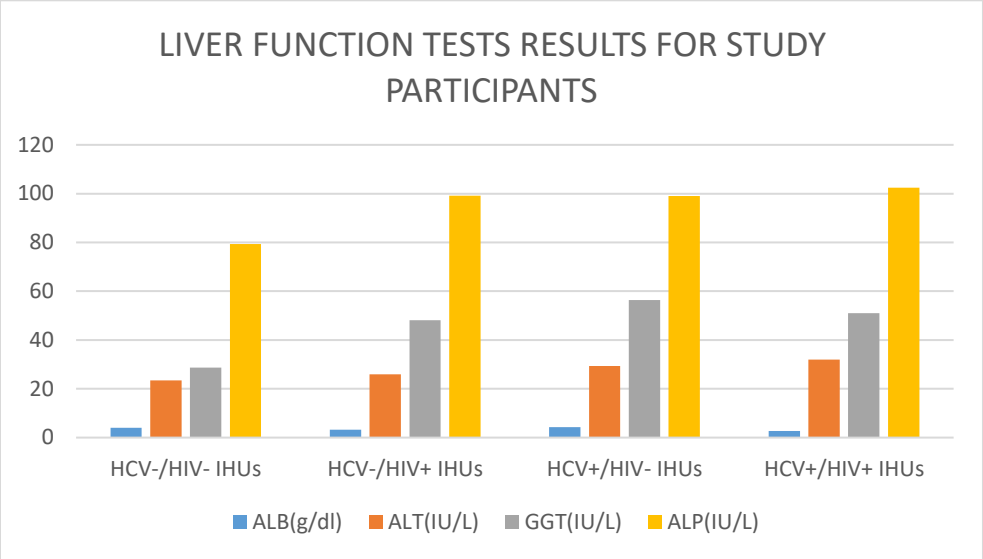


Figure 4.2 Liver function parameters

4.3.0 Metabolic Biomarkers

4.3.1 High Density Lipoprotein Cholesterol

The median HDL-C concentration was 1.4 mmol/L (1.2 – 1.6) among HIV/HCV uninfected IHUs, 1.2 mmol/L (1.1-1.3) in HIV monoinfected IHUs, 1.25 mmol/L (1.2 – 1.3) in HCV monoinfected IHUs, and 1.1 mmol/L (0.8 – 1.4) in HIV-1/ HCV co-infected individuals. Although values were lowest in co-infected IHUs, the differences did not reach statistical significance (P= 0.663). Nevertheless, HDL-C levels in all groups drifted toward the low –to- borderline range, suggesting impaired reverse cholesterol transport.

4.3.2 Low Density Lipoprotein Cholesterol

LDL-C levels were highest in HIV monoinfected IHUs (3.5 mmol/L, range

rCharacteristics	HCV-]/HIV[-] IHUs n = 128	HCV]/ HIV[+] IHUs n = 102	HCV[+]/HIV[-] IHUs n = 37	HCV+]/HIV[+] IHUs n = 22	P
Metabolic biomarkers					
HDL-C (mmol/l)	1.4(1.2-1.6)	1.2(1.1-1.3)	1.25(1.2-1.3)	1.1(0.8-1.4)	0.663
LDL-C (mmol/l)	3.0(2.6–3.4)	3.5(2.8 – 4.2)	2.6 (2.4 - 2.8)	2.2(21 – 2.3)	0.344
Tg (mmol/l)	1.5(1.2-1.8)	2.0(1.8 – 2.2)	4.2 (3.2 – 5.2)	5.0(4.6 – 5.4)	0.069

2.8 – 4.2), falling into the borderline – high category. HCV monoinfected IHUs exhibited reduced LDL-C (2.6 mmol/L, range 2.4 – 2.8; the optimal range), while HIV/HCV co-infected participants showed the lowest levels (2.2 mmol/L, range 2.1 – 2.3; below optimal). Despite the downward trend in LDL-C with HCV exposure, the differences across groups were not significant (P= 0.344).

4.3.3 Triglycerides

For Triglycerides, a progressive elevation was observed from uninfected IHUs (1.5 mmol/L, range 1.2 – 1.8; normal) to HIV-1 monoinfected (2.0 mmol/L, range 1.8 – 2.2

Table 4.3: Metabolic biomarkers of study participants

Data is presented as median (ranges) for continuous variables of study subjects. Low density lipoprotein, High density lipoprotein and Triglycerides were all measured in mmol/L. Statistical evaluations were con-

ducted by use of Fisher's for 2-by-2 cross-tabulations. HIV, human immunodeficiency virus. HCV, Hepatitis C virus, IHUs, injectable Heroin users, HDL-C, High density lipoprotein-levels of below 1.0 mmol/L (men) were considered to be low, below 1.3 mmol/L (women) low, above 1.0 mmol/L (men) acceptable, above 1.3 mmol/L (women) acceptable and levels above 1.55 mmol/L were considered high, LDL-C, Low density lipoprotein cholesterol-levels below 2.6 mmol/L were considered to be optimal, 2.6 – 3.3 mmol/L above optimal, 3.4 – 4.1 mmol/L borderline high, 4.1 – 4.9 mmol/L high and levels above 4.9 mmol/L were considered to be very high . Tg, Triglycerides- Levels below 1.7 mmol/L were considered to be normal, 1.7 – 2.2 mmol/L borderline high, 2.3 – 5.6 mmol/L high and levels above 5.6 mmol/L were considered to be very high.

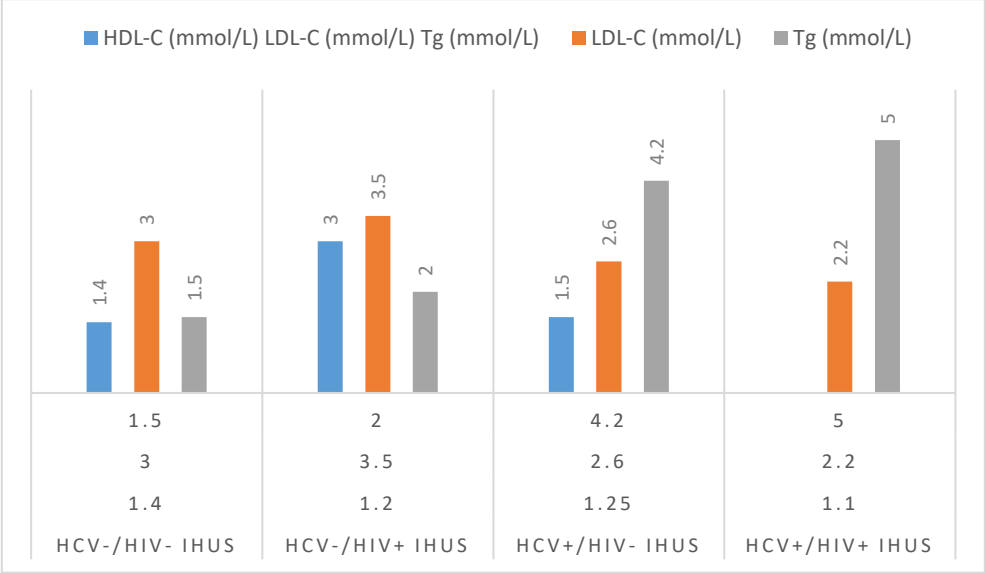


Figure 4.3 Metabolic biomarkers of study participants

4.4.0 Anthropometric characteristics

4.4.1 Heights

Median heights across groups were comparable: median 1.7m, range (1.5 – 1.9) in uninfected IHUs, median 1.7m, range (1.5 – 1.9) HIV-1 monoinfected IHUs 1.8m (1.6 – 1.9) in HCV monoinfected IHUs and 1.7m (1.5 – 1.9) in co-infected IHUs. Although the differences were small, statistical analysis showed significance (***P*=0.035**), suggesting that height distribution varied slightly between groups, with HCV monoinfected participants tending to be taller.

4.4.2 Weight

Uninfected IHUs had the highest median weight (74.0 kg, range 40.0 - 108.0), whereas HIV monoinfected (56.5kg, range 39.0 – 74.0), HCV

mono infected (55.5kg, range 42.0 – 69.0), and HIV-1/HCV co-infected (54.5kg, 39.0 – 71.0) participants had markedly lower weights. Despite this downward trend, the differences were not statistically significant (P=0.743).

4.4.3 Body Mass Index (BMI)

The median BMI was highest in HIV/HCV uninfected IHUs (22.7, range 12.1 – 33.3), consistent with normal weight. HIV monoinfected (19.6, range 13.5 – 25.6), HCV monoinfected (18.6, range 14.5 – 22.8), and HIV/HCV co-infected (18.4, 15.0 – 21.9) IHUs all exhibited reduced BMIs, approaching the underweight threshold. However, these differences were not statistically significant (P= 0.550) Table 4.4 Figure 4.4. Study findings show marked dysregulation in lipid metabolism.

Table 4.4: Anthropometric characteristics of study participants

Characteristics	HCV[-]/HIV[-] IHUs n = 128	HCV[-]/HIV[+] IHUs n = 102	HCV[+]/HIV[-] IHUs n = 37	HCV[+]/HIV[+] IHUsn= 22	P
Anthropometric parameters					
Height(m)	1.7(1.5-1.9)	1.7(1.5-1.9)	1.8(1.6-1.9)	1.7(1.5-1.9)	**0.035**
Weight(Kg)	74.0(40.0-108.0)	56.5(39.0-74.0)	55.5(42.0-69.0)	54.5(39.0-71.0)	0.743
BMI(Kg/m^2)	22.7(12.1-33.3)	19.6(13.5-25.6)	18.6(14.5-22.8)	18.4(15.0-21.9)	0.550

Data is presented as median (range) for height, weight and BMI. Body Mass Index (BMI) was measured in kg/m^2 while weight was measured in Kg. Height was measured in Meters. Statistical evaluations were conducted by use of Fisher's for 2-by-2 cross-tabulations. BMI of < 18.5 was considered underweight, BMI 18.5-24.9, Normal weight, BMI of 25-29.5, overweight while BMI $\geq$30 was considered Obesity. Figures in bold indicate significant P-values.

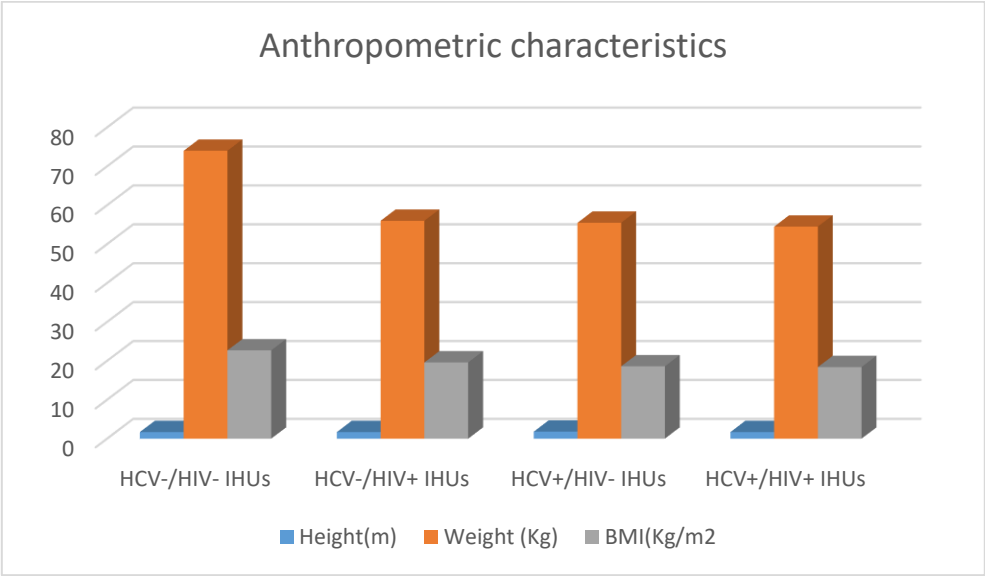


Figure 4.4 Anthropometric characteristics of study participants

4.5 Vitamin D3 and Calcium status

4.5.1 Vitamin D3

Uninfected IHUs had the highest reference Vitamin D_3 levels (25.4 ng/ml), with wide variability (3.0 – 44.0). HIV mono infection IHUs showed significantly reduced vitamin D3 levels (median 15.9 ng/ml), suggesting that HIV infection is strongly associated with Vitamin D depletion. HCV monoinfected (26.3 ng/ml) and HCV/HIV-1 co-infected (25.6 ng/ml) groups had vitamin D_3 values comparable to uninfected group, indicating that HCV infection alone or together with HIV did not further depress Vitamin D_3 levels. Statistical significance (**P= 0.0001**) confirms that HIV status is a major driver of vitamin D_3 alterations in this cohort. Vitamin D_3 deficiency (<10ng/ml) was relatively low across groups. Insufficiency (10 – 20 ng/ml) dominated in all groups, particularly among HIV-1 mono infection (76.7%) and co-infected individuals (100%). There were no participants in the HCV positive groups (with or without HIV-1) who fell into

the ‘deficiency’ category, but all were classified as insufficient. This pattern suggests that HIV infection increases the risk of Vitamin D_3 deficiency, while HCV infection is associated more with insufficiency rather than outright deficiency.

4.5.2 Ionized Calcium

Calcium levels were relatively uniform across all groups (22.2 – 22.7 mg/dl), with no significant differences (P= 0.350). This indicates that HIV-1 or HCV infection alone or co-infection does not substantially alter circulating ionized calcium levels in this population. Hypocalcemia was observed in 26.3% of uninfected participants, 35.6% of HIV-1 monoinfected, 42.1% of HCV monoinfected and 35.3% of co-infected individuals. Normocalcemia predominated across all groups (58.8% - 66.7%). The lack of statistical difference (P= 0.957) implies that calcium homeostasis is relatively preserved despite viral infections, though a considerable proportion still presented with hypocalcemia.

Table 4.5 Clinical information of study respondents

Characteristic	**HCV[-]/HIV[-] IHUs n = 177**	**HCV[-]/HIV[+]IHUs n = 136**	**HCV[+]/HIV[-] IHUs n = 37**	**HCV[+]/HIV[+] IHUs n = 22**	**P**
Vitamin D_3 (ng/ml)	25.4(3.0-44.0)	15.9 (3.1-35.1)	26.3(7.3-45.9)	25.6 (7.2-48.9)	**0.0001**
Deficiency n (%)	3.0 (9.1)	7.0 (13.3)	-	-	0.401
Insufficiency n (%)	19.0 (86.4)	23.0 (76.7)	1.0 (100.0)	8.0 (100.0)	
Ionized Calcium(mg/dl)	22.7 (11.6-28.9)	22.3 (12.5-35.8)	22.2 (16.2-24.7)	22.3 (18.0-27.0)	0.350
Hypocalcemia n (%)	46.0 (26.3)	30.0 (35.6)	8.0 (42.1)	7.0 (35.3)	0.957
Normocalcemia n (%)	76.0 (66.7)	43.0 (58.9)	11.0 (57.9)	10.0 (58.8)	0.957

Data are presented as numbers and proportions (%) of subjects. Statistical computations were conducted using the Fisher’s exact test for 2-by-2 cross-tabulations. HIV, human immunodeficiency virus. HCV, Hepatitis C

virus. IHUs, injection Heroin users. Figures in bold indicate significant *P*-values. Vitamin D_3 deficiency referred to serum levels below 10ng/ml, insufficiency to serum levels between 10ng/ml to 20 ng/ml, while sufficiency to serum levels between 30ng/ml to 100ng/ml. Hypocalcemia referred to ionized serum calcium levels below 8.5mg/dl, Normocalcemia to serum levels between 8.5mg/dl to 10.5mg/dl and Hypercalcemia to levels above 10.5mg/dl.

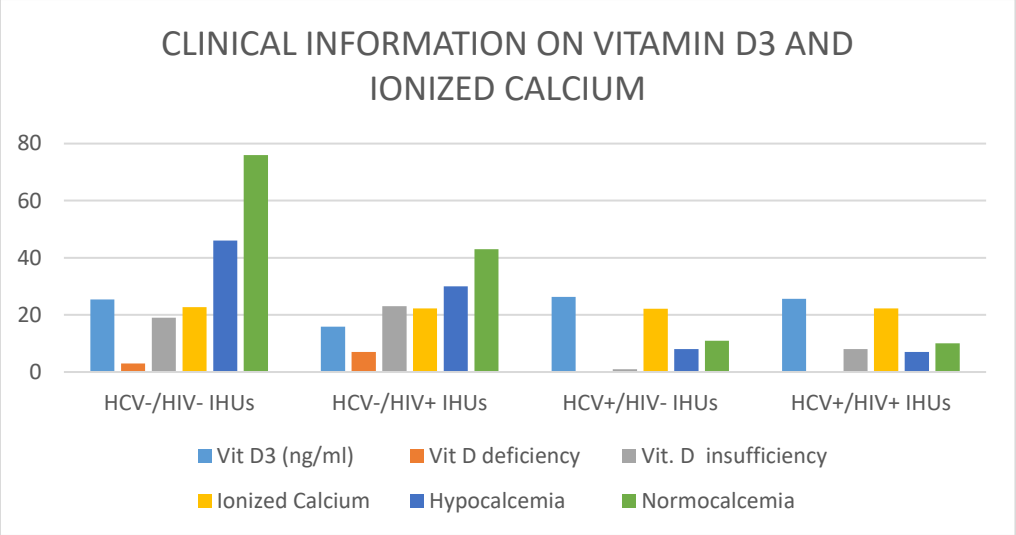


Figure 4.5 Clinical information of study respondents

4.6 CORRELATION ANALYSIS

4.6.1 Correlation of liver function with clinical outcome measures in HIV and HCV infected IHUs

4.6.1.1 Albumin with clinical outcome measures

Albumin showed weak positive correlation with BMI (ρ=0.113, P=0.476) and HIV viral load (ρ=0.158, P=0.318), but weak negative correlation with CD4 count (ρ= -0.214, P0.173). However, all did not reach the statistically significant threshold.

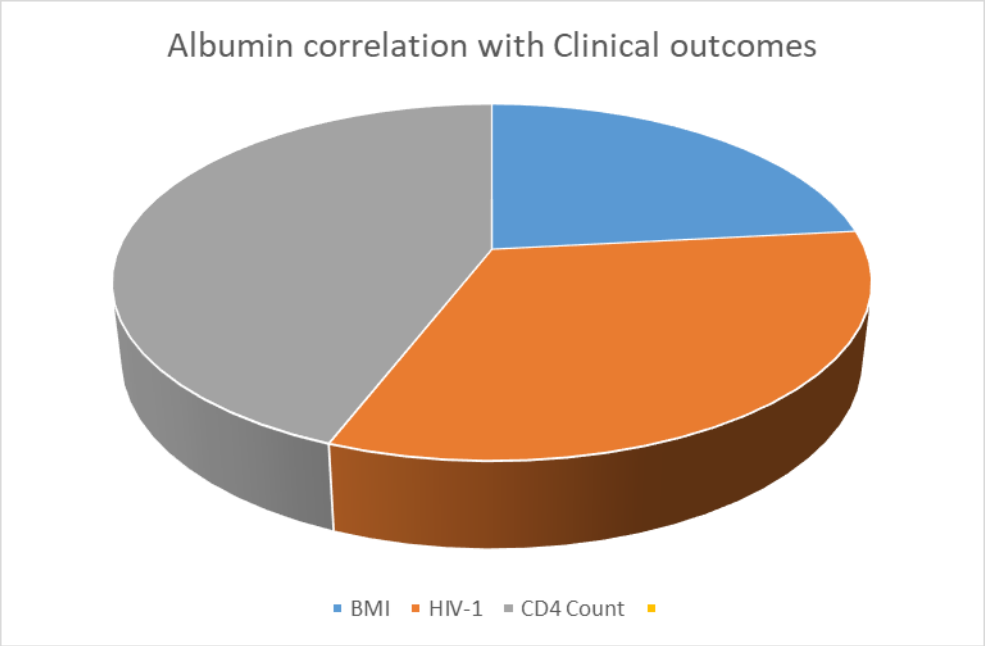


Figure 4.6.1.1 Albumin correlation with clinical outcome measures

4.6.1.2 Alanine Aminotransferase with clinical outcome measures

There was negative correlation between alanine aminotransferase and BMI (ρ=-0.054, P=0.735). This suggests that there is no meaningful link between body composition and hepatocellular injury. ALT showed weak positive correlation with viral load (ρ=0.164, P= 0.298), which implied that higher HIV replication might modestly increase ALT. Weak negative correlation was seen with CD4 (ρ=-0.211, P=0.179). However, this was not statistically significant.

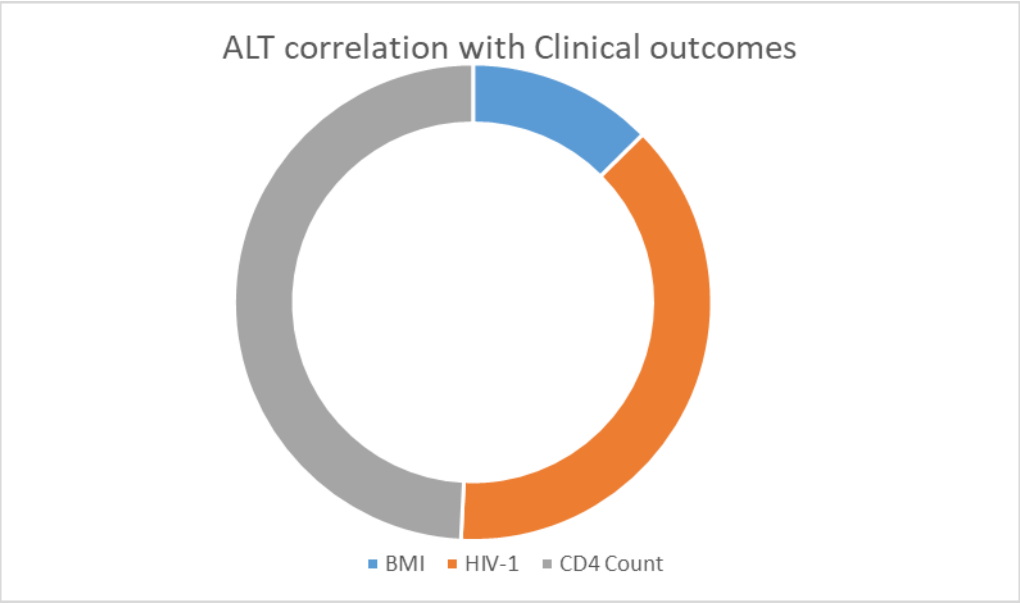


Figure 4.6.1.2 ALT with clinical outcome measures

4.6.1.3 Alkaline phosphatase with clinical outcome measures

ALP showed very weak correlation with BMI (ρ=-0.036, P=0.819). Positive correlation was seen with viral load (ρ=0.195, P=0.216), and negative correlation with CD4 (ρ=-0.169, P=0.285). All these were statistically non-significant.

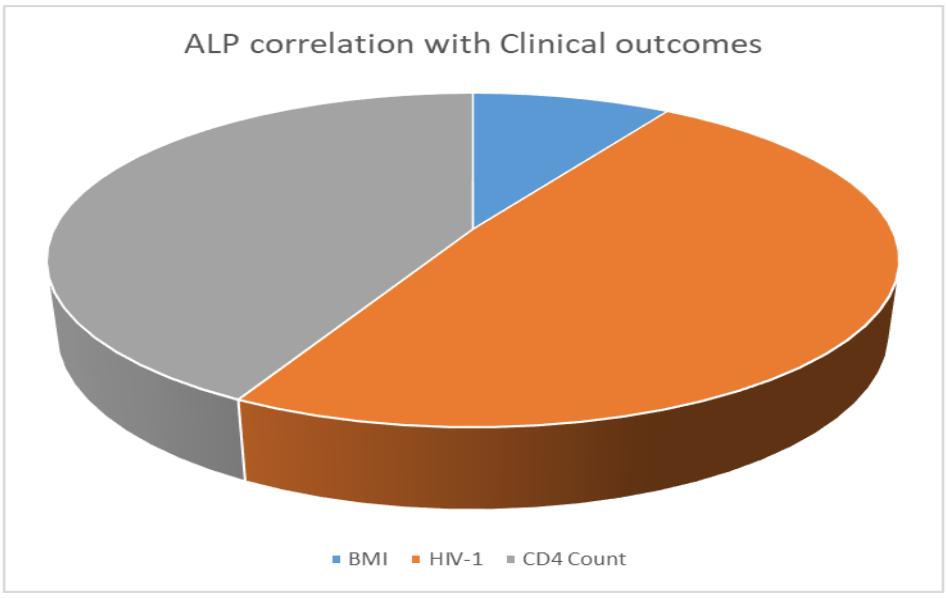


Figure 4.6.1.3 ALP correlation with clinical outcomes

4.6.1.4 Gamma-Glutamyl Transferase with clinical outcome measures

Gamma Glutamyl Transferase showed positive but nonsignificant correlation with BMI (ρ=0.129, P=0.414) and viral load (p=0.202, P=0.199). Negative correlation However, there was negative correlation with CD4 (ρ= -0.229, P=0.145).

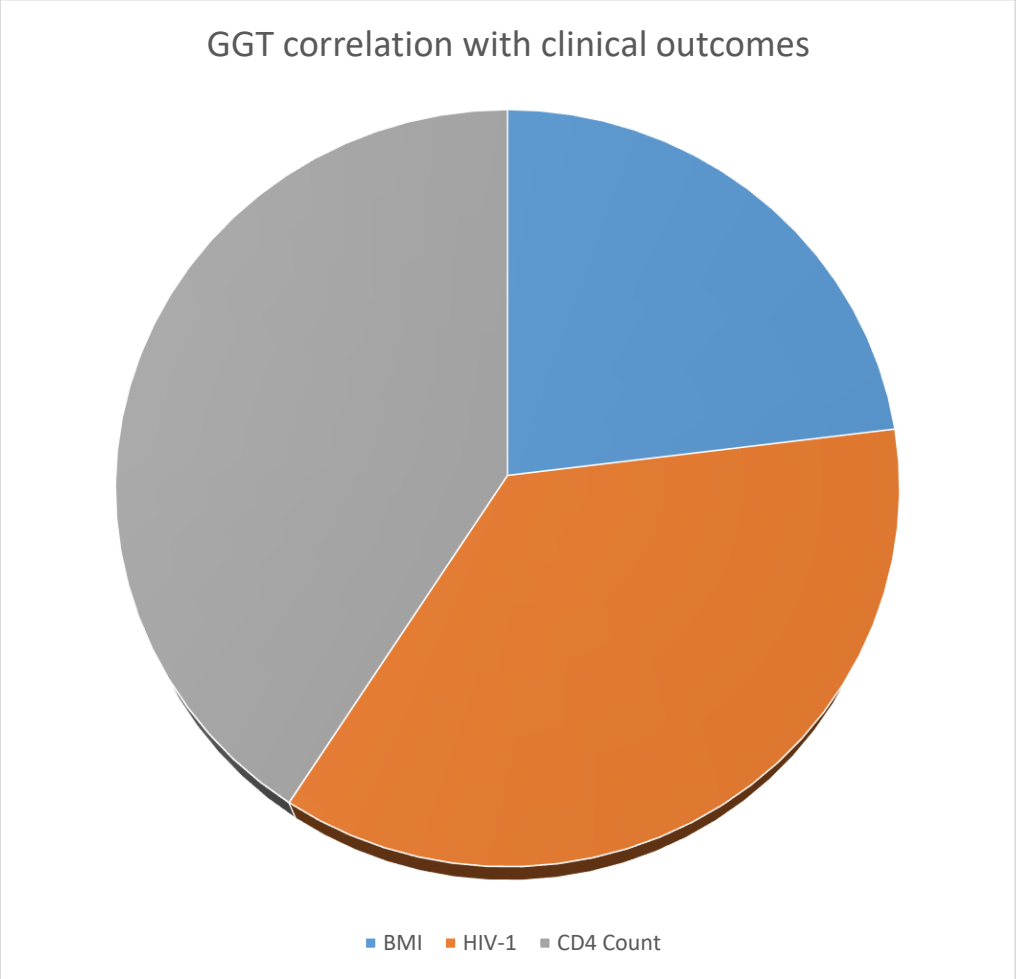


Figure 4.6.1.4 GGT correlation with clinical outcomes

Table 4.6 Correlation of liver function with Clinical outcome measures in HIV and HCV infected IHUs

Param-eter	BMI, kg		HIV-Viral loads		CD4+ count	
	ρ	P	ρ	P	ρ	P
Albumin	0.113	0.476	0.158	0.318	-0.214	0.173
ALT,(IU/L)	-0.054	0.735	0.164	0.298	-0.211	0.179
ALP,(IU/L)	-0.036	0.819	0.195	0.216	-0.169	0.285
GGT, (IU/L)	0.129	0.414	0.202	0.199	-0.229	0.145

Data presented are correlation coefficient (rho, ρ) with associated p-values. Statistical analysis was performed using Spearman's rank correlation test. BMI; body mass index; HIV, human immune virus; CD4, cluster of differentiation 4; Albumin, ALT; Alanine aminotransferase, ALP; alkaline phosphatase, GGT; gamma glutamyl transferase.

CHAPTER FIVE

DISCUSSION OF THE RESULTS

5.0 Overview

Clinical chemistry laboratory investigation is a major contributor to key steps in patient evaluation for initiating treatment and monitoring prognosis in HCV and HIV infections among people who inject heroin. The reproducibility, precision and accuracy of clinical chemistry analytes, however, is confounded by hard to understand interaction between use of injectable illicit drugs and individual life style. Hence, this clinical laboratory case control retrospective study determined the clinical chemistry biomarkers in HCV and HIV co-infected injection heroin users from across coastal region in Kenya. Investigation of clinical chemistry profiles in disease status is key in effective clinical patient management. The integrity of results outcome is complicated by co-infection and use of injection her-

oin, hence this case control retrospective study analyzed the clinical chemistry parameters in HCV and HIV-1 co-infected heroin users from Mombasa, Kenya. This study evaluated demographic, clinical and biochemical parameters among injectable heroin users with different HIV-1 and HCV infection statuses, focusing on hepatocellular injury and metabolic dysregulation. The findings reveal that HIV-1/ HCV co-infected individuals exhibit more profound alterations in liver function and lipid metabolism compared to mono-infected or uninfected heroin users.

5.1 Demographic characteristics

The demographic and behavioral profiles of IHUs in this cohort illustrate the interplay between drug-use patterns and blood-borne viral infections.

5.1.1 Gender distribution

The significant gender imbalance (P=0.0001) suggests women may be more represented among HIV-infected but less so among HCV monoinfected groups. This may reflect differences in transmission dynamics, where HIV is more often linked to sexual exposure in women, while HCV transmission is more closely tied to direct blood contact from injection sharing.

This findings reflect the male-dominant profile of heroin injection populations, consistent with regional and global patterns (WHO, 2022) (ClinicalInfo(NIH), 2024). The slightly higher female representation in HIV groups suggests that women who inject heroin may have higher HIV risk, likely due to sexual vulnerability, transactional sex and needle sharing practices. The low female proportion in HCV monoinfected group

suggests that HCV transmission is more injection-driven, while HIV transmission combines both sexual and injection routes.

5.1.1 Age differences

The age differences (P=0.001) suggest that older IHUs carry a higher burden of HCV infection, consistent with cumulative exposure risk over time. This agrees with global epidemiology showing that HCV prevalence increases with injection duration. (UNAIDS, 2023).

The findings suggests age –related accumulation of risk. HCV infection, being strongly associated with longer injection duration, appears in older users who have had prolonged exposure. Co-infected individuals were the oldest group, reflecting cumulative risk and longer drug use histories. Younger HIV mono infected individuals indicate that HIV acquisition occurs earlier, possibly via sexual exposure and unsafe injection practices, while HCV infection peaks later due to cumulative parenteral exposure.

5.1.2 CD4 counts

As expected, CD4 counts differed markedly between groups, with the highest values seen among the uninfected group and the lowest values observed in HIV/HCV co-infected individuals. This is consistent with the well-documented immunosuppressive effect of HIV infection, which progressively depletes CD4+ T lymphocytes (Chen et al, 2021). The further reduction in CD4 counts among co-infected individuals suggests synergistic effects of HCV in aggravating immune dysregulation and accelerating HIV disease progression. Several studies have reported that co-infection leads to impaired immune recovery, increasing the risk of opportunistic infections and liver related morbidity (Chen et al, 2021). CD4 count was

measured in uninfected individuals to ascertain whether other comorbidities had detrimental effect on the immune system which could compromise the findings of this study. However, this calls for further longitudinal studies to be conducted.

5.1.3 Viral loads

Although viral loads did not differ significantly across infection categories, trends indicated that co-infected individuals carried higher HIV viral loads compared to monoinfected HIV^+ IHUs. Similarly, HCV burden remained comparable between mono and co-infected groups, reflecting independent viral replication dynamics. These findings align with studies showing that HIV does not necessarily suppress HCV replication, though co-infection may accelerate liver injury (Boateng et al, 2019).

5.1.4 Duration and frequency of injection use

The duration and frequency of injection use emerged as the strongest predictors of infection. Longer duration (>1 year) and high frequency (>1 injection/day) were overwhelmingly associated with HCV positivity (P=0.0001 and P=0.004, respectively). This emphasizes the critical role of behavioral risk in driving transmission, particularly in environments with limited harm-reduction measures, for example needle exchange programs. Together, these findings indicate that prolonged and frequent injection drug use increases vulnerability to HCV and HIV/HCV co-infection, with implications for hepatocellular injury and subsequent metabolic dysregulation. Prevention strategies targeting early-stage IHUs, harm reduction interventions, and gender sensitive HIV prevention programs are warranted.

5.2.0 Liver function parameters

The findings of this study demonstrate clear evidence of hepatocellular injury and liver dysfunction among injectable heroin users, accentuated by HIV and HCV infection.

5.2.1 Albumin and immune status

Albumin, a key marker of hepatic synthetic capacity, was significantly reduced in HIV and HIV/HCV co-infected IHUs compared to uninfected controls. Hypoalbuminemia may reflect impaired protein synthesis due to viral –induced hepatocellular injury, chronic inflammation and malnutrition common among heroin users (Huang R et al, 2024). The lowest albumin levels in the co-infected group highlight the addictive burden of dual infections.

5.2.2 Alanine aminotransferase (ALT)

As marker of hepatocellular damage, ALT levels rose progressively with HIV and HCV infection, reaching their peak in co-infected individuals. This pattern suggests cumulative hepatocyte injury, consistent with viral cytopathic effects, immune mediated hepatotoxicity and potential hepatotoxic contributions from heroin.

5.2.3 Alkaline phosphatase (ALP)

ALP levels were significantly higher among infected groups, pointing to biliary epithelial cell involvement. The trend is compatible with chronic liver disease progression, including fibrosis and cholestatic injury, particularly in HCV infection (YU N et al, 2025).

This study data highlight the synergistic hepatotoxic effect of HIV and HCV in the context of heroin use, even before overt clinical liver disease develops.

5.2.4 Gamma Glutamyl transaminase (GGT)

GGT elevations were pronounced in all infected groups. This enzyme is a sensitive marker of cholestasis and microsomal enzyme induction, and chronic heroin use likely contributes to these elevations, while viral infection amplifies the hepatobiliary stress.

5.3 Metabolic biomarkers

The observed lipid profiles reflect metabolic dysregulation linked to viral coinfections in the context of heroin use. The profiles of study participants showed varying alterations across the four groups, although statistical significance was not reached (all p-values >0.05).

5.3.1 High density lipoprotein cholesterol (HDL-C)

Levels were lowest among HIV+/HCV+ co-infected IHUs median 1.1mmol/L (0.8-1.4) compared to HIV-/HCV- individuals median 1.4 mmol/L (1.2-1.6). Low HDL-C is a hallmark of both HIV infection and chronic HCV due to persistent inflammation and immune activation related dyslipidemia. This reduction in protective cholesterol may accelerate cardiovascular risk in coinfected individuals, consistent with previous reports that HIV and HCV synergistically worsen lipid metabolism (Yu N et al, 2025)

5.3.2 Low density lipoprotein cholesterol (LDL-C)

There was elevated LDL-C in HIV+/HCV- IHUs median 3.5 mmol/L (2.8-4.2), while HIV+/HCV+ co-infected had the lowest LDL-C median

2.2 mmol/L (2.1-2.3). HIV mono-infection is often associated with dyslipidemia, particularly increased LDL-C, due to drug induced metabolic effects. In contrast, HCV infection is known to lower LDL-C levels because HCV particles utilize LDL receptors for entry into hepatocytes, thereby depleting circulating LDL cholesterol (Chongo S et al, 2024). This explains the suppressed LDL-C levels in HCV-positive groups.

5.3.3 Triglycerides (Tg)

The most striking differences were seen in triglycerides, where HIV-/HCV- and HIV+/HCV+ groups had relatively normal levels median 1.5 and 2.0 mmol/L respectively, while HCV positive groups showed pronounced hypertriglyceridemia, with HIV-/HCV+ group median 4.2 mmol/L (3.2-5.2) and HIV+/HCV+ group median 5.0 mmol/L (4.6-5.4). Elevated triglycerides in HCV mono-infected individuals may be attributed to viral interference with hepatic lipid metabolism, impaired clearance of triglycerides-rich lipoproteins and the impact of chronic heroin use on metabolic pathways. High triglycerides have been associated with increased risk of hepatic steatosis and progression to liver fibrosis in HCV infection (Pascual-Oliver et al, 2024).

While the differences did not reach statistical significance, the observed patterns align with established metabolic dysregulation in HIV, HCV and coinfection. HIV tends to lower HDL-C and increase LDL-C, while HCV suppresses LDL-C but raises triglycerides. Co-infected individuals experience the combined impact, with the most adverse lipid profile i.e. low HDL-C, low LDL-C, and markedly elevated triglycerides. This metabolic

profile is strongly atherogenic and hepatotoxic, potentially accelerating both cardiovascular disease and liver injury in IHUs.

5.4 Anthropometric parameters

Anthropometric measurements provide important insights into the nutritional status and metabolic health of IHUs in the context of HIV and HCV infection. The profiles reveal that HIV and/or HCV infection among heroin users is associated with reduced body weight and lower BMI, reflecting poor nutritional status and possible wasting, though the differences were not statistically significant. Overall, the data underscore the role of malnutrition in compounding hepatocellular injury and metabolic dysregulation in this high-risk group.

5.4.1 Height differences

The statistically significant variation in height across groups ($p=0.035$) is unlikely to reflect the impact of infection status, as adult height is largely genetically determined and not significantly altered by viral infection. Instead, this may represent sampling variability or demographic differences between groups.

5.4.2 Weight decline with HIV/HCV infection

Injection heroin users with either HIV mono-infected, HCV mono-infected or co-infected consistently showed lower body weight compared to uninfected IHUs. This trend reflects the combined effect of chronic infection, heroin use and associated lifestyle factors, all of which are known to cause weight loss through anorexia, malnutrition, altered metabolism and catabolism.

5.4.3 Body Mass Index (BMI)

The reduced BMI in HIV- and/or HCV –infected IHUs (median ~18.4 - 19.6) suggests higher prevalence of undernutrition and wasting compared to uninfected controls (median 22.7, normal range). Although not statistically significant, this pattern aligns with clinical expectations, for example HIV infection is strongly associated with wasting syndrome, driven by increased energy expenditure, malabsorption and opportunistic infections (Yu N et al, 2025). HCV infection also contributes to metabolic disruption and malnutrition, particularly in advanced liver disease. Coinfection amplifies these effects, resulting in the lowest BMI values observed. The lower BMI in infected IHUs suggests nutritional vulnerability, which can worsen clinical outcomes, impair immune recovery, and accelerate liver disease progression (Karaca B et al, 2023). These findings highlight the importance of nutritional assessment and support in HIV/HCV care programs, especially for heroin users who face compounded risks of food insecurity and metabolic dysregulation.

5.5 Vitamin D_3 and Calcium levels

Study results demonstrate a consistent pattern of metabolic dysregulation among IHUs, with HIV infection exerting the most pronounced impact on Vitamin D_3 status.

5.5.1 Vitamin D_3 levels

The significant reduction in Vitamin D_3 among HIV mono-infected IHUs aligns with previous reports that HIV infection disrupts Vitamin D metabolism through chronic immune activation and nutritional deficiencies (Visuthranukul C et al, 2023). Interestingly, HCV mono and co-infected

IHUs had relatively preserved Vitamin D_3 levels compared to HIV mono infected IHUs, suggesting that hepatic Vitamin D hydroxylation may only be severely impaired in advanced stages of HCV infection (Ravaioli F et al,, 2022). The near universal Vitamin D insufficiency across groups highlights the combined effects of heroin use, poor dietary intake, and limited sun exposure, consistent with previous reports in substance-using populations (Jaimni et al, 2021). Deficient vitamin D status is clinically relevant because it worsens hepatocellular injury by promoting fibrosis (Anna et al, 2020), enhances immune dysregulation, and contributes to metabolic complications including insulin resistance and bone demineralization.

5.5.2 Ionized calcium

Although no significant differences in median calcium levels were observed, the higher prevalence of hypocalcemia in HIV and HCV-infected IHUs is consistent with studies reporting deranged calcium metabolism in chronic viral infection (Escobedo-Monge et al, 2024). Vitamin D deficiency likely exacerbates this imbalance by impairing intestinal calcium absorption. Hypocalcemia, even when subclinical, has important implications, as it predisposes to osteopenia, osteoporosis and increased fracture risk, which are well documented complications in HIV-infected populations (Reid et al, 2020).

5.6 Correlation of liver function with clinical outcomes

The absence of statistically significant correlations between liver function biomarkers and clinical outcomes in HIV/HCV co-infected heroin users

suggests that liver injury in this population is multifactorial and not directly explained by BMI, viral load and /or CD4 count alone (Khan N H et al, 2024).

5.3.1 Albumin and clinical outcomes

Albumin tended to be lower with reduced CD4 counts, which is biologically plausible, as immune suppression and advanced HIV disease are often associated with poor nutritional status and impaired hepatic protein synthesis. The lack of statistical significance, however, may reflect small sample size and wide inter-individual variability.

5.3.2 Alanine aminotransferase (ALT) and clinical outcomes

The weak positive association of ALT with viral load suggests that ongoing viral replication may contribute to hepatocyte damage, while its negative trend with CD4+ count implies that worsening immunosuppression could exacerbates liver injury. Yet, the effect was modest and non-significant, likely due to confounding by heroin use and co-existing comorbidities.

5.3.3 Alkaline phosphatase (ALP) and clinical outcomes

ALP showed trends similar to ALT i.e. positively linked with viral load and negatively with CD4+ count, suggesting possible biliary involvement in the setting of higher viral replication and immunosuppression. Still, the weak strength of association indicates that other factors like drug- induced hepatotoxicity, malnutrition and opportunistic infections may be more influential.

5.3.4 Gamma Glutamyl transaminase (GGT) and clinical outcomes

GGT showed the strongest negative correlation with CD4+ count (ρ= -0.229), hinting that more profound immune suppression may worsen cholestatic or hepatocellular stress. The positive correlation with viral load is consistent with viral load-driven hepatic inflammation. Even though not significant, these trends align with literature describing GGT as a sensitive early marker of HIV/HCV related hepatobiliary stress (YU N et al, 2025). These study findings in general suggest that clinical outcomes such as immune suppression and viral replication only partly explain hepatocellular injury in co-infected heroin users. The lack of statistical significance may be due to small sample size, high biological variability and confounding by heroin use and malnutrition. The patterns, though weak, are consistent with the hypothesis that HIV/HCV co-infection accelerates liver injury by reducing CD4+ counts, impairing albumin synthesis and increasing enzyme leakage into plasma.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study investigated hepatocellular injury and metabolic dysregulation of selected biomarkers in HIV and HCV co-infected heroin users, with particular focus on liver function parameters (Albumin, ALT, GGT, ALP) and their correlation with clinical outcomes (BMI, viral load, CD4+ count). The findings of this study demonstrate that HIV and HCV co-infection significantly exacerbates hepatocellular injury compared to mono infections or uninfected heroin users. This was evidenced by a marked reduction in albumin and elevated levels of ALT, GGT and ALP. The study revealed that HIV and HCV co-infected heroin users experience a dual burden of infection-related and lifestyle-related metabolic dysregulation, with vitamin D3 insufficiency, hypocalcemia and altered ALP activity being prominent disturbances. These imbalances may potentiate hepatocellular injury, accelerate fibrosis progression, impair immune recovery and

increase the risk of skeletal fragility. Synergistic effects of viral co-infection and heroin use contribute to the progression of hepatic dysfunction, likely through mechanisms involving immune suppression, oxidative stress and direct viral cytopathic effects.

Correlation analysis revealed weak but biologically consistent trends, for example albumin and liver enzymes tended to worsen with higher viral loads and lower CD4 counts, indicating that immune status and viral replication are important determinants of liver injury.

This study noted that BMI was not a reliable predictor of hepatic dysfunction in this cohort, suggesting that nutritional indices alone may be insufficient in assessing liver health among HIV/HCV co-infected heroin users. The results underscore that hepatocellular injury in HIV/HCV co-infected injection heroin users is multifactorial, arising from the combined impact of viral infection, immune suppression, metabolic dysregulation and drug-related hepatotoxicity. Monitoring and management of such patients require an integrated approach that goes beyond traditional clinical indicators.

In this cohort of IHUs with HIV and /or HCV infection, correlations between liver function (albumin, ALT, ALP, GGT) and BMI, HIV viral load, or CD4+ count are weak. However, observed trend directions are consistent with literature indicating that higher HIV viral load tends to go with more liver enzyme elevation. Lower CD4+ counts tend to be associated with poorer synthetic function and possibly more injury. These findings indicate that hepatocellular injury and metabolic dysregulation are multi-

factorial in HIV/HCV co-infected heroin users, with immune status markers only part of the story. Given similar findings in more powered studies, for example China cohort showing low CD4 is strongly linked to hepatic events (Lin W et al, 2022), results of this study support the hypothesis but highlight the need for more precise measurement and longitudinal design to capture the dynamics.

Anthropometric profiles reveal that HIV and /or HCV infection among heroin users is associated with reduced body weight and lower BMI (Karaca B et al, 2023), reflecting poor nutritional status and possible wasting, though the differences were not statistically significant. The data underscore the role of malnutrition in compounding hepatocellular injury and metabolic dysregulation in this high-risk group.

The findings from this study highlight a distinct dissociation between vitamin D and calcium homeostasis in HIV and HCV uninfected heroin users. Vitamin D3 alterations were strongly linked to HIV infection, with HIV+/HCV- individuals showing the most profound depletion. In contrast, HCV infection was associated primarily with insufficiency but did not independently lower vitamin D levels. Calcium balance appeared more resilient, although a notable proportion of participants exhibited hypocalcemia irrespective of viral infection. These results reinforce the concept of metabolic dysregulation in HIV/HCV-infected heroin users, with vitamin D deficiency emerging as a significant concern. Given the established links between vitamin D insufficiency and hepatocellular injury, impaired immune function and poor clinical outcomes, these findings underscore the

need for metabolic screening and nutritional interventions in this high-risk population.

The study reveals that HIV and HCV co-infected heroin users experience a dual burden of infection –related and lifestyle-related metabolic dysregulation, with vitamin D insufficiency, hypocalcemia and altered ALP activity being prominent disturbances. These imbalances may potentiate hepatocellular injury, accelerate fibrosis progression, impair immune recovery and increase the risk of skeletal fragility.

6.2 Recommendations

6.2.1 Clinical practice

Routine liver function monitoring (Albumin, ALT, ALP, GGT) should be integrated into the care of HIV-1/HCV co-infected heroin users to enable early detection of hepatic injury. Comprehensive clinical evaluation should prioritize viral load and CD4+ count monitoring, as these are more reliable predictors of hepatocellular dysfunction than BMI. Nutritional and metabolic support should complement anti-viral therapy, given the confounding role of malnutrition and heroin use in worsening outcomes. Integration of harm reduction strategies (e.g methadone maintenance therapy and safe injection programs) into HIV/HCV care to minimize ongoing liver injury from heroin use.

6.2.1.1 Routine Lipid Monitoring

Regular assessment of lipid profiles in IHUs with HIV and/or HCV should be integrated into clinical care to detect early dyslipidemia.

6.2.1.2 Cardiovascular risk management

Lifestyle counseling, smoking cessation and consideration of lipid-lowering therapies should be offered to high-risk IHUs, especially those co-infected with HIV and HCV.

6.2.1.3 ART optimization

Clinicians should consider ART regimens with lower metabolic side effects to minimize LDL-C elevation and further lipid disturbances in HIV-infected IHUs.

6.2.2 Policy

6.2.2.1 Integrated services

Health systems should strengthen multidisciplinary care models combining hepatology, infectious disease, psychiatry and addiction medicine for co-infected heroin users.

6.2.2.2 Screening programs

Targeted screening programs for HIV/HCV in populations of injecting heroin users should be scaled up to enable early diagnosis and management.

Policy frameworks should incorporate liver health monitoring as a standard of care in HIV-1 and HCV treatment programs for injection heroin users.

6.2.2.3 Harm-reduction integration

Harm-reduction programs for IHUs should incorporate metabolic health assessments, including lipid monitoring and nutritional support.

6.2.3 Research

Further longitudinal studies are required to elucidate the long-term progression of hepatocellular injury in HIV-1/HCV co-infected heroin users and the impact of anti-viral therapy.

Further work should investigate novel metabolic biomarkers (eg lipid profile, insulin resistance markers and oxidative stress indices) to better characterize metabolic dysregulation.

Studies evaluating the effectiveness of integrated addiction treatment and antiviral therapy on improving liver outcomes in this population are warranted.

By addressing both viral pathogenesis and metabolic dysregulation, clinical outcomes for this vulnerable population can be significantly improve

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APPENDICES

Appendix I: Testing and monitoring algorithm

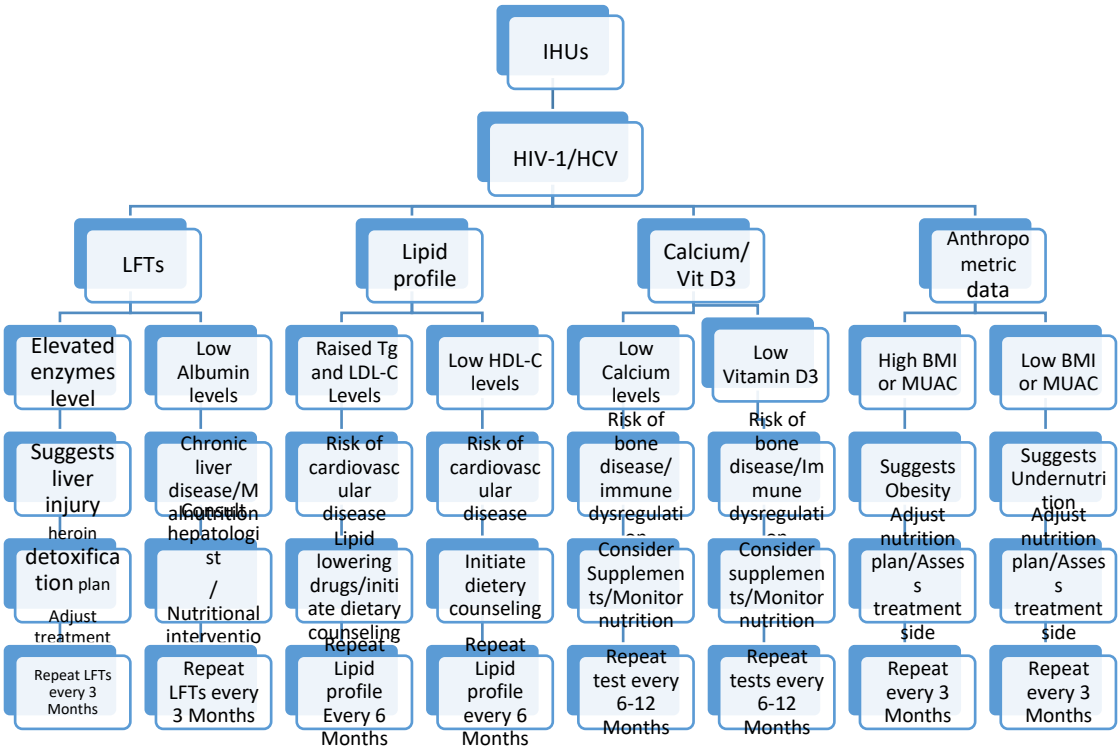
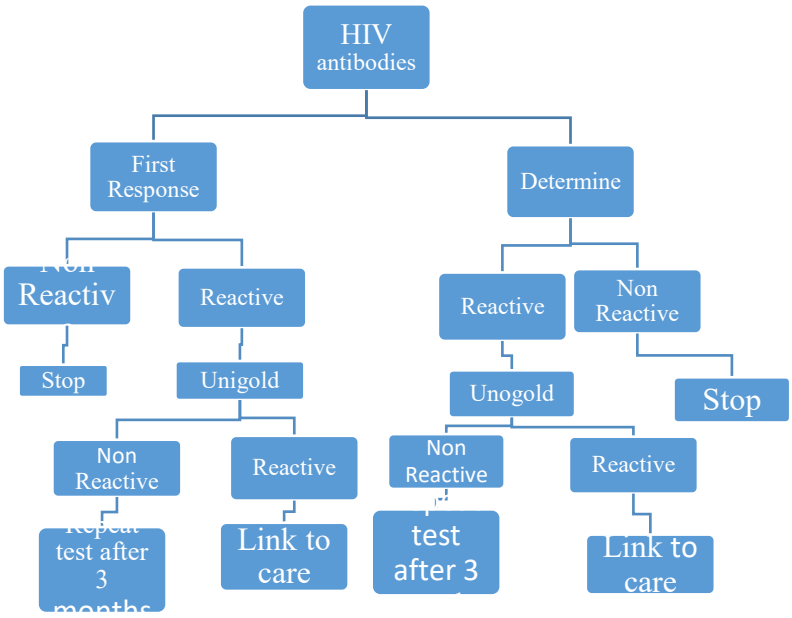


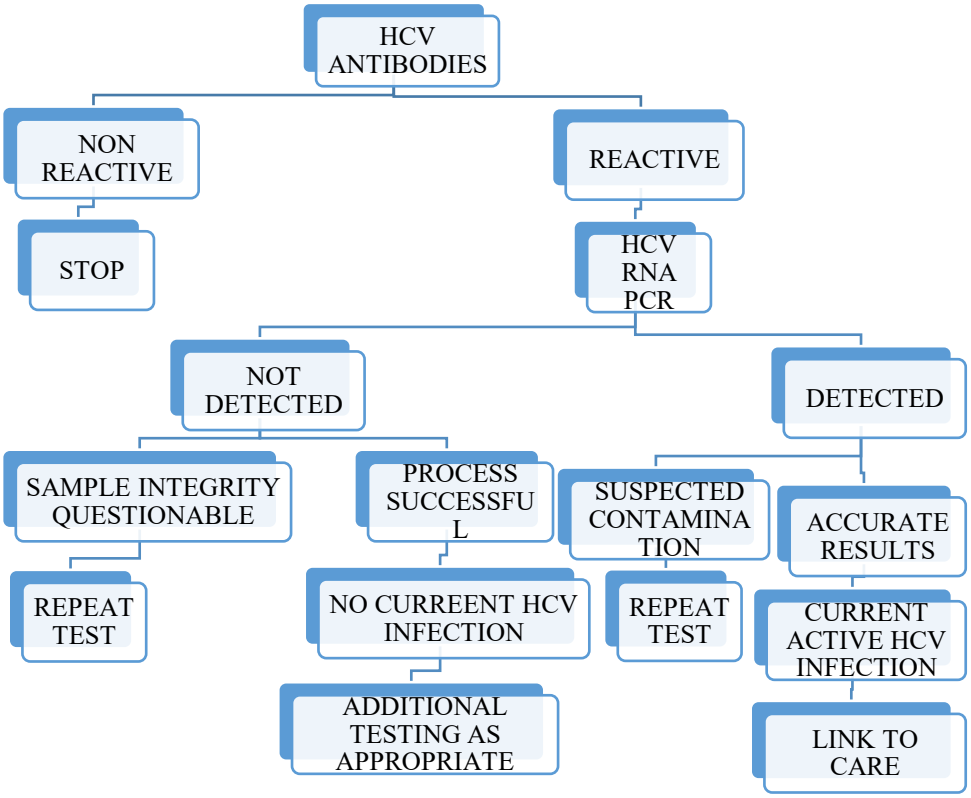
Figure I Testing algorithm

NB: If results are within normal range, continue routine monitoring of all parameters and maintain nutritional and substance abuse support programs.

Appendix II: HIV Testing and Monitoring Algorithm



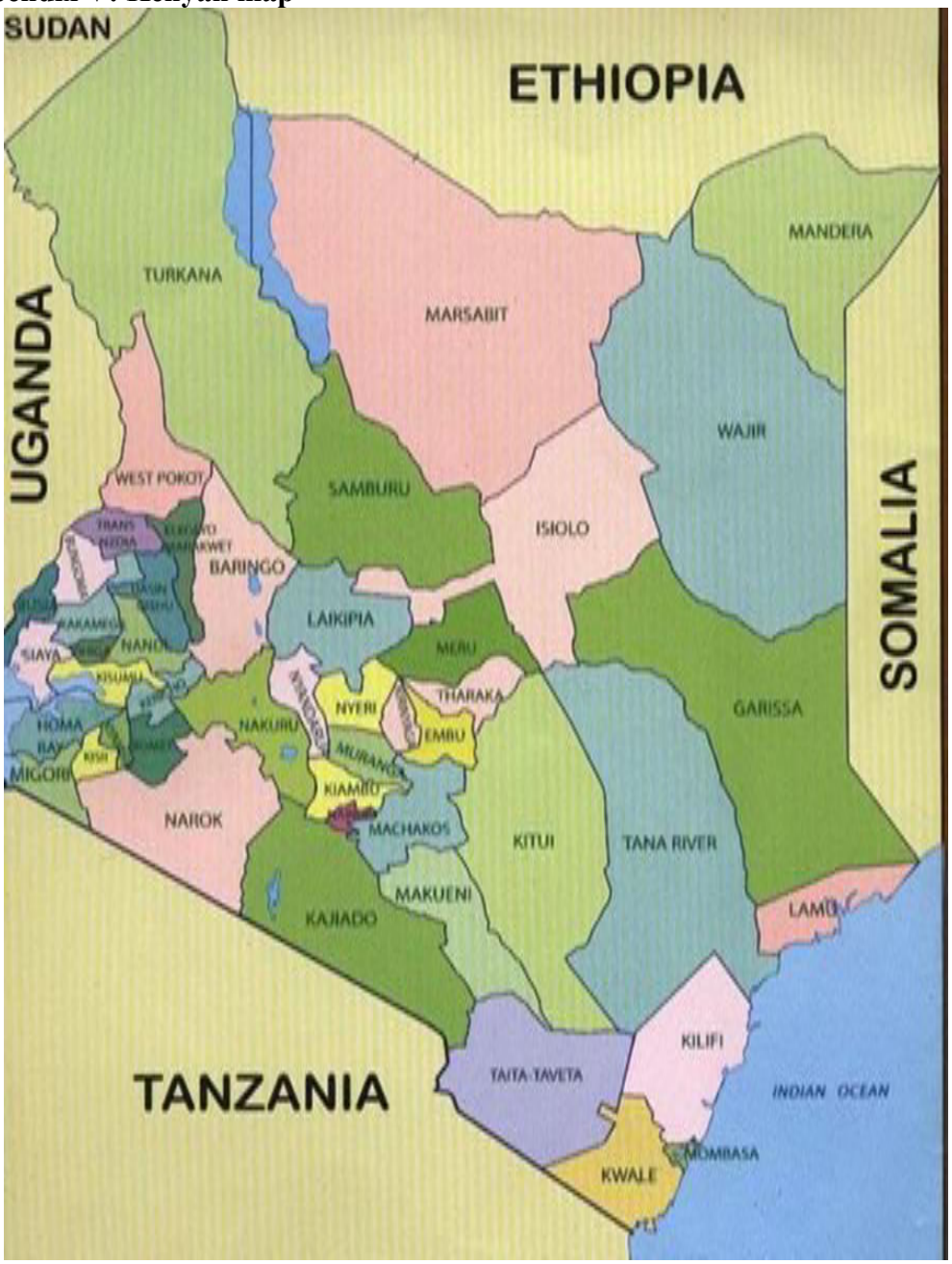
Appendix III: HCV testing algorithm



Appendix IV: Mombasa County map



Appendix V: Kenyan map



Appendix VI: Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **245330**

Date of Issue: **29/January/2024**

RESEARCH LICENSE



This is to Certify that Mr.. HAVILA LUKALO ORIEDO of Masinde Muliro University of Science and Technology, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Mombasa on the topic: LIPID PROFILE,LIVER FUNCTION,CALCIUM AND VITAMIN D3 IN HCV-HIV CO-INFECTED PEOPLE WHO INJECT HEROIN IN MOMBASA COUNTY-KENYA for the period ending : 29/January/2025.

License No: **NACOSTI/P/24/32609**

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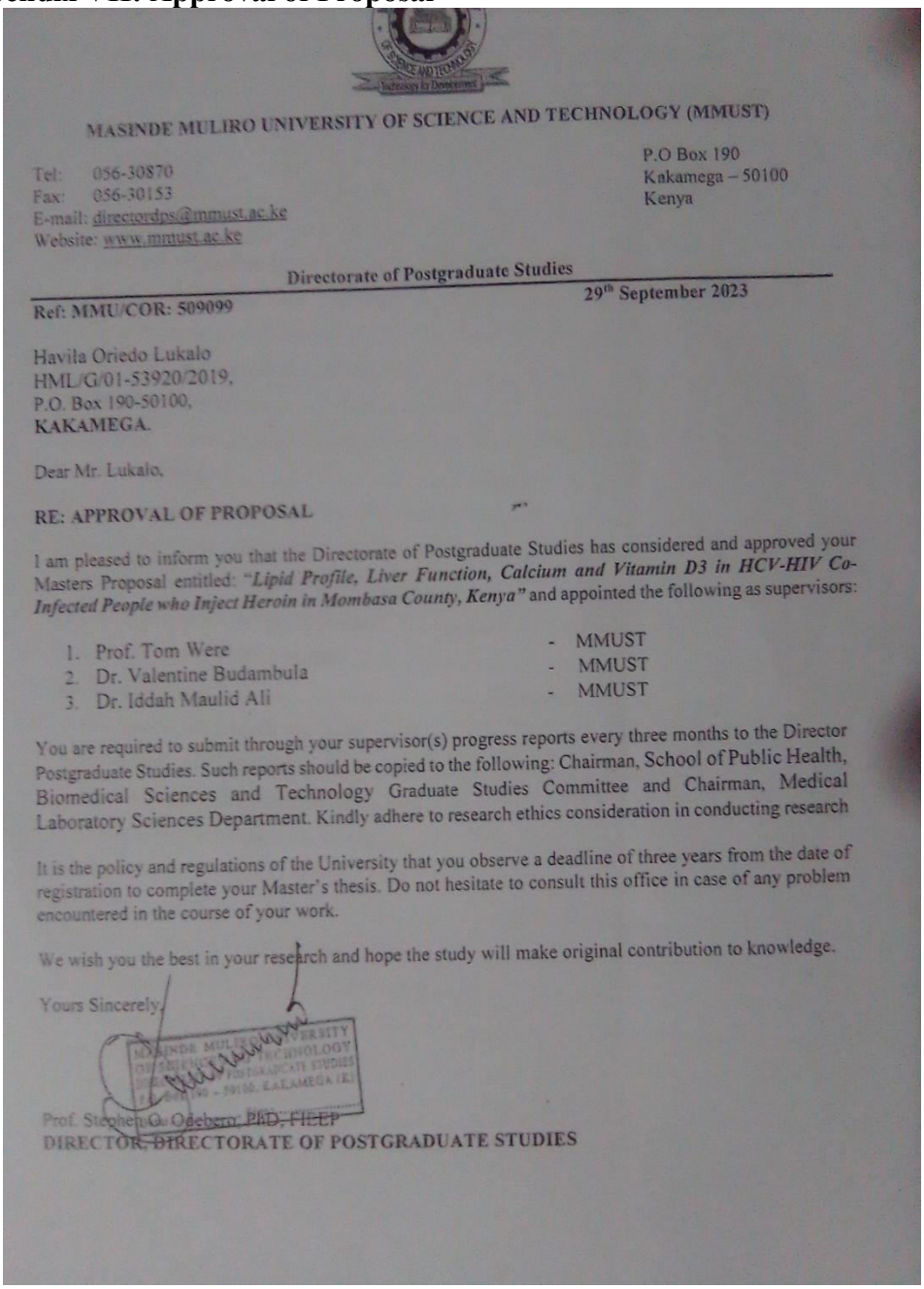
See overleaf for conditions

Appendix VII: Approval of Proposal



MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY (MMUST)

Tel: 056-30870
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E-mail: directordps@mmust.ac.ke
Website: www.mmust.ac.ke

P.O Box 190
Kakamega – 50100
Kenya

Directorate of Postgraduate Studies

Ref: MMU/COR: 509099

29th September 2023

Havila Oriedo Lukalo
HML/G/01-53920/2019,
P.O. Box 190-50100,
KAKAMEGA.

Dear Mr. Lukalo,

RE: APPROVAL OF PROPOSAL

I am pleased to inform you that the Directorate of Postgraduate Studies has considered and approved your Masters Proposal entitled: *"Lipid Profile, Liver Function, Calcium and Vitamin D3 in HCV-HIV Co-Infected People who Inject Heroin in Mombasa County, Kenya"* and appointed the following as supervisors:

1. Prof. Tom Were - MMUST
2. Dr. Valentine Budambula - MMUST
3. Dr. Iddah Maulid Ali - MMUST

You are required to submit through your supervisor(s) progress reports every three months to the Director Postgraduate Studies. Such reports should be copied to the following: Chairman, School of Public Health, Biomedical Sciences and Technology Graduate Studies Committee and Chairman, Medical Laboratory Sciences Department. Kindly adhere to research ethics consideration in conducting research

It is the policy and regulations of the University that you observe a deadline of three years from the date of registration to complete your Master's thesis. Do not hesitate to consult this office in case of any problem encountered in the course of your work.

We wish you the best in your research and hope the study will make original contribution to knowledge.

Yours Sincerely,

Prof. Stephen O. Odebero, PhD, FIEP
DIRECTOR, DIRECTORATE OF POSTGRADUATE STUDIES

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY
Tel: 056-31375
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Website: www.mmust.ac.ke
P. O. Box 190,
50100,
Kakamega,
KENYA

Institutional Scientific and Ethics Review Committee (ISERC)

REF: MMU/COR: 403012 Vol 6 (01) Date: October 11th, 2023

To: Mr. Havila Lukalo Oriedo,

Dear Mr.

RE: LIPID PROFILE, LIVER FUNCTION, CALCIUM AND VITAMIN D 3 IN HCV-HIV CO-INFECTED PEOPLE WHO INJECT HEROIN IN MOMBASA COUNTY-KENYA.

This is to inform you that the ***Masinde Muliro University of Science and Technology Institutional Scientific and Ethics Review Committee (MMUST-ISERC)*** has reviewed and approved your above research proposal. Your application approval number is **MMUST/IERC/195/2023.** The approval covers for the period ***October 11th, 2023 to October 11th, 2024.***

This approval is subject to compliance with the following requirements;

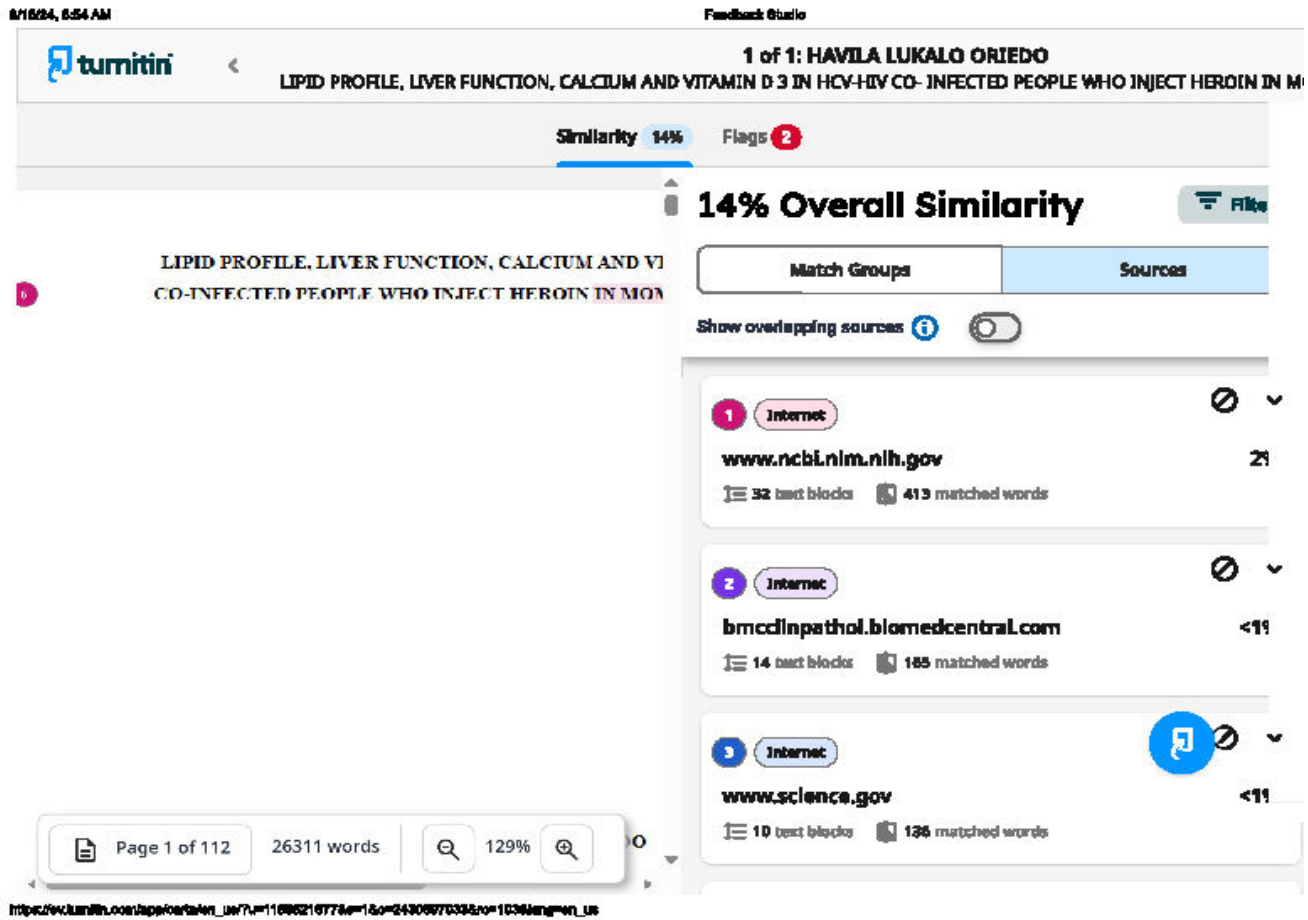
i. Only approved documents including informed consents, study instruments, MTA will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***MMUST-ISERC.***
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***MMUST-ISERC*** 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 ***MMUST-ISERC*** 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 ***MMUST-ISERC.***

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

Yours Sincerely,

Prof. Gordon Nguka (PhD)
Chairperson, Institutional Scientific and Ethics Review Committee

Copy to:
- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)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1 of 1: HAVILA LUKALO ORIEDO
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