Hepatocellular injury and metabolic dysregulation of selected biomarkers in Human Immune Virus-1 - Hepatitis C Virus co-infected individuals using heroin in Mombasa County, Kenya

Havila Oriedo Lukalo[1]
Tom Were[2]
Iddah Maulid Ali[3]
Valentine Budambula[4]
Brian Mutuma[5]
Abel Onyango[6]

[1]oriedohavila@gmail.com (+254721415227)
[2]mugogwe@yahoo.com (+254720326127)
[3]iali@mmust.ac.ke (+254722400223)
[4]valbudambula@gmail.com (+254722822448)
[5]brianmutumaz7@gmail.com (+254711939377)
[6]aonyango1967@gmail.com (+254703703783)

[1,2,3,5]Masinde Muliro University of Science and Technology, [4]Technical University of Mombasa, [6]Kakamega Teaching and Referral Hospital, [1,2,3,4,5,6] Kenya

https://doi.org/10.51867/scimundi.5.2.31

ABSTRACT

Hepatocellular injury and metabolic dysregulation are significant complications in individuals co-infected with human immunodeficiency 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 contributes 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. This case-control retrospective study was conducted targeting injection heroin users stratified into HIV-1 and HCV co-infected, mono-infected, and uninfected groups. A total of 289 samples from persons aged between 18 and 65 years were analyzed for liver enzymes (alkaline phosphatase (ALP), alanine aminotransferase (ALT), and gamma-glutamyl transaminase (GGT)), albumin, metabolic indicators (high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, calcium, and vitamin D3), and virological parameters (HIV ribonucleic acid (HIV RNA), HCV ribonucleic acid (HCV RNA), and CD4 count). Statistical analyses included group comparisons and correlation modeling to evaluate biomarker association with disease severity. The co-infected group exhibited significantly elevated liver enzymes compared to mono-infected and uninfected participants (ALT $P = 0.003$; GGT $P < 0.0001$; ALP $P < 0.0001$) and significantly low albumin levels in co-infected group 2.7 (1.8-3.6). Although none of the correlations reached statistical significance ($P>0.05$), there were marked associations between GGT and viral load (positive), GGT and CD4+ (negative), and albumin and CD4+ (negative), and trends were consistent with expected directions of liver injury in HIV/HCV co-infection. The findings of this study demonstrate a clear pattern of progressive hepatocellular injury and dysfunction in relation to HIV-1 and HCV status among injection heroin users (IHUs). The pattern aligns with prior evidence that HIV accelerates HCV-induced liver damage, leading to faster progression to cirrhosis, hepatocellular carcinoma, and liver-related mortality; hence, the need to develop a biomarker-based diagnostic and monitoring algorithm to guide targeted clinical intervention. The findings of this study underscore the importance of integrated clinical care, such as antiretroviral therapy, nutritional support, and harm reduction measures, to mitigate liver injury and metabolic complications in this high-risk population. To improve on clinical management of this population, health systems should strengthen multidisciplinary care models combining hepatology, infectious disease, psychiatry, and addiction medicine for co-infected heroin users.

Keywords: Biomarkers, Diagnostic Algorithm, HIV-1-HCV Co-Infection, Hepatocellular Injury, Injection Heroin Use, Liver Function, Lipid Profile, Metabolic Dysregulation

I. INTRODUCTION

Chronic hepatitis C infection and Acquired immunodeficiency syndrome together are immunosuppressive diseases caused by hepatitis C virus (HCV) and human immune virus (HIV) respectively. This is complicated by illicit psychoactive injection substances, which increases public health concern globally [WHO-2024]. Worldwide, approximately 11.2 million people inject drugs with approximately 1.4 million diagnosed with HIV-1, half live with hepatitis

C virus and 1.2 million live with both [UNAIDS-2024]. In Africa, use of injectable heroin is rising amongst persons above 18 years, mostly in informal settlements of urban centers [Monroe-Wise et al, 2024]. Majority of illicit substance users' advance from taking non-injectable to injectable drug use [Oguya et al, 2021], which amount to worldwide burden of injection drug use with Africa carrying substantial number of affected persons [UNAIDS-2024] and heroin is key, in addition to marijuana and cocaine [Degenhardt et al, 2023] [Cartwright & Patel, 2024]. Kenya is registering increased cases of drug use with approximately 37.1 % of subjects confessing to use injectable heroin (National Authority for the Campaign Against Alcohol and Drug Abuse [NACADA-2022), which began at the coast and infiltrated across the country [WHO-2024]. Poverty and lack of employment remain key factors determining health status of illicit drug users [Budambula et al, 2024].

Opioids are associated with persistent immune stimulation, inducing chronic inflammation [Reece & Hulse, 2018] with HIV-1 infected people who inject heroin (PWIH) developing marked inflammation of hepatocytes, assessed by determination of hepatic functionality. Injectable illicit drug use causes chronic liver deterioration and severe derangement in hepatic synthetic capabilities [TB HIV Care-2023/2024]. 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 IHUs.PWIH are key to HCV elimination, because they constitute the group with highest HCV spread across many countries and often associated with risk factors for HCV transmission [Monroe-Wise et al, 2024].

Sub-Saharan Africa is inhabited by large numbers of people who live with HCV [Colledge-Frisby et al, 2023]), although little information is in public domain about PWIH and the degree of HCV prevalence among them in the region. PWIH in the lower part of Africa are among populations with highest risk for HCV and HIV-1 infections, and comprise substantial proportion of HCV and HIV-1 recorded transmissions in the region [Sonderup et al, 2017]. Majority of HCV infected persons suffer extra-hepatic symptoms associated with fatigue, joint disorders, depression, insulin resistance, hyperglycemia, nephropathy and lymphoproliferative disorders which cause hospitalization of 15% HCV patients per year [Ugbesean et al, 2023] [Mazzaro et al, 2021] [Cartwright et al, 2024].

Evidence shows that globally approximately five million HIV-1 infected individuals have episodes of chronic HCV infection [WHO-2024], becoming cause of death in HIV infected individuals in economically stable countries [Singh et al, 2022] [Ugbesean et al, 2023]. Monitoring of HCV-HIV-1 co-infection is paramount among PWIH because HIV infection cause reduction in HCV clearance and increased rate of HCV disease progression [Vergara-Samur et al, 2023] [Gardner et al, 2023] complicating pathogenicity of these conditions. This calls for evaluation of lipids and liver enzymes in resource limited settings. In Kenya, data on widespread HCV infection in HIV-1 infected individuals constitute 18% to 32%, and its effect on long term clinical successes limited [Boateng et al, 2019] [NASCOP-2021].

Scarce documented evidence on clinical chemistry biomarkers in IHUs exist, hence the need to monitor liver and lipids biomarkers in PWIH for proper clinical management. Evaluation of structural and functional hepatic biomarkers, lipid biosynthesis, calcium and 25-hydroxyvitamin D_3 gives a clear prognosis picture of HIV-1 and HCV co-infection in PWIH [UNAIDS-2024] [Fiamenghi et al, 2021] [Obare et al, 2024] [Onyango et al,2024].

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.

1.1 Research Objectives

1.1.1 Broad objective

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

1.2 Specific objectives

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

II. METHODOLOGY

2.1 Study Design

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.

2.2 Study Site and Population

This study was undertaken in Mombasa County in Kenya which has approximately 37 000 PWID. Bomu, a community hospital and a rehabilitation center for PWID was an ideal testing site, with daily turnover of 850 both in and out patients. Common ailments are malaria and filariasis but bigger portion of the population are involved with heroin use. This has resulted to increased prevalence of HIV (7.5%) [Golabi et al, 2023] [UNAIDS-2025] and HCV (66 of 131 participants) [WHO-2024], raising HIV-1-HCV concurrent infection index among coastal population. Given this raise in number, the study was viable.

Key population were injection heroin users. The control group was HIV-1 -ve - HCV -ve persons.

2.3 Inclusion and Exclusion Criteria

Enrolled were adults between 18 to 65 years, who confessed to have injected an illicit drug and were able to provide written informed consent. Excluded were HIV-1-HCV co-infected persons who tested positive of Hepatitis B surface Antigen and those on antiretroviral therapy. Persons who had underlying liver diseases and those with pre-existing cardiovascular conditions were excluded.

2.4 Demographic and Anthropometric Measures

Demographic and anthropometric data was extracted from archives. BMI was categorized into underweight (<18.5 kg/m^2), normal (18.5 – 24.9 kg/m^2) and overweight (>25.0 kg/m^2) based on World Health Organization (WHO) adult nutrition status.

2.5 Sample Collection and Processing.

Four milliliters venous blood was drawn from brachial vein in the antecubital region of the arm into serum separator tubes (SST) using the closed mode system. Samples were centrifuged at 3000 revolutions/minute to get clear serum for storage. Samples were analyzed within 24 hours after thawing. Serum for clinical chemistry tests was aliquoted into separate 2mls cryovials for storage at -80^0 C.

2.6 Clinical Chemistry Measurements

Clinical chemistry tests were performed on auto analyzer (Roche COBAS 6000, Lausanne, Switzerland). Automatic pipettes measuring 10µl to 200µl and 100µl to 1000µl were used to aliquot serum samples. Samples were refrigerated at -25 to 8^o C pending analysis. Samples stored for longer periods were kept at -80^0 C, and analyzed within 24 hours after thawing. Regional reference ranges were used [Sing'oei et al., 2021].

2.6.1 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.

2.6.2 HIV-1 Viral Load

HIV-1 viral copies were determined by Abbott m2000 molecular analyzer following 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 amplified with HIV-1-specific amplifying kit and internal control primers. Fluorescence intensity of HIV-1 probe was detected and converted into viral load copies/ml of blood by the analyzer.

2.6.3 HCV Antibody Testing

Detection of HCV infection was performed using single-step anti-HCV immunochromatographic diagnostic tests (Healthaw Medical limited, Hangzhou, China). Clear serum samples were tested for presence of antibodies against anti-HCV markers and reactive samples were considered seropositive.

2.6.4 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. For discordant results, Unigold was used as a tie breaker.

2.7 CD4 Cell Count

CD4 cell count data was extracted from the laboratory archived results, and were reported as CD4 Cell count/milliliter of whole blood. 50 µL of whole anticoagulated blood was dispensed into Trucount tube. 20µL of fluorescent antibodies were added, vortexed and incubated at room temperature for 15 minutes in the dark. 450µL of lysing solution was added, vortexed and further incubated at room temperature for 15 minutes in the dark. The sample then was analyzed on a flow cytometer Facs Calibur platform.

2.8 Determination of Body Mass Index (BMI)

Anthropometric measurements were documented for each individual at enrolment following CDC guidelines. Height (m) was determined by 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). BMI was calculated using the height and weight measurements from 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.

2.9 Statistical Analysis

Data analysis was done by IBM1 SPSS 1 statistical software version 20 (SPSS Inc. Chicago, USA). Continuous data was summarized as medians and percentages. Where there was no normal distribution, comparison across study groups was done with non –parametric ANOVA (Kruskal Wallis) test, and Dunn's post hoc test for multiple comparison. Differences in distribution of biochemistry parameters was compared by Chi square test. Categorical data was presented in tables as frequencies and percentages. Spearman's rank correlation test was used to determine association of clinical chemistry measurements within study groups. A two sided probability value of ≤ 0.05 was considered statistically significant.

2.10 Ethical Consideration

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

III. FINDINGS & DISCUSSION

3.1 Findings

3.1.1 Demographic and Clinical Information of Study Participants

Study samples were from persons above 18 years. Total of 289 samples were analyzed (**Table 1**). The samples were from PWIH and were categorized as HIV-HCV –ve (n=128) uninfected controls, HCV-ve- HIV +ve (n=102) HIV only infected, HCV +ve HIV –ve (n=37) HCV only infected and HCV+ naïve HIV +ve (n=22) co-infected. Gender distribution differed significantly across groups (P=0.0001). The unaffected group was predominantly male (89.8%), while females accounted for 47% of HIV-1 mono-infected participants and 18.2% of co-infected participants. In HCV monoinfected IHUs, almost all (97.3%) were male, suggesting a gender related vulnerability to HCV mono-infection in this population. Age distribution also differed significantly (P=0.001). The median age of uninfected participants was 34.9 years (range: 18.7 – 51.1), and HIV-1 mono-infected were slightly younger median: 34.4 years (range: 21.9 47.0). Contrastingly, HCV mono-infected median: 39.3 years (range 23.1-55.5) and the co-infected median: 40.3 years (range: 23.4-57.2) groups were older, reflecting a possible cumulative risk of HCV acquisition and co-infection with prolonged drug use.

3.1.2 CD4 Count

CD4 count differed significantly across groups (**P<0.001**). Median CD4 counts were lower among HIV-1 infected IHUs (650cells/µL, range 250-800 and 320cells/µL, range 60 - 580). The lowest counts were seen in co-infected individuals (320 cells/µL), showing marked immunosuppression.

3.1.3 Viral Load Patterns

Viral loads showed expected patterns. The HIV-1 mono infected had median HIV-RNA levels of 2.2 log10 copies/ml (range: 1.6 – 2.8), while the co-infected participants had higher HIV-1 RNA (3.0 log10, range: 1.8 – 4.2) and HCV-RNA (3.7 log10, range: 2.5 – 4.8) loads. HCV-RNA in monoinfected IHUs was higher (3.9 $\log_{10,}$ range: 2.2 – 5.7). Duration of injection heroin use was markedly associated with infection status (P=0.0001). Most uninfected IHUs reported injection duration <1 year (85.2%), whereas the majority of HCV monoinfected participants (89.1% for HCV monoinfected and 77.3% for the co-infected) reported injection for >1year. This highlights prolonged exposure as a key risk factor for HCV acquisition. Frequency of injection was significantly higher among HCV infected individuals (P= 0.004). In the HCV mono infected group, 75.6% injected more than once daily, and this proportion was similar among the co-infected individuals (82.0%). Contrastingly, only 14.0% of uninfected IHUs reported >1injection daily, emphasizing the dose –dependent risk of viral acquisition through high frequency exposure.

Table 1

Demographic and Characterized Clinical Information of Study Individuals

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 (89.8)	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.

3.1.4 Liver Function

This study assessed liver function biomarkers among injecting heroin users (IHUs) stratified by HIV-1 and HCV infection status (Table 2). The parameters evaluated included albumin, alanine aminotransferase (ALT), Gamma-Glutamyl transferase (GGT) and alkaline phosphatase (ALP). Albumin levels differed significantly across groups (P<0.0001). HIV-1/HCV uninfected IHUs exhibited a median of 4.0 g/dl (range 3.1 to 4.9). HIV-1 mono infected IHUs showed significantly reduced albumin levels (median 3.2g/dl, range 2.8 to 3.6), whereas HCV mono infected IHUs recorded levels of 4.3 g/dl, (range 2.7 to 5.9). The low albumin levels were observed among HIV/HCV co-infected IHUs (median 2.7g/dl, range 1.8 to 3.6). ALT levels were low in uninfected IHUs (median 23.4IU/L, range 9.4 to 37.4) and increased in HIV mono infected (median 26.0 IU/L, range 15.6 to 36.4), HCV mono infected (median 29.4 IU/L, range 14.8 to 43.9), and HIV/HCV co-infected IHUs (median 32.0 IU/L, range 18.7 to 45.3) suggesting synergistic hepatotoxicity from dual viral replication and heroin use. The overall difference was statistically significant (P=0.003). GGT levels showed a progressive increase across groups (P<0.0001). Uninfected IHUs had median (28.7 IU/L range 9.4 to 48.0), while 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

HIV/HCV co-infected IHUs (51.0 IU/L, range 26.4 – 75.6) all demonstrated elevated values. ALP levels were similarly elevated in infected groups (**P<0.0001**). The lowest median was recorded in uninfected IHUs (79.4 IU/L, range 43.1 to 115.6) and the highest in HIV/HCV co-infected IHUs (102.5 IU/L, range 72.2 to 132.8) reflecting biliary tract involvement and hepatocellular injury.

Table 2
Liver Function Parameters of Study Participants

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(72.2-132.8)	**<0.0001**

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. **Table 3**) HDL-C levels were numerically lower in HIV/HCV co-infected participants (median 1.1, range 0.8-1.4) compared to HIV-1 or HCV mono infected groups. (Median 1.2, range 1.1-1.3; median 1.25, range 1.2 -1.3) and uninfected IHUs (median 1.4 range 1.2 – 1.6), although the difference did not reach statistical significance (P=0.663).

Table 3
Metabolic Biomarkers of Study Participants

Characteristics	**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 (2.1 – 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

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 conducted 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.

3.1.5 Vitamin D and Calcium Status

Vitamin D3 levels differed significantly across groups (P=0.0001) (Table 4). The median serum vitamin D3 concentration was high among HCV mono infected IHUs (median 26.3ng/ml, range: 7.3 - 45.9) and co-infected indi-

viduals (median 25.6ng/ml, range: 7.2 – 48.9). In contrast, HIV mono infected participants had low median levels (median 15.9ng/ml, range: 3.1 – 35.1), while uninfected individuals had intermediate values (median 25.4ng/ml, range: 3.0 – 44.0).

Vitamin D_3 deficiency did not differ significantly between groups (P=0.401). Deficiency was observed in 9.1% of uninfected individuals and 13.3% of mono infected IHUs, while none of the HCV- positive participants presented with deficiency. Vitamin D_3 insufficiency (20-29ng/ml) was highly prevalent across all groups. It affected 86.4% of uninfected IHUs, 76.7% of HIV-1 mono infected and 100% of both HCV mono infected and co-infected IHUs. Ionized calcium levels did not differ significantly (P=0.350). Median concentrations ranged narrowly between 22.2 (range 16.2 – 22.7) mg/dl across the four groups. Hypocalcemia (<8.5mg/dl) prevalence was relatively high but statistically insignificant across groups (P= 0.957). It was reported in 26.3% of uninfected, 35.6% of HIV-1 mono infected, 42.1% of HCV mono infected and 35.3% 0f co-infected individuals.

Table 4

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 (%), medians and ranges 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 20ng/ml, insufficiency to serum levels between 20ng/ml to 29 ng/ml, sufficiency to serum levels between 30ng/ml to 100ng/ml while vitamin toxicity to levels above 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.

3.1.6 Correlation Analysis

Albumin correlated weakly and positively with BMI (*p=0.113*, P=0.476) and viral loads (*p*=0.158, P=0.318). Albumin also showed weak negative correlation with CD4+ count (*p*=-0.214, P=0.173). Alanine aminotransferase (ALT) had a weak negative correlation with BMI (*p*=-0.054, P= 0.735). ALT also showed weak positive correlation with viral load (*p*=0.164, P=0.298) and weak negative correlation with CD4+ count (*p*=-0.211, P=0.179). Alkaline phosphatase (ALP) showed a weak negative correlation with BMI (*p*=-0.036, P=0.819), weak positive correlation with viral load (*p*=0.195, P=0.216) and weak negative correlation with CD4+ (*p*=-0.169, P=0.285). ALP correlated negatively with age (p=-0.483, P=0.049). BMI showed a negative non-significant association with ALP (p=-0.389, P=0.123), while ALP also showed weak inverse correlation with height (p=-0.241, P=0.352). However, Gamma-Glutamyl transferase showed a weak positive correlation with BMI (*p*=0.129, P=0.414), weak positive correlation with viral load (*p*=0.202, P= 0.199), and weak negative correlation with CD4+ (*p*=-0.229, P=0.145) (Table 5)

Table 5

Correlation of Liver Function with Clinical Outcome Measures in HIV-1 Infected IHUs

Parameter	**BMI, kg**		**HIV-1 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.

3.2 Discussion

This study registered small sample size in the co-infected (n=22) and mono-infected (n=37) groups, what may have had an impact on correlation analysis, leading to reduction in statistical power. The small sample size can be attributed to the exclusion of the anti-retroviral therapy (ART)-Treated individuals who may suffer ART related hepatocellular injury other than that caused by illicit drug use, contributing to derangement of hepatic functional and structural biomarkers. This calls for more longitudinal studies to be carried out on this particular population.

3.2.1 Demographic and Clinical Information

The demographic and behavioral profiles of IHUs in this study population illustrate the interplay between heroin use patterns and blood borne viral infections (Table 1). The significant gender imbalance (P=0.0001) suggests women may be more represented among HIV-1 infected but less so among HCV mono infected groups. This may reflect differences in transmission dynamics, where HIV-1 is more often linked to sexual exposure in women as documented by a previous researcher [Monroe-Wise et al., 2024], while HCV transmission is more closely tied to direct blood contact from injection sharing.

Age differences (P=0.001) suggest that older IHUs carry a higher burden of HCV infection, consistent with cumulative exposure to risk over time. This agrees with global epidemiology showing that HCV prevalence increases with injection duration [WHO-2024]. Evidence of immunosuppression among HIV infected respondents was eminent with reduced CD4 counts (P<0.001), marked reduction in albumin levels and elevated ALT levels. This is biologically plausible as immune suppression and advanced HIV disease are often associated with poor nutritional status and impaired hepatic protein synthesis. Lower CD4 counts, reduced albumin and elevated ALT/GGT were consistent with the hypothesis that immunosuppression accelerates hepatocellular dysfunction as also noted in a previous study [Lin et al., 2022].

3.2.2 Viral Load Patterns

Although viral loads did not differ significantly across infection categories, trends indicate that co-infected individuals carried higher HIV viral loads compared to mono infected HIV IHUs. Nevertheless, HCV burden remained comparable between mono and co-infected groups, reflecting independent viral replication dynamics. These findings align with previous studies [Marin et al, 2025] showing that HIV-1 does not necessarily suppress HCV replication, though co-infection may accelerate liver injury.

3.2.3 Duration and Frequency of Heroin Injection

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 like needle exchange programs [WHO-2024]. These findings indicate that prolonged and frequent injection heroin 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.

3.2.4 Liver Function

The findings of this study demonstrate a clear pattern of progressive hepatocellular injury and dysfunction in relation to HIV-1 and HCV status among IHUs (Table 2). Albumin reduction highlights impaired liver synthetic function in co-infected individuals, which is consistent with progression towards liver fibrosis or cirrhosis, as documented by a previous researcher [Ugbesean et al, 2023]. ALT elevations are more pronounced in HCV –infected groups, confirming that HCV is the primary driver of hepatocellular necroinflammation. GGT and ALP elevations indicate cholestasis, drug- induced hepatotoxicity and metabolic dysregulation, which may be compounded by heroin metabolism. The co-infected group (HIV-1/HCV) consistently shows the most deranged liver profile, supporting the synergistic hepatotoxicity of dual infection and heroin use. This pattern aligns with prior evidence that HIV accelerate HCV induced liver damage, leading to faster progression to cirrhosis, hepatocellular carcinoma and liver related mortality, as noted by Marin et al., 2025).

3.2.5 Metabolic Biomarkers of Study Participants

The lipid profiles of the study participants showed varying alterations across study groups (Table 3), although statistical significance was not reached (all P>0.05).

Vitamin D and Calcium Status: This study demonstrates a significant alteration in vitamin D_3 levels among IHUs, particularly in HIV infected individuals (Table 4). The finding that HIV-1 mono infected participants had low vitamin D_3 levels is consistent with studies carried out in Australia, suggesting that HIV infection disrupts vitamin D_3 metabolism due to immune activation, malnutrition and drug interference with hepatic vitamin D hydroxylation, hence accelerating deficiency [Reece & Hulse, 2018] [Kettritz, 2020]. In contrast, HCV infected IHUs demonstrated higher vitamin D_3 levels compared to HIV-1 mono infected. This may be due to compensatory hepatic adaptation in chronic HCV infection or population specific dietary/ sunlight factors. The strikingly high prevalence of vitamin D insufficiency (>76% across all groups) highlights a widespread nutritional/metabolic disturbance among IHUs, regardless of viral status. This aligns with studies in sub Saharan Africa showing high background rates of vitamin D insufficiency in HIV infection [Muriuki et al., 2021] [Escobedo-Monge et al, 2024].

Calcium levels were not significantly different across groups. However, high prevalence of hypocalcemia (26 – 42%) was observed. Hypocalcemia in IHUs may reflect vitamin D-related impaired calcium absorption, heroin-induced metabolic dysregulation, renal dysfunction or drug associated metabolic bone disease. Previous studies have linked hypocalcemia to both HIV and chronic HCV infection due to systemic inflammation, liver dysfunction and endocrine disruption [Degenhardt et al, 2023] [WHO-2024].

Correlation analysis: This study examined correlations (Spearman's ρ) among liver function biomarkers and clinical outcome measures (BMI, HIV viral load and CD4 count) in IHUs (Table 5). The correlation analysis revealed weak, non-significant associations between liver function biomarkers and clinical outcome measures in HIV/HCV-infected IHUs. Albumin, a marker of hepatic synthetic function, showed a negative correlation with CD4+ count. This suggests that immune suppression may be associated with declining liver synthetic function, although the association was not statistically significant. Prior studies have demonstrated that persistently low CD4+ counts increase the risk of hepatic events and mortality in HIV/HCV- co-infected patients [Lin et al., 2022].

ALT levels demonstrated a positive trend with HIV-1 viral load, consistent with findings that uncontrolled HIV-1 replication exacerbates hepatocellular injury through immune activation, oxidative stress and drug-related hepatotoxicity [Gobran et al., 2021]. This trend, though non-significant, aligns with clinical observations that patients with unsuppressed HIV viral loads experience higher liver enzyme elevations.

ALP and GGT showed positive but weak correlations with HIV viral load and negative correlations with CD4+ count. These patterns suggest that poorer immune status and higher viral replication may contribute to cholestatic liver injury. Marin et al, 7 May 2025 reported weak but significant associations between CD4+ counts and liver function in HIV/HCV co-infected patients, underscoring the complex interplay of viral co-infection and immune suppression.

These findings emphasize that hepatocellular injury and metabolic dysregulation in HIV/HCV co-infected IHUs are multifactorial [Jerkeman et al., 2020]. Beyond immune markers and viral replication, factors such as heroin-induced hepatotoxicity, nutritional deficiencies and viral-specific mechanisms, e.g. HCV's use of LDL-C receptors for hepatocyte entry, contribute significantly to hepatocellular injury [Gobran et al., 2021] [Lin et al., 2022].

V. CONCLUSION & RECOMMENDATIONS

4.1 Conclusion

This study demonstrates that HIV and HCV co-infection in injection heroin users significantly contributes to hepatocellular injury and metabolic dysregulation compared to mono infection or absence of infection. The findings underscores the importance of integrated clinical care that includes antiviral therapy, nutritional support and harm reduction measures to mitigate liver injury and metabolic complications in this high-risk population. The study provides a foundation for the development of a diagnostic algorithm which may enhance patient management, guide timely interventions, and improve survival outcomes in this high-risk population (Figure I). Although not statistically significant, the observed correlation patterns suggest that viral load and CD4 count may influence liver injury trends in HIV/HCV co-infected heroin users. This underscores the complexity of hepatocellular injury in this population, where metabolic dysregulation arises from the combined effects of viral infection, immune compromise, drug toxicity and lifestyle factors.

4.2 Recommendations

Health systems need to strengthen multidisciplinary models of care that bring together experts in hepatology, infectious diseases, psychiatry, and addiction medicine to better support heroin users who are co-infected with HIV and HCV. However, screening programs targeting HIV and HCV among people who inject heroin should be expanded so that early diagnosis and timely treatment can be achieved. Furthermore, policy frameworks should ensure that routine

liver-health monitoring becomes a standard part of HIV-1 and HCV treatment programs for people who use injection heroin.

Harm-reduction programs for injecting heroin users should also include assessments of metabolic health, such as regular lipid monitoring and appropriate nutritional support. Nevertheless, long-term studies are needed to clarify how hepatocellular injury progresses in individuals co-infected with HIV-1 and HCV, as well as to determine how antiviral therapy influences this progression. Further research should explore new metabolic biomarkers such as lipid profiles, markers of insulin resistance, and oxidative-stress indicators to better understand metabolic disturbances in this group. Studies assessing how integrated addiction treatment together with antiviral therapy affect liver outcomes are of great importance.

Routine liver-function tests, regular lipid monitoring, and comprehensive cardiovascular risk management should be incorporated into routine clinical care to identify and reduce liver damage, dyslipidemia, and cardiovascular complications among injecting heroin users.

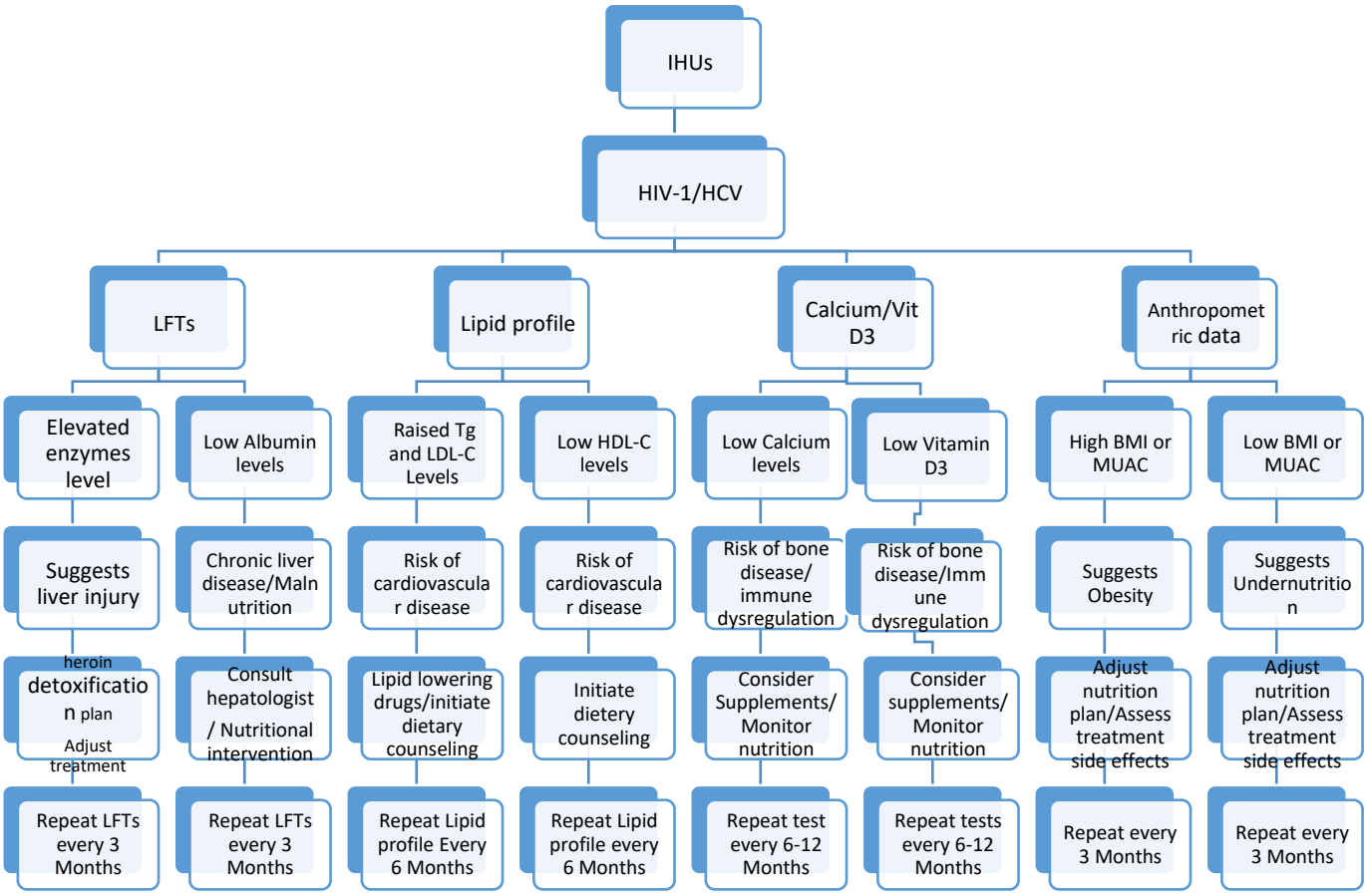

Figure I

Testing Algorithm

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

REFERENCES

Boateng, R., Mutocheluh, M., Dompreh, A., Obiri-Yeboah, D., Odame Anto, E., Owusu, M., & Narkwa, P. W. (2019). Sero-prevalence of hepatitis B and C viral co-infections among HIV-1 infected ART-naïve individuals in Kumasi, Ghana. *PLoS ONE*, *14*(4), e0215377.

Budambula, V., Ngari, M., & Were, T. (2024). Nutritional status of PWID in Coastal Kenya. *BMC Nutrition*, *10*, 55.

Cartwright, E. J., & Patel, P. R. (2024). Opportunities for enhanced prevention and control of HCV through improved screening and testing. *Journal of Infectious Diseases*, *229*(Suppl. 3), S350–S356. https://doi.org/10.1093/infdis/jiad199

Degenhardt, L., Peacock, A., Colledge, S., et al. (2023). Epidemiology of injecting drug use and prevalence of HIV/HCV/HBV among PWID: Updated global systematic review. *The Lancet Global Health*, *11*(5), e673–e683. https://doi.org/10.1016/S2214-109X(23)00058-X

Eckart, A., Struja, T., Kutz, A., Baumgartner, A., Baumgartner, T., Zurfluh, S., Neeser, O., Huber, A., Stanga, Z., Mueller, B., & Schuetz, P. (2019). Relationship of nutritional status, inflammation, and serum albumin levels during acute illness: A prospective study. *American Journal of Medicine, 6*(3), 56. https://doi.org/10.1016/j.amjmed.2019.10.031

Escobedo-Monge, M. F., Bahillo-Curieses, P., Parodi-Román, J., Escobedo-Monge, M. A., Alonso-López, P., & Marugán-Miguelsanz, J. M. (2024). Calcium, phosphate, and vitamin D in children and adolescents with chronic diseases: A cross-sectional study. *Nutrients, 16*(9), 1349.

Fiamenghi, V. I., Indicatti, V., & de Mello, E. D. (2021). Vitamin D deficiency in children and adolescents with obesity: A meta-analysis. *Journal of Pediatrics (Rio de Janeiro), 97*(3), 273–279. https://doi.org/10.1016/j.jped.2020.08.006

Gardner, A. R., Ma, Y., Bacchetti, P., Price, J. C., Kuniholm, M. H., French, A. L., & Tien, P. C. (2023). Longitudinal assessment of the enhanced liver fibrosis score in the era of contemporary HIV and hepatitis C virus treatment. *Journal of Infectious Diseases, 227*(11), 1274–1281.

Gobran, S. T., Ancuta, P., & Shoukry, N. H. (2021). Immunological insights into HCV/HIV coinfection. *Frontiers in Immunology, 12*, 726419. https://doi.org/10.3389/fimmu.2021.726419

Golabi, P., Younossi, Z. M., & Paik, J. M. (2023). The global epidemiology of NAFLD and NASH: A systematic review. *Hepatology, 77*(4), 1335–1347. https://doi.org/10.1097/HEP.0000000000000004

Jerkeman, A., Lagging, M., Norkrans, G., Lidman, C., Frimand, J., Simons-Berg, C., Kakko, J., Widell, A., & Björkman, P. (2020). Chronic hepatitis C in Swedish subjects receiving opiate substitution therapy: Factors associated with advanced fibrosis. *Scandinavian Journal of Gastroenterology, 55*(5), 574–580. https://doi.org/10.1080/00365521.2020.1754456

Kettritz, R. (2020). Hyperkalzämie [Hypercalcemia]. *Deutsche Medizinische Wochenschrift, 145*(3), 171–174. https://doi.org/10.1055/a-0851-5200

Lin, W., Zhong, H., & Wen, C. (2022). Persistently low CD4 cell counts are associated with hepatic events in HCV/HIV-coinfected patients. *Chinese Medical Journal, 135*(22), 2699–2705. https://doi.org/10.1097/CM9.0000000000002502

Marin, R. C., Marin, R.-C., Tit, D. M., Bungău, G., & Moleriu, R. D. (2025). The impact of hepatitis B and/or C on liver function in HIV patients. *Pharmaceuticals (Basel), 18*(5), 688. https://doi.org/10.3390/ph18050688

Mazzaro, C., Quartuccio, L., Adinolfi, L. E., Roccatello, D., Pozzato, G., Nevola, R., ... & Gattei, V. (2021). Extrahepatic manifestations of chronic hepatitis C virus infection and the impact of DAA therapy. *Viruses, 13*(11), 2249.

Monroe-Wise, A., Mbogo, L., Sambai, B., et al. (2024). Assisted partner services for PWID in Kenya increase HIV/HCV case-finding. *The Lancet Global Health, 7*(8), 34–38.

Muriuki, J. M., Mentzer, A. J., Webb, E. L., Kimita, W., Macharia, A. W., Ndungu, F. M., Cutland, C. L., Sirima, S. B., Diarra, A., Tiono, A. B., Lule, S. A., Madhi, S. A., Sandhu, M. S., Prentice, A. M., Bejon, P., Pettifor, J. M., Elliott, A. M., Adeyemo, A., Williams, T. N., & Atkinson, S. H. (2021). Prevalence and predictors of vitamin D deficiency in young African children. *BMC Medicine, 19*, 115. https://doi.org/10.1186/s12916-021-01985-8

NASCOP. (2021). *Guidelines for antiretroviral therapy in Kenya, 2021*. Ministry of Health.

National Authority for the Campaign Against Alcohol and Drug Abuse. (2022). *Rapid situation assessment of the status of drug and substance abuse*. NACADA.

Obare, L. M., Temu, T., Mallal, S. A., & Wanjalla, C. N. (2024). Inflammation in HIV and its impact on atherosclerotic disease. *Circulation Research, 134*(11), 1515–1545.

Oguya, F. O., Kenya, P. R., Ongecha, F., Mureithi, P., Musyoka, H., Muraguri, N., & Abdool, R. (2021). Rapid situational assessment of PWID in Nairobi and Coastal Kenya. *BMC Public Health, 21*, 1549.

Onyango, A. O., Shaviya, N., & Were, T. (2024). 25-OH vitamin D, calcium, and ALP in people with and without HIV using injecting drugs in Kenya. *BMC Infectious Diseases, 24*, 703.

Reece, A. S., & Hulse, G. K. (2018). Vitamin D metabolism in opioid dependence: A longitudinal study. *BMJ Open, 8*(1), e016806.

Singh, G. P., Late, S., Swu, A. K., Virk, N. S., Singh, J., & Thakkar, S. (2022). Prevalence of HIV, HCV and dual HIV–HCV infection in prison inmates: A retrospective study. *Journal of Family Medicine and Primary Care, 11*(10), 6250–6254.

Sing'oei, V., Ochola, J., Owuoth, J., Otieno, J., Rono, E., Andagalu, B., & Polyak, C. S. (2021). Clinical laboratory reference values in adults in Kisumu County, Kenya. *PLoS ONE, 16*(3), e0249259.

Sonderup, M. W., Afihene, M., & Ally, R. M. W. (2017). Hepatitis C in sub-Saharan Africa: Current status and recommendations. *The Lancet Gastroenterology & Hepatology, 2*, 910–919.

TB HIV Care. (2024, May–September). High HIV and hepatitis infections thwart 95-95-95 goals: Bio-behavioural survey among people who inject drugs, South Africa [Press release]. TB HIV Care.

Ugbesean, M. A., Gilmore, C. L., & Kasikara, C. (2023). Hepatitis C virus–lipid interplay: Pathogenesis and clinical impact. *Biomedicines, 11*(2), 271.

UNAIDS. (2024). *Global AIDS update 2024: People who inject drugs (PWID chapter/report with policy context and service coverage)*. UNAIDS.

UNAIDS. (2025). *Global HIV & AIDS statistics: Fact sheet 2024/2025 (Fact Sheet 2025; data through 2024)*. UNAIDS.

Vergara-Samur, H., Martínez-Vernaza, S., De la Hoz, A., Barahona-Correa, J., Ortiz, J. P., Gualtero-Trujillo, S., ... & Valderrama-Beltrán, S. (2023). Direct-acting antiviral treatment outcomes in HIV–HCV co-infection: A Colombian study. *Infezioni in Medicina, 31*(3), 374–383.

World Health Organization. (2024a). New report flags major increase in STIs—amidst challenges in HIV and hepatitis (news release synthesizing 2024 surveillance). World Health Organization.

World Health Organization. (2024b). *Global hepatitis report 2024: Action for access in low- and middle-income countries*. Geneva: WHO. https://iris.who.int/handle/10665/376461