EFFECTS OF HEPATITIS B VIRUS CO-INFECTION ON IMMUNE BIO-MARKERS AMONG HIV INFECTED PATIENTS ATTENDING COMPREHENSIVE CARE CLINICS IN MAKUENI COUNTY

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DOCTOR OF PHILOSOPHY

(Epidemiology)

JOMO KENYATTA UNIVERSITY

OF

AGRICULTURE AND TECHNOLOGY

2023

Effects of Hepatitis B Virus Co-Infection on Immune Bio-Markers among Hiv Infected Patients Attending Comprehensive Care Clinics in Makueni County

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Epidemiology of the Jomo Kenyatta University of Agriculture and Technology

2023

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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DEDICATION

I dedicate this thesis to my family and parents for been my source of encouragement throughout the process of conducting the entire study.

ACKNOWLEDGMENTS

I would like to acknowledge my supervisors Prof. Gideon Kikuvi, Dr. Peter Wanzala, and Dr. Fredrick Kirui who skillfully guided me through the difficult process and encouraged me, especially when the task seemed impossible. My lecturers at ITROMID also deserve a mention. Each of you brought unique qualities into the process, made me search for a deeper understanding, and provided invaluable insights as I worked on my coursework. My appreciation goes to National Research Fund Kenya (NRF) for financially supporting me to carry out this study. This work would not have been possible without the dedicated efforts by the site teams who participated in conducting interviews, specimen collection, and analysis. I would also like to thank the study subjects from the study sites for their participation in the study. Finally, I thank the KEMRI Scientific Steering Committee (SSC) and Ethical Review Committee (ERC) for granting me the permission to carry out the project and the College of Health Sciences of Jomo Kenyatta University of Agriculture and Technology for the opportunity to study and assigning me the University supervisors.

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ABBREVIATIONS AND ACRONYMS

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ART	Antiretroviral therapy
ARVS	Antiretroviral drugs
AST	Aspartate transaminase
C.C.C	Comprehensive Care Clinic
CDC	Centers for Disease Control and Prevention
DBS	Dry Blood Specimen
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme linked Immunosorbent Assay
FGD	Focused Group Discussions
HAART	Highly Active Antiretroviral Therapy
HBs Ag	Hepatitis B surface Antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV-	Hepatitis C virus

HIV	Human Immunodeficiency Virus
KAIS	Kenya Aids Indicator Survey
KEMRI	Kenya Medical Research Institute
LFTs	Liver function Tests
pVL	-Plasma Viral load
RNA	Ribonucleic Acid
SPSS	Statistical Package for Social Science
WHO	World Health Organization

ABSTRACT

Hepatitis B virus (HBV) and human immunodeficiency virus (HIV) co-infection represents a considerable health burden worldwide. Combination antiretroviral therapy (cART) has greatly improved survival in HIV infected people however HBV has emerged as a major cause of morbidity and mortality in this group. It is estimated that 5%–20% of the 35 million people living with HIV are also infected with HBV. Sub-Saharan Africa has the highest burden of HIV/HBV co-infection. The broad objective of this study was to determine the influence of Hepatitis B virus co-infection on immune biomarkers among HIV infected persons attending comprehensive care clinics in Makueni County. The study also assessed risk factors for HIV/HBV co-infection and its influence on liver functions (ALT).It was carried out in three selected comprehensive care clinics in Makueni County and a total 258 patients participated. This was a prospective case-control study comprised of two arms of participants, HIV infected (129) and HIV/HBV co-infected (129). It was six months follow-up study adopting the quantitative methodology. Quantitative data was collected using structured questionnaires and collection of laboratory data was done with the help of research assistants. The participants included all new HIV-infected persons enrolled in these facilities and met the inclusion criteria for the study. Participants were interviewed and blood samples collected from them for analysis of CD4 count, viral load, Alanine aminotransferase levels, and Hepatitis B at baseline and follow-up after giving informed consent. Both HIV infected patients and HIV/HBV co-infected patients were started on ARVS and enrolled in the study then followed up for six months for comparison between the two groups. Influence of co-infection with HBV was then determined and the association between the biomarkers and HBV co-infection established. Analysis was done using the statistical package for the social sciences (SPSS). There were more females 164 (63.6 %) than males 94 (36.4 %) participants in the study. The average age of the participants in the study was 31±0.402years. The mean viral load at the beginning of our current study was (30,169&21860copies/ml) while at the sixth month was (1731&1689) copies/ml for HBV co-infected and HIV mono-infected respectively. The risk factors found to be associated with HBV co-infection were having multiple sexual partners, alcohol intake, and not using condoms while having sexual intercourse. There was a significant drop in the viral load when compared at the beginning and the end of the study among the HIV/HBV co-infected and mono-infected at $p<0.001$. After enrolling them into ART treatment program and six months follow-up there was a significant increase in CD4 count for both the HIV and HIV/HBV positive patients at $p<0.001$ however there was no statistical significance between ALT at baseline and sixth month for the two groups at $p=0.388$. Hepatitis B virus has shown to influence CD4 count and viral load levels among HIV/HBV co-infected more than mono-infected and marked improvement is shown in co-infected individuals after ART initiation. ALT was slightly high among co-infected compared to mono-infected. Vaccination on Hepatitis B should be done on all HIV patients after undertaking the test since their immune system is weak and are at risk of contracting the virus. Molecular technologies for identification of HBV RNA levels should be introduced to determine the progression on treatment

since the rapid method only detects the antibodies present and more education should be done to the general population not only HIV positive patients on transmission, prevention, and treatment of Hepatitis B to avoid a further epidemic of the disease.

CHAPTER ONE

INTRODUCTION

1.1 Background information

In areas of low endemicity, such as North America, Australia, and Europe, HBV and HIV infection are usually acquired in adulthood through sexual or percutaneous transmission and the prevalence of chronic co-infection is around 5-7% (Alter et al., 2006). In countries where the HBV endemicity is high or intermediate, the primary HBV transmission routes are perinatal or early childhood. The HBV co-infection rates in these nations are 10-20% (Lee et al., 2005, Nyirenda et al., 2008; Diop et al., 2008). Reports indicate that the rate of progression and complications from viral hepatitis is higher in patients who are co-infected with HIV (Puoti et al., 2006; Thio et al., 2009). HIV/HBV co-infected individuals are 6 times more likely to develop chronic hepatitis B than HIV-negative individuals. In addition, HIV-infected individuals are more likely to lose previously developed protective anti-HBs antibody and develop acute hepatitis B infection.

The association between HIV, HBV and sexually transmitted disease (STDs) has been established. According to Desalegn *et al.*,(2016) and Shaw and Locarnini (2004), the Hepatitis B Virus (HBV) and Human Immunodeficiency Virus (HIV) are viruses that share certain epidemiological characteristics such as risk populations and transmission routes. This puts HIV-positive individuals at risk of co-infection with hepatitis B. For HIV and HBV co-infection (HIV/HBV), the sero-prevalence is between 6.3% 39% (Uneke *et al*., 2005; Mendes *et al*., 2000). Human immunodeficiency virus type 1 (HIV-1) and hepatitis B virus (HBV) exact a high toll worldwide. Both can lead to chronic disease, cancer, and death and neither can be eradicated with the use of current therapies.

Antiviral drug resistance often develops after patients have received treatment for some time and is usually followed by the loss of clinical benefit (Hoffman *et al*., 2007). Co-

infection with the two viruses exacerbates the negative effects. Worldwide, HBV is the leading cause of chronic liver disease and a leading cause of death, accounting for up to half of all hepatocellular carcinoma and cirrhosis cases (Hoffman *et al*., 2007). An estimated 400 million people are infected with HBV (Hoffman *et al*., 2007) with the majority of cases occurring in regions of Asia and Africa where the virus is endemic. Studies have shown that up to 70% of adults show serologic evidence of current or prior infection and 8 to 15% have chronic HBV infection (Hoffman *et al*., 2007).

Co-infection with Hepatitis B (HBV) is a major concern in HIV/AIDS patients (Sharma *et al*., 2005). HIV and HBV share modes of transmission and hence co-exist in the same host at significantly high rates (Kottilil *et al*., 2005). Moreover, co-infections with HIV have become a major health care catastrophe. Hence, it is key to detect them early for reduced morbidity, delayed mortality, and enhanced quality of life among HIV/AIDS patients (Rewari *et al*., 2003).

Hepatitis B virus (HBV) co-infections contribute significantly to HIV associated morbidity and mortality, but the burden of these diseases is not fully appreciated in sub-Saharan Africa, as prevalence data are scarce (Zoufaly *et al*., 2012). Both infections often remain undiagnosed in resource-limited settings because routine testing is not a part of most of the national guidelines. Epidemiological studies provide important information on prevalence and risk factors for such co-infections and can provide guidance for clinical management and the development of test strategies (Zoufaly *et al*., 2012).

HIV-1 and Hepatitis B virus co-infection are common in Sub-Saharan Africa due to similar routes of transmission and high levels of poverty. Most studies on HIV-1 and Hepatitis B virus have occurred in hospital settings and blood transfusion units. Data on Hepatitis B virus and HIV-1 co-infection in informal urban settlements in Kenya are scanty, yet they could partly explain the disproportionately high morbidity and mortality associated with HIV-1 infections in these slums (Kerubo *et al.,* 2015). The HIV prevalence in these informal settlements suggests a higher rate than what is observed

nationally. The prevalence rates of HBV are significantly higher in the HIV-1 positive and negative populations (Kerubo *et al*., 2015). This indicates the need for HIV-1 control programmes and hepatitis B virus vaccination to be promoted through public awareness as a preventive strategy (Kerubo *et al*., 2015). Therefore, this research further investigated the effects of Hepatitis B co-infection on immune biomarkers among HIV patients.

1.2 Statement of the Problem

Hepatitis B virus (HBV) is one of the major causes of acute and chronic hepatitis worldwide. Co-infection with HBV and human immunodeficiency virus (HIV) is frequent as both share the same routes of transmission. In the general population, the risk of developing hepatitis is 6%, but it can reach 10–20% in HBV/HIV co-infected patients, besides these HBV/ HIV patients present a higher level of HBV replication, and the potential of transmission is increased. It has been observed that HBV/HIV co-infection leads to increased morbidity and mortality as compared to HIV or HBV mono-infection (Thio *et al*., 2009). The ever-increasing burden of these infections has become a growing concern. Studies show that HIV co-infection adversely impacts the natural history of HBV Ranjbar *et al*., (2010) by accelerating progression to chronic liver disease due to drug-related hepatotoxicity and hepatitis reactivation (Benhamou *et al*., 2001). In Kenya, the HIV-1 epidemic has been well documented. However, little data exists on HBV co-infection among HIV-1 positive patients and its effects on immune progression markers. A study carried out by Muriuki *et al*., (2013) on the prevalence of HBV/HIV co-infections in Nairobi found out that 6% of the participants had HIV-1 and HBV infections.

Makueni County has an HIV prevalence of 5.6% (adults) however how CD4 count levels, viral load, ALT (liver functions) respond to people co-infected with HBV compared to those not co-infected with HBV has not been established despite its importance in the successful clinical management of these patients. Data on risk factors related to HIV/HBV co-infections is also scarce within this population in Makueni

County. To ensure success in the management of HIV patient's proper data and management of HBV co-infection is required and the results obtained from this study will help provide information to fill the gaps identified.

1.3 Justification of the study

HBV infection has been associated with more rapid progression to AIDS, explained by an increased expression of HIV infected cells and a faster decrease in CD4 lymphocytes (Cornejo-Juarez *et al.,* 2006). When compared to the general population, the response rate to HBV vaccine in HIV-infected patients is diminished (40–60% vs 60–80%) (Cornejo-Juarez *et al.,* 2006). This lower response is related to a CD4 count of less than 500 cells/mm^3 and has also been found with other antigens like influenza or pneumococcal vaccines. HIV treatment and management is coupled with a lot of challenges ranging from lack of drug adherence, loss to follow up, treatment failure, and occurrence of co-infections which increases chances of mortality and morbidity. However, healthcare providers will advocate for people to know their HIV status or fund HIV testing activities and linkage to care to access drugs in the management of the disease. The biggest challenge remains retention into the care of these individuals and prevention and management of co-infections which causes 90% of the mortalities (Cornejo-Juarez *et al*., 2006). These co-infections may result in continued immune suppression coupled with drug adherence challenges which are critical in maintaining a strong immune system for successful treatment. Results from this study will address the risk factors for co-infection with HBV among HIV patients and its influence on immune progression markers for patients attending comprehensive care clinics in Makueni. The recommendations drawn from this study will also help formulate new strategies in the successful management of HIV patients with HBV co-infection and reduce their predisposing factors to mortality and morbidity due to these co-infections.

1.4 Research Questions

1. What is the effect of Hepatitis B virus co-infection on viral load levels among HIV infected persons attending comprehensive care clinics in Makueni County?
2. What is the effect of Hepatitis B virus co-infection on CD4 count among HIV infected persons attending comprehensive care clinics in Makueni County?
3. What is the effect of Hepatitis B virus co-infection on liver functions (ALT) among HIV infected persons attending comprehensive care clinics in Makueni County?
4. What are the risk factors for HIV/HBV co-infection among HIV-infected persons attending comprehensive care clinics in Makueni County?

1.5 Broad objective

To determine the effect of Hepatitis B virus co-infection on immune bio-markers among HIV infected persons attending comprehensive care clinics in Makueni County.

1.5.1 Specific objectives

1. To determine the effect of Hepatitis B virus co-infection on viral load levels among HIV-infected persons attending comprehensive care clinics in Makueni County.
2. To determine the effect of Hepatitis B virus co-infection on CD4 count among HIV infected persons attending comprehensive care clinics in Makueni County.
3. To determine the effect of Hepatitis B virus co-infection on liver functions (ALT) among HIV infected persons attending comprehensive care clinics in Makueni County.
4. To determine the risk factors for HIV/HBV co-infection among HIV infected persons attending comprehensive care clinics in Makueni County.

1.6 Limitation of the study.

This study was carried out during the outbreak of COVID-19 and flow of clients to the facility was limited hence it took some time before we achieved our required sample size. We could not make to do several follow-ups for our study participants though this could not be possible because of the limited time and financial constraints.

CHAPTER TWO

LITERATURE REVIEW

2.1 Human Immunodeficiency Virus

HIV is a virus spread through certain body fluids that attacks the body's immune system, specifically the CD4 cells, often called T cells. Human immunodeficiency virus (HIV) is the virus that causes acquired immunodeficiency syndrome, also known as AIDS. Over time, HIV can destroy numerous cells that the body cannot fight off infections and diseases. These special cells help the immune system fight off infections. Untreated, HIV reduces the number of CD4 cells (T cells) in the body (CDC 2014). The virus gains entry to the cells by attaching to the CD4 receptor and a co-receptor (CCR5 or CXCR4) via its envelope glycoproteins. It is called a retrovirus because it encodes the enzyme reverse transcriptase, allowing a DNA copy to be made from viral RNA.

The reverse transcriptase enzyme is inherently error-prone, resulting in a high rate of HIV mutation, which can rapidly lead to viral resistance in those on treatment (Gupta *et al*., 2008). Once integrated into the cellular DNA the provirus resides in the nucleus of infected cells and can remain quiescent for extended periods. Alternatively, it can become transcriptionally active (especially where immune activity is occurring) and can use the human host cell machinery to replicate itself. Viral RNA is then spliced singly or multiply to make a variety of structural and regulatory and accessory proteins (Gupta *et al*., 2008).

Viral proteases further process proteins and mature viral particles are formed when the virus buds through the host cell membrane (Gupta *et al*., 2008). Within a few weeks of infection, there is a high level of viral replication in the blood that can exceed 10 million viral particles per microlitre of plasma. There is a concomitant decline in CD4 T cells. However, an immune response to HIV develops that curtails viral replication, resulting in a decrease in viral load and a return of CD4 T-cell numbers to near-normal levels. The

immune control is thought to be dependent on killer T cells and neutralizing antibodies. Depending on how effective this control is, the viral load is known as the set point and this is thought to be prognostic of natural history outcomes for the infected person (Morris *et al*., 2005).

2.1.1 Incubation Period of HIV Infection

The length of time can vary widely between individuals. Left without treatment, the majority of people infected with HIV will develop signs of HIV-related illness within 5–10 years, although this can be shorter. The time between acquiring HIV and an AIDS diagnosis is usually between 10–15 years, but sometimes longer. Antiretroviral therapy (ART) can slow the disease progression by preventing the virus from replicating and therefore decreasing the amount of virus in an infected person's blood (known as the 'viral load') (WHO, 2016).

2.1.2 Symptoms of HIV infection

For many people, early infection is asymptomatic. Within a month or two of exposure, one may display signs of fever, headache, fatigue, and enlarged lymph nodes. These symptoms usually fade for as little as a few months to up to 10 years depending on the individual. After this asymptomatic period, as the immune system is further broken down by the HIV more persistent symptoms such as energy and weight loss, fevers and sweats, persistent yeast infections, skin rashes or flaky skin, pelvic inflammatory disease, short term memory loss, and severe herpes infection appear (NIAID, 2005).

2.1.3 Prevention and Management of HIV Infection

Antiretroviral drugs are used to treat and prevent HIV infection. They fight HIV by stopping or interfering with the reproduction of the virus in the body, reducing the amount of virus in the body (WHO, 2016).

2.2 Hepatitis B Virus Infection

HBV may be directly cytopathic to hepatocytes. Nonetheless, Keeffe *et al.* (2008) note the immune system-mediated cytotoxicity assumes a central role in causing liver damage. The immune assault is driven by human leukocyte antigen (HLA) class I–restricted CD8 cytotoxic T-lymphocytes which identify hepatitis B core antigen (HBcAg) and hepatitis B e antigen (HBeAg) on the infected hepatocytes' cell membranes (Keeffe *et al*.,2008).

2.2.1 Acute Hepatitis B Virus Infection

The HBV incubation period is 40-150 days (around 12 weeks on average). Similar to the acute HAV infection, the clinical illness linked to acute HBV infection may span from mild disease to severe illnesses such as fulminant hepatic failure (< 1% of patients) (Sorrell *et al*.,2009). Following the resolution of acute hepatitis resolves, approximately 5-10% of infected infants and 95% of adult patients ultimately develop antibodies against hepatitis B surface antigen (HBsAg) including anti-HBs—clear HBsAg (and HBV virions), and recover fully. About 5% of adult patients and 90-95% of infected infants develop chronic infection (Keeffe *et al*., 2008; Sorrell *et al*., 2009). After some years, some of these people may get into the immune-active phase of the disease. The HBV DNA may significantly high due to the active inflammation of the liver and fibrosis. During this period, the ALT levels are notably elevated (Keeffe *et al*., 2008).

2.2.2 Chronic Hepatitis B Virus Infection

The 10-30% of HBsAg carriers who develop chronic hepatitis present with some symptoms including fatigue. Occasional acute disease flares also occur, presenting with acute hepatitis' symptoms. The extra-hepatic manifestations of the disease (such cryoglobulinemia, polyarteritis nodosa, and glomerulonephritis) may occur (Lok *et al*., 2009). Chronic hepatitis B patients have abnormal liver chemistry results, inflammatory or fibrotic activity on liver biopsy, and blood test evidence of active HBV replication.

Patients with chronic hepatitis may be regarded as HBeAg-negative or HBeAg-positive (Keeffe *et al*., 2008).

2.3 Influence of Hepatitis B virus co-infection on CD4 and viral load in HIV patients

Impact of Hepatitis B Virus Infection on Human Immunodeficiency Virus Response to Antiretroviral Therapy in Nigeria was studied among 1564 HIV-infected patients in Jos, Nigeria, who initiated ART. Participants with HIV-HBV co-infection had hepatitis B e antigen (HBeAg) and HBV DNA status determined. $CD4^+$T cell count and HIV load at ART initiation were compared between individuals with HIV mono-infection and those with HIV-HBV co-infection with the use of univariate methods. Regression analyses were used to determine if HBeAg status or HBV DNA at ART initiation were associated with baseline HIV parameters or ART response. The median $CD4^+$T cell count of the 262 participants with HIV-HBV co-infection (16.7%) was 107 cells/mL, compared with 130 cells/mL for participants with HIV mono-infection at ART initiation.

The participants with HIV-HBV co-infection also had higher HIV loads than did patients with HIV mono-infection. Higher HBV DNA and detectable HBeAg levels were independently associated with lower $CD4^+$T cell counts at ART initiation but not with higher HIV loads. In a multivariable model, HBeAg-positive patients were less likely than HBeAg-negative patients to suppress HIV replication to $\leqslant$400 copies/mL at 24 weeks, but they had similar $CD4^+$T cell increases. At 48 weeks, there was no significant effect of HBeAg status on ART response**.** Among HIV-infected Nigerian individuals, HBV co-infection, especially among those with high levels of HBV replication, was associated with lower $CD4^+$T cell counts at ART initiation, independent of HIV RNA level. Patients with HBeAg-positive status had a slower virological response to ART, compared with HBeAg-negative patients. Further work is needed to understand the effects of HBV on $CD4^+$T cells (John *et al*., 2009).

A study by Summer *et al*., (2013) was carried out to determine the prevalence, clinical, and virologic outcomes of chronic HBV infection, including HBV resistance to lamivudine, in a cohort of HIV-1 seropositive Kenyan women on long-term ART. In this prospective cohort study, HIV-1 seropositive women initiated three-drug ART regimens that included lamivudine as the single drug active against HBV. Archived samples were tested for HBsAg, with further testing to determine HBeAg sero-prevalence, HBV DNA suppression, and lamivudine resistance. Prevalence of chronic HBV was estimated and examined associations between HBV co-infection and clinical and virologic outcomes.

In a cohort of 159 women followed for a median of 3.4 years (interquartile range 1.4–4.5), 11 (6.9%; 95% CI 3.1–10.7) had chronic HBV infection. Of these, 9 (82%) achieved undetectable plasma HBV DNA levels. One woman developed lamivudine resistance, for an incidence of 3 per 100 person-years. The HBV co-infected women were at greater risk for abnormal ALT elevations compared to HIV-1 mono-infected women (HR 2.37; 95% CI 1.1–5.3). There were no differences between HBV-infected and uninfected women in mortality, CD4 count, or HIV-1 RNA suppression (Summer *et al*., 2013).

A study carried out in Botswana was an effort to determine the response to Truvada-based first-line combination antiretroviral therapy (cART) in HIV mono--infected patients verses HIV/HBV-co-infected. The determination of Hepatitis B virus surface antigen (HBsAg), HBV e antigen (HBeAg), and HBV deoxyribonucleic acid (DNA) load was from baseline and follow-up visits in a longitudinal cART cohort of Truvada-based regimen. Logical regression techniques established that the predictors of HBV serostatus and viral suppression were (undetectable HBV DNA). There was a reduced $CD4^{+}$ T-cell gain in HIV/HBV co-infected in comparison to HIV-mono-infected patients. Hepatitis B virus surface antigen loss was 38% while HBeAg was 60%, at two years following cART initiation. The HBV DNA suppression rates surged with time on cART from 54% in 6 months to 75% in 24 months, (Motswedi *et al*., 2016).

Prospective research of HIV-positive people in the USA and Europe in The Center for AIDS Research Network of Integrated Clinical Systems and the HIV-CAUSAL Collaboration and was conducted to demonstrate a way of comparing CD4 cell count as well HIV-RNA monitoring approaches in HIV-positive people on antiretroviral therapy (ART) (Caniglia *et al*., 2016). Within 12 months, antiretroviral-naive people who started ART and became virologically suppressed were followed from the suppression date. The researchers compared 3 HIV-RNA monitoring strategies and CD4 cell count: once every (1) 9-12 ± 1 months (2) 6 ± 1 months, and (3) 3 ± 1 months. Inverse-probability weighted models were used in comparing the clinical, immunologic, and virologic results.

In 39,029 participants, 265 deaths and 690 AIDS-defining morbidities and mortalities were reported. The mortality hazard ratios (95% CIs) were 0.82 (0.46 to 1.47) for the 9-12 month strategy and 0.86 (0.42 to 1.78) for the 6 months relative to the three-month strategy. Caniglia *et al. (*2016) further notes that the respective 18-month risk ratios (95% CIs) of virologic failure (RNA >200) were 0.74 (0.46 to 1.19) and 2.35 (1.56 to 3.54) and 18-month mean CD4 differences (95% CIs) were -5.3 (-18.6 to 7.9) and -31.7 (-52.0 to -11.3). The estimates for the 2-year risk of AIDS-defining mortality and morbidity were the same across the approaches.

Human Immunodeficiency Virus/Hepatitis B Virus (HIV/HBV) co-infection in Nigerian children has emerged as a major concern with the advent of HAART and its impact on the immune system and liver has not been extensively studied in children. A study was conducted in Nigeria and it included consecutive HIV-positive children aged two months to seventeen years on HAART constituted the study population. Age and gender; CD4+ count, ALT, creatinine, and HBsAg were tested and documented at enrolment and 12months. The results from this study showed no significant effect of HBV status on the elevation of ALT levels after 12 months of HAART. Co-infected patients had an odds ratio of achieving immune response of 0.14 (95% CI 0.02–0.79).HIV/HBV co-infection rates in the children are comparable to other localities and

ALT levels did not worsen with HAART and the immune response of the co-infected children on HAART is lower (Ikpeme *et al*., 2013).

A cross-sectional study was conducted at the University of Gondar Teaching Hospital, Northwest Ethiopia to assess the HBV and HCV seroprevalence and how they correlate with liver enzyme and CD4 levels among HAART naive HIV-positive individuals. The standard procedures were used to assess the HBV and HCV serological tests and liver enzymes as well as CD4 T cell level determination. Study participants with HIV-HBV-HCV, HIV-HCV, and HIV-HBV co-infection have relatively raised mean liver enzyme levels (ALT, AST, and ALP) than HIV mono-infected individuals. Furthermore, they had lower mean CD4 levels compared to the participants who were HIV mono-infected (Yitayih *et al*., 2013). The males had a lower mean CD4 value compared to the females. These findings highlight the significance of screening all HIV-positive people before putting them on antiretroviral treatment.

HIV/HBV co-infection has been linked to less immune recovery and mortality, but tenofovir has been shown to improve outcomes.

At baseline, there was hepatitis B surface antigen (HBsAg) screening among the participants which is indicative of the current hepatitis B virus infection. The prescription of ART was informed by the World Health Organization (WHO) and Kenyan guidelines, starting threshold of < 200 cells/mm^3 until 2007 when it was increased 250 until 2010 and to 350 afterward. A total of 6214 participants were put on ART, among them were 3125 on tenofovir-containing regimens (Wondimeneh, Alem, Asfaw, & Belyhun, 2013). For about 1.75 years, the patients were observed every three months. The virological and immunological responses to ART and clinical outcomes were compared between HIV/HBV co-infected and HIV mono-infected participants. The tenofovir-containing ART impact was determined in an analysis adjusting for confounding factors including baseline CD4 count, baseline creatinine level, sex age, and calendar year.

In comparison with HBsAg-negative patients, HBsAg-positive patients had significantly impaired immunological recovery with median CD4 count surges of 110 versus 135 cells/mm^3 during the initial treatment year notwithstanding similar rates of HIV viral suppression (90 versus 89%). Baseline ALT levels –a liver inflammation indicator – were elevated HBsAg-positive patients (27 versus 23 IU/L), but with clinically insignificance difference. Severe liver disease was not evident among HBsAg-positive individuals during the initial year on ART. The mortality rate during the initial year on ART was significantly higher among HBsAg-positive relative to HBsAg-negative participants (9.3% vs 5.3%, correspondingly) (Liz *et al*., 2016). Being HBsAg positive was related to a notably increased risk of death. Hepatitis B co-infection was linked to compromised immunological reactions to ART and amplified mortality risk in this large cohort of Kenyans commencing ART. Regardless of sufficient HIV virological suppression and lack of evidence for severe liver disease, the researchers concluded the adoption of tenofovir-containing regimens reduced mortality risk in HIV/HBV co-infected patients significantly (Liz *et al*., 2016).

2.4 Influence of Hepatitis B virus co-infection on Liver function in HIV Patients

Olawumi *et al*., (2014) studied the prevalence of HBV infection among HIV-infected HAART naive patients and investigate the effect of co-infection on CD4 count and liver function. This was a hospital-based descriptive cross-sectional study of one hundred consecutive therapy-naive HIV-infected individuals. The CD4 count, Hepatitis B surface antigen. Serum albumin, total protein, and liver enzymes were determined using standard techniques. The prevalence of HIV and HBV co-infection was 37%. The mean serum ALT and ALP were significantly higher in the co-infected patients.

The mean CD4 count of the mono-infected patients was significantly higher. The mean serum ALT, AST, and ALP of mono and co-infected patients with CD4 count<200/µl were significantly higher than those with count ≥ 200 cells/µl. The mean ALT and AST of the co-infected patients and all patients with CD4 count <200 cells/µl were higher than the normal reference range. Approximately one-third of HIV-positive patients had

hepatitis B virus co-infection. Co-infection and CD4 count <200 cells/µl are likely to result in abnormal ALT and AST. The study recommended those co-infected patients and those with CD4 count <200cells/µl should be given non-hepatotoxic antiretroviral drug (Olawumi *et al*., 2014).

The occurrence of HBV/HIV co-infection by laboratory values in Roma, Lesotho was carried out to assess the co-infection status of patients with human immunodeficiency virus (HIV) and hepatitis B virus (HBV) in Lesotho, and this has been rarely reported (Eltony *et al*., 2015). This was a retrospective study, in a laboratory setting, on HBV/HIV co-infection among 304 HIV-positive patients who were screened for HBsAg in St Joseph's Hospital records between March 2011 and December 2013. In this study, 10.5% of 304 HIV-positive patients had HBV/HIV co-infection. The increased levels of ALT and AST were significantly associated with HBV/HIV co-infection status. Gender and liver function tests are important predictors for HBV/HIV co-infection. Screening for HBV co-infection in HIV-positive patients is recommended (Eltony *et al*., 2015).

A study carried out in Nigeria determined Liver function test abnormalities in Nigerian patients with human immunodeficiency virus and hepatitis B virus co-infection. Data on baseline hepatic function of HIV and hepatitis B virus (HBV) co-infected patients are limited in sub-Saharan Africa. The assessment of liver function test (LFT) abnormalities in Nigerian patients with HIV/HBV co-infection was done to highlight the impact of HIV on HBV-related liver disease in sub-Saharan Africa. The blood testing for HIV antibodies, CD4+ cell count, hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), LFTs, platelet count, fasting blood glucose and lipid profile were carried out. The co-infected patients had deranged liver enzymes more than the controls. LFT abnormalities are common in Nigerians with HBV infection and co-infection with HIV negatively impacts hepatic function (Iroezindu *et al*., 2013).

Patients with a co-infection of HIV and Hepatitis B or C are at risk of getting a liver injury from antiretroviral drugs because the co-infections accelerate liver injury that may lead to cirrhosis or hepatocellular carcinoma. The risk of liver damage for those with a

mono-infection of HIV alone is lower than in co-infections. This review explores risk factors for hepatotoxicity, its hepatotoxic antiretroviral drugs, and the mechanisms of toxicity. It is meant to highlight the hepatotoxic potential of different antiretroviral drugs currently in use by HIV-infected individuals (Wambani *et al*., 2015).

Serum of 200 patients positive for HIV was screened for HBsAg by ELISA test and LFTs were performed in first, second, and third week in serum of patients co-infected with HIV- HBV, and HBV alone. Prevalence of HBV was found in 15 out of 200 HIV positives with the maximum in age group 21-40yrs 60% of the cases (Tamal *et al*., 2013). In HIV-HBV co-infected patients the amount of total bilirubin, ALP, and ALT was found to be considerably lower as compared to HBV infected persons only and the difference was statistically significant. From the study, it is fairly clear that the co-infection of HIV and HBV is an emerging problem that should be addressed immediately. Hepatic damage in the case of co-infected patients should not be assessed only based on serum liver enzyme estimation as their rise is not significant enough in these cases. A liver biopsy accompanied by a liver function test provides a clearer picture of necro inflammation. Such co-infected individuals also face an increased risk of hepatotoxicity from anti-retroviral therapy. Individuals with HIV-HBV co-infection should have both the infections completely assessed to decide on the best therapeutic option for both viruses (Tamal *et al*., 2013).

A study by James *et al.*, (2007) looking at virology and clinical management of Hepatitis B co-infection among HIV co-infected found out that HIV can impact the outcome of HBV infection, liver damage quietly however yet it progresses with time and with the introduction with HAART liver disease has emerged as one of the leading causes of the HIV/HBV co-infected patients. A cross-sectional study was conducted in 15 government clinics in Lusaka including 5436 adult patients who initiated antiretroviral therapy between 2011 and 2013. Cases were described as HIV-positive patients who tested HBsAg-positive and controls as HIV-positive patients who tested HBsAg-negative. HIV-HBV co-infection was defined as the number of patients who tested HBsAg-positive divided by the total tested (with 95% CI). Laboratory measures

of CD4 and ALT were categorized in the analysis. Elevated ALT was defined as ALT≥66 IU/ml. CD4 cell count was dichotomized CD4 of >200 cells/µl.

The median age was 35 (29–41) years. The median CD4 cell count was 202 (102–305) cells/µl with the median ALT being 20 (14–30) IU/ml. HIV−HBV prevalence was 12.3% (95% CI: 11.4−13.1). Elevated ALT was reported in 11.1% cases and 4.7% in controls (p-value <0.001). The adjusted odds ratio (OR) of experiencing elevated ALT before ART initiation for HI-HBV patients was 2.4 (95% CI: 1.8−3.2) compared to their HIV-mono-infected counterparts. Of the cases, 53.5% had a CD4<200 while only 48.9% of controls had CD4 <200 before ART initiation (p-value 0.026). Patients infected with HBV are at increased risk of experiencing elevated alanine transaminase enzyme (ALT) and HIV-HBV co-infection may lead to further reduced CD4 cell count before initiating antiretroviral therapy (ART) (Musukuma *et al.*,2016).

2.5 Risk factors for Hepatitis B virus co-infection among HIV patients

A Study by Weldemhret *et al.,* (2016) found that socio-demographic characteristics like age, occupation, education, residence, and marital status lacked a significant association with HBsAg positivity. Likewise, the history of blood transfusion, unsafe injection, tooth extraction, history of surgery, having a history of family liver disease, catheterization, abortion, tattooing and lacked a statistically significant relationship with HBV infection. These findings corroborated the results from a study done in Goba, Ethiopia by Erena *et al.,* (2014) revealed that males were 2.59 times more predisposed to HBV exposure compared to females. Similarly, the males were more likely to a history of having multiple sexual partners (6.9 %) compared to females (3.9 %). The probable rationale for this finding is, in developing nations, particularly the semi-urban and rural communities, males travel more often than females due to the nature of their jobs. This was relative to the research conducted in Gondar teaching hospital, Ethiopia, Pasteur institute, Pakistani Punjab, and Morocco (Wondimeneh *et al.*,2013; Chen *et al.*,2013; Baha *et al* 2013; Khan *et al.*,2013; Lok *et al* .,2007).

HBsAg seropositivity in people whose CD4 count was below 200 cells/µl was significantly higher compared to those with a CD4 count above 200 cells/µl. The AZT-3TC-EFV also had higher HBsAg seropositivity compared to those with TDF-3TC-EFV combination ART therapy. The possible justification is the TDF, a nucleotide analog, which inhibits the viral polymerase and replication cycle more efficiently compared to the AZT. The study also revealed that males were regularly infected with HBV compared to females. The researchers also note a larger HBV prevalence among people who had multiple sexual partners' histories. Therefore, this demonstrates the need to reinforce and incorporate HBV screening and treatment with the current HIV/AIDS Prevention and Control Policies (Weldemhret *et al.*, 2016).

Findings by Apedichkul *et al.,* (2016) suggested that some of the HIV/HBV co-infection protective factors included having a good education and a good immune status. Therefore, providing broad access to general and sex education on HIV, and HBV risk and prevention as well as promoting HBV immunization are considered as a viable solution. It was found that education and CD4 level had statistically significant associations with HIV/HBV co-infection. In this study, participants who had no education were at a significantly greater association with HIV/HBV co-infection. This study found HIV-infected patients with a CD4 cell count of ≥ 201 cells/mm3 had a greater association with HIV/HBV co-infection with a statistical significance while compared to those who had CD4 ≤ 200 cells/mm3. This might be due to those subjects who had a CD4 ≤ 200 cells/mm3 had to meet more often with health personnel and getting more opportunity to be suggested for living in healthy behaviors. Therefore, it made less the opportunity to get an infection of HBV. Moreover, it could be the impact of losing the amount of CD4 after getting an HIV infection. Therefore, HBV infections later may not completely produce an individual immune reaction. However, the mean of CD4 cell count in cases was higher than in controls (308.70 cells/mm3 *vs.*255.97 cells/mm3), which was not statistically significant (Apedichkul *et al.,* 2016).

A study by Rodrigue *et al.,* (2019) was conducted to evaluate the seroprevalence and risk factors of HIV, HBV in Ottou village, a communal area on the outskirts of

Yaounde. This was a cross-sectional study carried out from May to September 2018 at "Sainte Monique", a pediatric and gynecologic health center. Associations between AIDS and HBV knowledge, demographics, behavior factors, and blood transfusion, and HIV and HBV infection were analyzed. The prevalence of HIV, HBV, and the co-infection HIV/HBV was respectively 11.11% (17/153), 14.37% (22/153) and1.3% (2/153). The study found out that females were more infected by HIV than men (13.3% vs. 6.3%). Contrariwise, men were more infected by HBV (16.7% vs. 11.4%) however, the differences were not statistically significant. Univariate analysis identified that multiple sexual partners and the lack of awareness on HBV were associated risk factors to contracting HBV whereas having multiple sexual partners was the only identifiable risk factor for HIV. Their study suggested that targeted, tailored, and comprehensive interventions are urgently needed to prevent HIV and HBV infections in this locality.

A study by Omatola *et al.,* (2019) determined the prevalence of hepatitis B surface antigenemia among HIV-positive patients on an anti-retroviral treatment program in Anyigba, Kogi State, North-Central Nigeria. Sera samples obtained from consented HIV patients were screened for HBsAg using the commercial rapid test membrane-based qualitative immunoassay. A structured questionnaire was used to collect information on patients' demographic variables and probable risk factors for HBV transmission. Overall, 3.5% of HIV patients were seropositive to HB sAg, and the difference between seroprevalence rates and patients' age as well as gender was not statistically significant. There was a significant difference between patients' demographic variables such as marital status and educational level and HBsAg seropositivity. Patients with a history of surgical applications and who indulged in alcoholism significantly had higher rates of concomitant HIV/HBV infection in the study area.

A cross-sectional study on the HBV and HDV infection prevalence, genotype distribution, risk factors, and genotype distribution was conducted in Mato Grosso do Sul, Central Brazil among 848 HIV-infected patients (Freitas *et al*., 2014). Serum samples of participants were tested for hepatitis surface antibody (anti-HBs), hepatitis B core antibody (anti-HBc), and hepatitis B surface antigen (HbsAg). The statistical

analysis methods used to analyze the association between HBV positivity (defined as anti-HBc and/or HBsAg positivity) and risk factors included student's t-test, Fisher's exact test, and chi-square test. The findings indicate that 222 among the 848 HIV-infected patients investigated had HBV infection serological markers. There was a 2.5% prevalence rate of HIV-HBV co-infection. Among the HIV-HBV co-infected patients, there was only one (0.1%) anti-HCV-positive case and no cases of anti-HDV-positive (Freitas *et al*., 2014). Family history of hepatitis, use of an illicit drug, male gender, homosexual activity, and increasing age were independent factors linked to HBV exposure.

2.6 Conceptual frame work

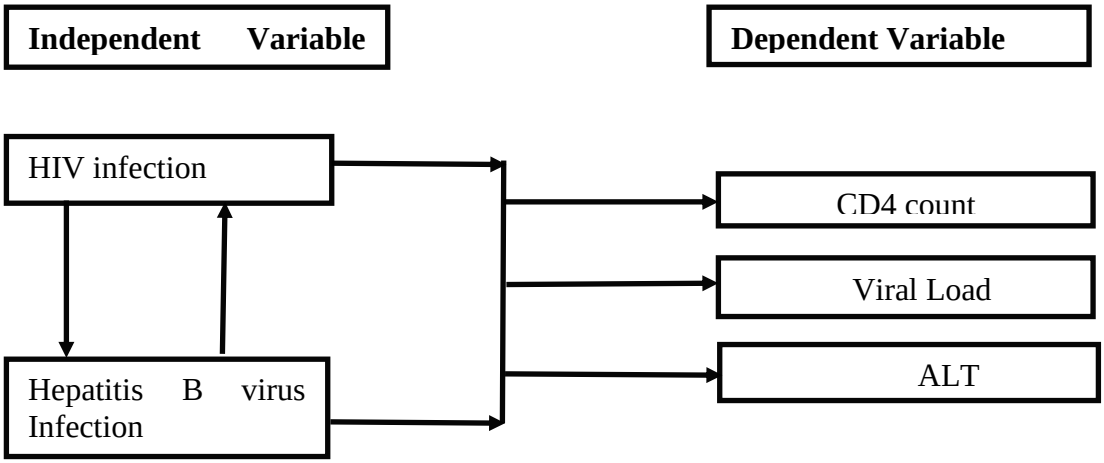


Figure 2.1: Conceptual framework

HBV and HIV have a mutually detrimental impact in that HIV infection accelerates HBV-related liver damage, leading to earlier cirrhosis and end-stage liver disease and the presence of HBV infection complicates the management of HIV infection by impairing CD4 and Viral load recovery thus accelerating immunologic progression, and increases the morbidity and mortality of HIV-infected patients**.**

2.7 Summary and gaps identified from literature review.

Most studies have been carried out on factors responsible for HIV/HBV co-infection among people visiting comprehensive care clinics in various parts of the world however few studies have been carried on what Hepatitis B virus impacts on immune bio-markers among people living with HIV. Studies done by various authors in this area are majorly cross sectional in nature.

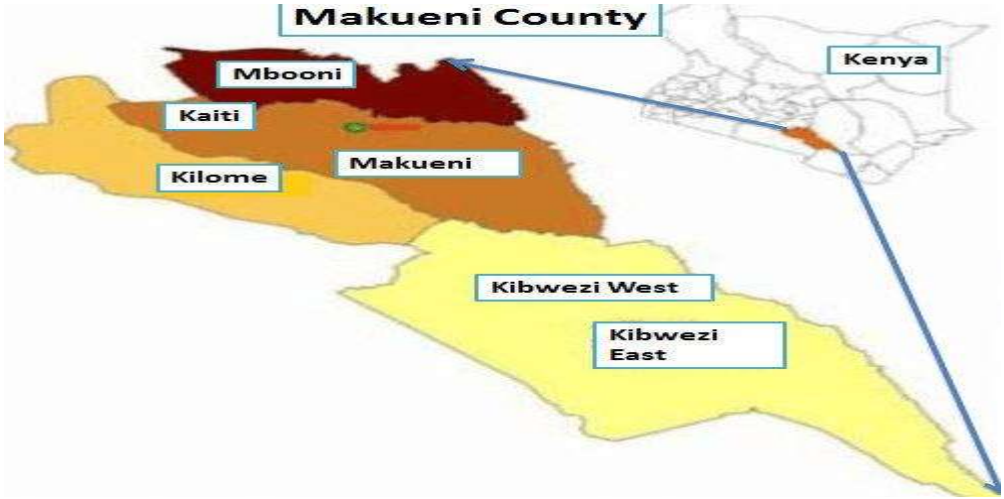
CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was carried out in three selected comprehensive care clinics in Makueni County which is 144 KM south of Nairobi county located in the southern part of eastern province and borders four counties with Kitui to the east, Taita Taveta to the south, Kajiando to the west and Machakos to the north with a current prevalence of HIV been 5.6%. It covers an Area of 8169.8km^2.According to the 2019 Kenya population and housing census the population was 987,653 with a population density of 121 people per KM^2 and an annual growth rate of 2.8%.Agriculture is the predominant economic activity in the county. Makueni County has six (6) constituencies, which are further divided into 30 electoral wards. These include Mbooni, Kaiti, Kibwezi east, Kibwezi west, Kilome and Makueni constituency. Temperatures range from between 120c to maximum of 28^{o}c.Rainfall ranges from 150mm to 650 mm per annum.Poverty levels urban (34%), rural (67%) of population live below poverty. Main economic activities include subsistence agriculture, beekeeping, small scale trade, dairy farming and limited coffee growing, Eco tourism and commercial businesses.

3.1.1 Figure showing Makueni County Map



3.2 Study design

This was a prospective case-control study among HIV mono-infected (controls) and HIV/HBV co-infected (cases) persons. The study looked at the difference in CD4 count, ALT, and viral load between the two groups at baseline (enrollment) and follow-up which was conducted once at six months to ascertain if there is any association with HBV co-infection. At enrollment, both groups were initiated on ARVS and their CD4 count, ALT, and viral load taken whereby these tests were also done during follow-up.

3.3 Study population

HIV infected persons aged 18 years and above seeking services in the three comprehensive care clinics in Makueni County.

3.4 Target population

HIV infected persons attending comprehensive care clinics in Makueni County.

3.4.1 Inclusion criteria

1. HIV infected persons registered at the facility who have not started ARVS.
2. Newly diagnosed HIV infected persons co-infected with HBV.
3. Newly diagnosed HIV infected persons with no HBV co-infection.
4. Should be willing to participate and ready to give informed consent.
5. Should be 18 years and above

3.4.2 Exclusion criteria

1. HIV infected persons on antiretroviral drugs
2. Those who are very sick
3. Previously vaccinated for HBV
4. Transfer in HIV infected persons already on ARVS

3.5 Sample size determination and sampling techniques

3.5.1 Sample size determination

The sample size formula comparing two proportions by Casagrande *et al*.,(1978) was applied to obtain the minimum sample size.

$$N = \frac{\left\{ Z_{\alpha}\sqrt{2\overline{pq}} + Z_{\beta}\sqrt{p_1\left[1+R-p_1\left(1+R^2\right)\right]} \right\}^2}{\left\{p_1\left(1-R\right)\right\}^2}$$

Where;

N is the sample size for each group

$\overline{p} = 1/2 p_1(1+R)$, $\overline{q} = 1 - \overline{p}$

P_1=is the anticipated incidence of the factor of interest (outcome) among the unexposed (expressed as a proportion), i.e., 0.55

P_2 =is the anticipated incidence of the factor of interest (outcome) among the exposed (expressed as a proportion), i.e., 0.75

R= is the anticipated relative risk of having the factor of interest (outcome) ($R= P_2/ P_1$)

0.75/0.55 = 1.36

$Z_{\alpha=}$ is the standard normal deviate for a given level of significance (1.962 for 5% level of significance)

$Z_{\beta=}$ is the standard normal deviate for a given power (0.84 for a power of 80%)

$$\bar{p} = \frac{1}{2}\{0.55(1 + 136)\} = 0.65;\ \bar{q} = 1 - 0.65 = 0.35$$

$$N = \frac{\{1.96\sqrt{2} * 0.65 * 0.35 + 0.84\sqrt{0.55[1+1.36-0.55(1+1.36^2)]^2}}{\{0.55\ (1-0.65)\}^2} = 107$$

NOTE: Exposure variable = Hepatitis B

The minimum sample size per group was calculated as 107. Allowing for 20% non-completeness/loss to follow up; the sample size was adjusted upwards to 129. The study targeted to recruit a minimum of 129 HIV infected persons with HBV co-infection and 129 HIV infected persons without HBV co-infection making a total of 258.

3.5.2 Sampling procedure and sampling techniques

The sampling frame consisted of HIV infected persons seeking services in comprehensive care clinics in Makueni County and of 18 years and above. Makueni

County has 57 comprehensive care clinics and three facilities were selected purposively to participate in the study. Makueni county referral hospital, Makindu hospital, and Emali clinic were used for this study. These facilities were selected because they have a high number of HIV-infected persons seeking services and can produce a representative sample of all comprehensive care clinics in Makueni county both for people coming in rural and urban areas. This was guided by epidemiological data submitted to the county. The study was composed of two arms of patients, newly HIV diagnosed with HBV co-infection (cases) and those infected with HIV only (control). These were newly tested HIV infected persons in the facility or recently linked and had not started ARVS. Equal numbers were allocated to controls and cases Makueni county referral has approximately monthly new HIV enrollments of 60 patients, Makindu hospital has 50, and Emali clinic has 35. The sample size was proportionately allocated to the three sites depending on the new HIV monthly testing data from each hospital. Fifty-three, forty-five, and thirty one patients for each of the two arms were recruited from Makueni county referral, Makindu hospital, and Emali clinic respectively. Following this distribution the three hospitals produced 129 HIV mono-infected participants and 129 HIV/HBV co-infected participants producing a total of 258 study participants for both arms. Consecutive sampling was used to recruit participants from each facility. The group found to be negative for HBV was vaccinated for Hepatitis B after enrollment in the study. All these groups were initiated ARVS after enrollment and followed up for six months. In the two groups, baseline information was collected at enrollment to the study, their CD4, ALT, and viral load tests, and recorded before ARVS initiation. The follow-up of six months was scheduled for the study participants and information was collected for CD4, ALT, and viral load tests were done. Individual data was collected using a semi-structured questionnaire with the help of a trained research assistant.2mls of venous blood was collected in an EDTA bottle at each visit to perform CD4, HBV test and prepare the sample for viral load testing using DBS papers which were done at KEMRI, while 2mls of blood was collected in plain bottle for doing ALT test with the help of qualified laboratory technologists. The samples were tested in these hospitals participating in the study since they have the equipment required to support the tests. The laboratory results

were then recorded to the participant's questionnaire and then entered into the computer as part of the quantitative data.

3.7 Determination of HIV and HBV status, CD4 count, ALT, and Viral load

Hepatitis B, HIV, ALT, and CD4 count screening for each participant was done at the same facility where the study was taking place and laboratory results were entered into the participants' questionnaire the same day. Later, the viral load results were entered into the participants' database after receiving them from KEMRI where analysis was done. Both HIV and HBV were conducted using rapid test methods. Blood was stored at room temperature and analysis for CD4 was done using Partec Cyflow machine while HBV was detected from serum using an advanced quality one-step rapid test kit at the facility since all these facilities have laboratories that can carry out these tests. The dried blood spot (DBS) samples for viral load were collected and kept at room temperature and taken to the nearby G4S (courier) office for transportation to KEMRI (Centre for virus Research) Nairobi for processing. Individuals with less than 1000 copies/*ul* were considered to have achieved HIV viral load suppression.

3.8 Data collection

Quantitative data was collected using questionnaires after participant's informed consent with the help of a healthcare provider recruited to be part of the study. This comprised of HIV infected persons seeking comprehensive care services in that facility where the study was conducted. Hepatitis B screening for each participant, CD4 count and ALT was done at the same facility where the study was taking place and laboratory results entered into the patient's questionnaire the same day while viral load results were later entered into the participants' database after receiving them from KEMRI. This data was collected at baseline and during follow-up.

3.9 Recruitment and training of interviewers

The minimum requirements for interviewers were form four education levels, fluent in English, Kikamba, and Kiswahili languages. Interviews were conducted and successful candidates formed part of the study team. The training was organized and conducted and took three days. The training was conducted in English, Kiswahili, and the local language. The tools were reviewed during the training and the interviewers were given guidelines on ethical issues governing the study. These included confidentiality of information obtained and the rights of the interviewees. The interviewees were randomly selected to participate in the study.

3.10 Pre-testing of research tools

After the training on the research tools, the questionnaires were pre-tested in Sultan Hamud Sub-county comprehensive care clinic. This was done to check the ability of the interviewers and the quality of the tools. Based on the results corrections were done and harmonized. This was done for quality control and to understand the intricacies of the research.

3.11 Data management and analysis

A double data entry method was used to ensure quality and consistency. Data cleaning was undertaken to identify and correct errors made during questionnaire filling and data entry. It was done daily after data collection in the evenings as feedback from the enumerators. Data processing and analysis were done using the Statistics Package for Social Science (SPSS) software. Descriptive data was presented using frequency tables and cross tabulation involving Chi-square test was used to compare variables. P values $\leq$ 0.05 were considered significant.

3.12 Ethical considerations

The scientific and ethical research committee of Kenya Medical Research Institute granted ethical approval to carry out the study. The clearance to go carry out the study was given by the board of postgraduate of Jomo Kenyatta University of Agriculture, Science, and technology. The permission was sought from leaders in the County Ministry of Health Department of HIV/AIDS and the Medical Superintendent of the facilities where the study took place. The participants' informed consent was sought and codes were used instead of their real names. Consent was also sought from the participants to collect 4ml of blood using a vacutainer at recruitment and endpoint of the study for testing HBV, ALT, CD4 count, and viral load. Patients were at liberty to refuse consent with or without explanation, and penalty or prejudicial action towards them. Samples from this study were only identified by assigned identification numbers and were not linked to client identifiers, and thus ensuring confidentiality. The data was consolidated into aggregate spreadsheets which contained no individual identifiers. Data analysed was not therefore linked with identifiable human subjects. All clients were given a patient information sheet detailing all aspects of the study including the title of the research project, invitation to participate in the research, purpose, and significance of the research, time commitments, termination of participation, indication voluntary contribution, risks involved, costs and compensation and anonymity and confidentiality. The information sheet also included the contact details of the study principal investigator whom the study participant may contact should they have any questions. No direct compensation in the form of salary was paid for participating in the study and no special incentive was offered to persuade persons to participate. A total of 258 participants gave informed consent and were recruited to the study.

CHAPTER FOUR

RESULTS

4.1 Socio-demographic and socio-economic characteristics of the study participants

Majority (64%) of the study participants in both HIV/HBV and HIV arms of the study were females. The mean age of the participants was 31±0.402 years and most(33%) of them in the HIV/HBV cohort were aged 26-30 years while in the HIV cohort most(26%) were aged 31-35 years. More than seventy-eight percent of the participants in each of the groups were married. Among them, 77% in the HIV/HBV and 98% in the HIV group were in monogamous marriage. More than half of the study participants in each of the groups had attained a secondary level of education. In both groups, more than a third of the study participants were in formal employment, and the majority (>50%) lived in rural areas. There were no significant differences($p>0.05$) in the characteristics of the participants in the two groups. (Table 4.1)

Table 4.1: Study participant's characteristics

Characteristic	Category	Presence of co-infection		
		HBV/ HIV (n) (%)	HIV (n) (%)	Total (n) (%)
Gender	Male	47(36.7)	47(36.2)	94(36.4)
	Female	82(63.3)	82(63.8)	164(63.6)
	Total	129(100.0)	129(100.0)	258(100.0)
Age	21-25	28(21.9)	24(19.2)	52(20.5)
	26-30	42(32.8)	33(25.4)	75(29.1)
	31-35	30(23.4)	34(26.2)	64(24.8)
	36-40	18(14.1)	26(20.0)	44(17.1)
	41-45	6(4.7)	8(6.9)	15(5.8)
	46-50	3(2.3)	1(0.8)	4(1.6)
	51-55	2(0.8)	2(1.5)	4(1.2)
	Total	129(100.0)	129(100.0)	258(100.0)
Marital Status	Married	103(79.7)	102(78.5)	205(79.1)
	Single	19(14.8)	22(17.7)	41(16.3)
	Divorced	4(3.1)	4(3.1)	8(3.1)
	Windowed	3(2.3)	1(0.8)	4(1.6)
	Total	129(100.0)	129(100.0)	258(100.0)
Education	Primary	28(21.9)	29(22.3)	57(22.1)
level	Secondary	69(53.1)	68(52.3)	136(52.7)
	Tertiary (college/University)	29(22.7)	32(25.4)	62(24.0)
	No formal education	3(2.3)	0(0.0)	3(1.2)
	Total	129(100.0)	129(100.0)	258(100.0)
Employment	Self employed	45(35.2)	46(35.4)	91(35.3)
status	Employed	48(36.7)	50(39.2)	98(38.0)
	Not employed	36(28.1)	33(25.4)	69(26.7)
	Total	129(100.0)	129(100.0)	258(100.0)
Residence	Urban area	63(49.2)	51(40.0)	114(44.6)
	Rural area	66(50.8)	78(60.0)	144(55.4)
	Total	129(100.0)	129(100.0)	258(100.0)

4.2 Risk factors for HIV/HBV co-infection among study participants

Ninety-nine (38.4 %) of the participants reported using condoms while having sex compared to (61.6 %) of them who did not. Condom use showed a statistically significant association with HIV/HBV co-infection at p=0.038. Among the participants, 188(72.9%) reported only having one sexual partner 69(26.7%) reported two sexual partners while the remaining reported more than two sexual partners. Having more than one sexual partner was associated with HIV/HBV co-infection ($p<0.001$). History of family members having Hepatitis B before, blood transfusion, and having been screened before for Hepatitis B did not show a relationship of one acquiring the disease however taking alcohol or smoking showed a significant relationship with acquiring the Hepatitis B virus co-infection at $p<0.001$.

Table 4.2: Risk factors for HIV/HBV co-infection among study participants

Risk Factors	Characteristics	Presence of co-infection			
		HBV/ HIV (n) (%)	HIV (n) (%)	Total (n) (%)	p-value
Use condoms	Yes	41(32.0)	58(44.6)	99(38.4)	0.038
while having	No	88(68.0)	71(55.4)	159(61.6)	
sex	Total	129(100.0)	129(100.0)	258(100.0)	
Number of	One	80(62.5)	108(83.1)	188(72.9)	0.001
Sexual	Two	48(36.7)	21(16.9)	69(26.7)	
Partners	More than two	1(0.8)	0(0.0)	1(0.4)	
	Total	129(100.0)	129(100.0)	258(100.0)	
Screened for	Yes	4(3.1)	3(2.3)	7(2.7)	0.686
Hepatitis B	No	125(96.9)	126(97.7)	251(97.3)	
	Total	129(100.0)	129(100.0)	258(100.0)	
Smoke or take	Yes	80(62.5)	45(34.6)	125(48.4)	0.001
alcohol	No	49(37.5)	84(65.4)	133(51.6)	
	Total	129(100.0)	129(100.0)	258(100.0)	
Family	No	55(43.0)	58(44.6)	113(43.8)	0.790
member(s) had	I don't know	74(57.0)	71(55.4)	145(56.2)	
Hepatitis B	Total	129(100.0)	129(100.0)	258(100.0)	
infection					
Done Blood	Yes	2(1.6)	0(0.0)	2(0.8)	0.152
Transfusion	No	127(98.4)	129(100.0)	256(99.2)	
	Total	129(100.0)	129(100.0)	258(100.0)	
Vaccinated	No	129(100.0)	129(100.0)	258(100.0)	
against	Total	129(100.0)	129(100.0)	258(100.0)	
Hepatitis B					

4.3 Baseline laboratory results for Viral load, CD4, and Alanine Aminotransferase (ALT)

The study revealed that the HIV/HBV co-infected participants had a mean blood viral load of 30169±103692copies/ml at baseline compared to 21860±77690 copies/ml mean viral load for HIV patients.HIV/HBV participants had a mean CD4 count of 327±136cells/*ul* and ALT of 20±7*u/l* compared to 421±121cells/*ul* mean CD4 and ALT of 18±6*u/l* count for those who had no HBV in their blood.ALT levels and viral load levels were high in HIV/HBV co-infected and CD4 levels were seen to be low in the same cohort compared to HIV mono-infected patients as shown in Table 4.3 below.

Table 4.3: Baseline laboratory results for study participants

Laboratory Results	HIV/HBV Status in the Blood specimen	N	Mean	Std. Deviation	Std. Error Mean
Viral load (copies/ml)	HIV/HBV	129	30169.69	103692.763	9198.677
	HIV	129	21860.25	77690.345	6789.055
CD4 Count (cells/ul)	HIV/HBV	129	327.60	136.825	12.063
	HIV	129	421.88	121.898	10.667
ALT (u/l)	HIV/HBV	129	20.59	7.967	0.706
	HIV	129	18.37	6.285	0.552

4.4 Gender and laboratory baseline viral load levels among study paricipants

Majority of the study participants 171 (66.3 %) in the survey had high viral and 85 (32.9%) in the survey had suppressed viral load, among these 2 (0.8%) of them had undetectable viral load levels load at the beginning of the study. Females had a high viral load suppression rate compared to their male counterparts. Out of the 85 participants with suppressed viral load, 32 (37.6%) were males and 53 (62.4%) were females.

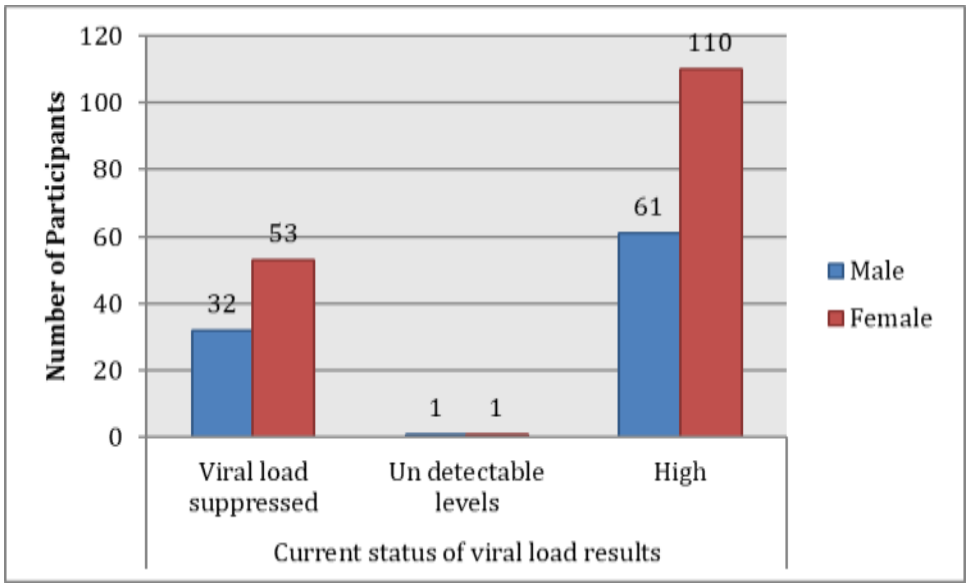


Figure 4.1: Gender and viral load levels baseline laboratory results for HIV/HBV and HIV participants.

4.5 Follow up laboratory results for viral load, CD4, and Alanine Aminotransferase (ALT)

After six months participants with Hepatitis B virus had a mean viral load of 1731±8286 copies/ml compared to 1689±5118 copies/ml for those who were not co-infected with the virus. Participants with Hepatitis B virus had a mean CD4 count of 459±127cells/*ul* compared to 437±117cells/*ul* for those who had no HBV virus. Improvement on immune markers progression (CD4 and Viral load) was seen to be good among HIV/HBV compared to HIV patients this was after the introduction of ARVS among the two groups and follow-up tests conducted in the sixth month. The same was also observed for ALT. The participants with Hepatitis B virus had a mean ALT of 18±*7u/l* compared to 17±6*u/l* for those who had no virus.

Table 4.4: Follow up Laboratory Results for study participants

Laboratory Results	HIV/HBV Status in the Blood specimen	N	Mean	Std. Deviation	Std. Error Mean
Viral load	HIV/HBV	129	1731.40	8286.449	729.581
	HIV	129	1689.23	5118.269	450.638
CD4 Count	HIV/HBV	129	459.26	127.342	11.212
	HIV	129	437.23	117.804	10.372
ALT	HIV/HBV	129	18.37	7.301	0.650
	HIV	129	17.37	6.010	0.529

4.6 Comparison of viral load status among HIV/HBV co-infected and HIV participants at baseline and follow up

At baseline, none of the participants with HBV co-infection had undetectable viral load status and only two participants among the HIV mono-infected group had undetectable levels. Viral load status did not show statistical significance at baseline(p=0.342). After the introduction of ARVS to both groups and at six months follow up there was a great improvement for both HIV/HBV co-infected and HIV mono-infected. The biggest improvement in viral load status was observed among HIV/HBV co-infected clients; however, this did not show any statistical significance at p=0.292.

Table 4.5: Viral load levels among HIV/HBV co-infected and HIV participants at baseline and follow up

	Current status of viral load results(*copies/ml*)	HIV/HBV (Cases) (n) (%)	HIV (Controls) (n) (%)	Total (n) (%)	p-value	Kappa
Baseline	Viral load suppressed (50-999)	40(48.2)	45(51.8)	85(100)	0.342	-0.001
	Un detectable levels (Less than 50)	0(0)	2(100)	2(100)		
	High (More 1000)	88(50.9)	83(49.1)	171(100)		
Follow up	Viral load suppressed (50-999)	60(49.2)	62(50.8)	122(100)	0.292	-0.037
	Un detectable levels (Less than 50)	38(57.6)	28(42.4)	66(100)		
	High (More 1000)	31(44.3)	39(55.7)	70(100)		

4.7 Risk factors relative to Viral load, CD4, and ALT among study participants

Participants who reported using condoms while having sex had a low viral load, similar CD4 count, and ALT levels compared to those who didn't however this was not statistically 8significant. The participants who smoked and drunk alcohol had higher viral load levels than those who did not and participants who reported having only one sexual partner had significantly increased CD4 count compared to those who had more than one partner at p=0.003. Participant's history of blood transfusion before or having a history of any of their family members have hepatitis B did not have a significant change on ALT, CD4, and Viral load.

Table 4.6: Risk factors in relation with CD4, ALT, and Viral load among study participants

Risk Factor	Laboratory Results	Participants Response	N	Mean	Std. Deviation	df	F	Sig.
Condoms use	Viral load	Yes	39	1268.18	1494.055	2	0.174	0.678
		No	90	1932.12	9882.644	127		
		Total	129	1731.40	8286.449	129		
	CD4 Count	Yes	39	459.51	118.432	2	0.000	0.988
		No	90	459.16	131.655	127		
		Total	129	459.26	127.342	129		
	ALT	Yes	40	17.00	6.617	5	2.014	0.158
		No	89	18.99	7.543	124		
		Total	129	18.37	7.301	129		
Number of partners	Viral load	One	81	1978.80	10384.838	2	0.193	0.661
		More than one	48	1313.90	1775.942	127		
		Total	129	1731.40	8286.449	129		
	CD4 Count	One	81	484.36	134.161	2	8.981	0.003
		More than one	48	416.92	103.001	127		
		Total	129	459.26	127.342	129		
	ALT	One	79	18.56	7.782	5	0.139	0.710
		More than one	50	18.06	6.512	124		
		Total	129	18.37	7.301	129		
Smoke or take alcohol	Viral load	Yes	78	2413.38	10602.753	2	1.340	0.249
		No	51	688.35	907.434	127		
		Total	129	1731.40	8286.449	129		
	CD4 Count	Yes	78	454.17	126.812	2	0.314	0.576
		No	51	467.06	129.016	127		

		Total	129	459.26	127.342	129		
	ALT	Yes	76	17.22	6.898	5	4.894	0.029
		No	53	20.12	7.615	124		
		Total	129	18.37	7.301	129		
Family members had Hepatitis B infection?	Viral load	Yes	1	1721.00		2	0.889	0.413
		No	54	2876.00	12696.418	127		
		I don't know	74	896.28	1299.348	129		
		Total	129	1731.40	8286.449			
	CD4 Count	Yes	1	395.00		2	.480	.620
		No	54	448.65	116.599	127		
		I don't know	74	467.88	135.454	129		
		Total	129	459.26	127.342			
	ALT	Yes	3	23.00		5	0.204	0.815
		No	54	18.41	7.681	124		
		I don't know	72	18.28	7.086	129		
		Total	129	18.37	7.301			
Had blood transfusion before?	Viral load	Yes	3	505.33	715.456	2	0.067	0.797
		No	126	1760.59	8382.605	127		
		Total	129	1731.40	8286.449	129		
	CD4 Count	Yes	3	542.00	53.113	2	1.300	0.256
		No	126	457.29	128.031	127		
		Total	129	459.26	127.342	129		
	ALT	Yes	5	14.33	5.686	5	0.940	0.334
		No	124	18.47	7.326	124		
		Total	129	18.37	7.301	129		

4.8 Association between risk factors and viral load status among HIV/HBV and HIV patients

Among the three risk factors identified to be associated with HBV co-infection among HIV patients, the number of sexual partners and smoking and talking alcohol was found to have statistical significance on the three viral load levels while the use of condoms did not show any association ap p=0.616.

Table 4.7: Risk factors versus viral load status among study participants

		Current status of viral load results				
Risk Factors	**Viral load levels(*copies/ml*)**	**HIV/HBV (n) (%)**	**HIV (n) (%)**	**Total (n) (%)**	**p-value**	**Kappa**
Use of condoms	Viral load suppressed	44(44.9)	78(48.8)	122(47.3)	0.616	0.0077
	Un detectable levels	24(24.5)	42(26.3)	66(25.6)		
	High	30(30.6)	40(25)	70(27.1)		
	Total	98(100)	160(100)	258(100)		
Number of sexual partners	Viral load suppressed	96(50.8)	26(37.7)	122(47.3)	0.001	0.006
	Un detectable levels	54(28.6)	12(17.4)	66(25.6)		
	High	39(20.6)	31(44.9)	70(27.1)		
	Total	189(100)	69(100)	258(100)		
Smoke or take alcohol	Viral load suppressed	51(42.5)	71(51.4)	122(47.3)	0.030	-0.012
	Un detectable levels	27(22.5)	39(28.3)	66(25.6)		
	High	42(35)	28(20.3)	70(27.1)		
	Total	120(100)	138(100)	258(100)		

4.9 Relationship between baseline and follow-up Laboratory results for HIV/HBV patients and HIV patients

At baseline, the mean viral loads were 30169±103693 copies/ml and 21860±77690 copies/ml in the HIV/HBV co-infected and HIV mono-infected patients respectively. During follow-up at the sixth month and introduction of ARVS both groups of patients showed reduced viral load levels which were statistically significant at ($p<0.001$), highest change in viral load levels was noticed among the HIV/HBV co-infected patients. A similar trend was observed for CD4 count among the groups where there was statistical significance between baseline and follow-up at $p<0.001$. The mean CD4 count increased in the two groups however highest improvement was noticed among the HIV/HBV co-infected individuals compared to those with only HIV infection. The mean CD4 count change from baseline and follow-up for HIV/HBV co-infected was 132 cells/ul while that of HIV mono-infected was 16 cells/ul after ART initiation. At the baseline the mean ALT for HIV/HBV positive was 20*u/l* and for the HIV/HBV negative was 18.48*u/l*. After enrolling them into an ART treatment program and six months follow-up there was a significant drop in ALT for both the HIV/HBV negative and HIV/HBV positive patients. The mean ALT for the HIV/HBV positive dropped to 18.37*u/l* and HIV/HBV negative dropped to 17.37*u/l* as shown in Table 4.8 below.

Table 4.8: Baseline and follow-up laboratory results for study participants

Laboratory Test	Survey	Status	N	Mean	Std. Deviation	F	p-value	Partial Eta Squared
Viral Load (copies/ml)	Baseline	HIV/HBV	129	30169.69	103692.763	18.054	0.001	0.034
		HIV	129	21860.25	77690.345			
	Follow up	HIV/HBV	129	1731.40	8286.449			
		HIV	129	1689.23	5118.269			
CD4 Count (cells/*ul*)	Baseline	HIV/HBV	129	327.66	136.825	43.756	0.001	0.079
		HIV	129	421.88	121.898			
	Follow up	HIV/HBV	129	459.26	127.342			
		HIV	129	437.23	117.804			
ALT	Baseline	HIV/HBV	128	20.59	7.967	0.745	0.388	0.001
		HIV	129	18.53	6.285			
		Total	257	19.56	7.232			
	Follow up	HIV/HBV	126	18.37	7.301			
		HIV	129	17.37	6.010			

CHAPTER FIVE

DISCUSSIONS, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Influence of Hepatitis B virus co-infection on CD4 count among HIV clients

This study has shown that the CD4 count for the HIV mono-infected patients was significantly higher than that of the patients co-infected by HBV. This is in line with findings from a hospital-based descriptive cross-sectional study conducted in Nigeria by Olawumi *et al.,* (2014) to determine the effect of Hepatitis B virus co-infection on CD4 cell count and liver function of HIV infected patients. A study by Sarka *et al.,* (2016) reported that CD4 count was non-significantly lower in co-infected patients.

Mbae *et al.,*(2016) found that compared to HBsAg-negative patients, HBsAg-positive patients had significantly weakened immunological recovery with median CD4 count increasing with 110 vs 135 cells/mm^3 in the initial year of therapy notwithstanding the comparable HIV viral suppression rates (90 vs 89%, respectively). This is not consistent with this study where CD4 count was seen to improve significantly after ART initiation for both HIV/HBV mono and co-infected and follow-up of six months. ARVS are known to boost the immune system hence CD4 count is expected to be high after initiation with good adherence and nutritional support which was not outlined in their study. In our current study, the significance of improvement was more in HIV/HBV co-infected individuals. At the beginning of the study the mean CD4 Count for HIV/HBV positive was 327cells/ul and for the HIV/HBV negative was 421cells/ul. After enrolling them into an ART treatment program and six months follow-up, there was a significant increase in CD4 Count for both the HIV/HBV negative and HIV/HBV positive patients. The mean CD4 Count for the HIV/HBV positive increased to 459.26cells/ul and HIV/HBV negative increased to 437.23cells/ul at $p<0.001$. Significant recovery is more likely to be in patients with dual infection of Hepatitis than those only with HIV since

HBV is a viral infection and can impair the immune system. However, with the introduction of ARV drugs, this is taken care of. A study by Otedo *et al.,* (2004) conducted in Kisumu district hospital found that the mean CD4 cell count for patients with co-infection was lower, (120 (+/-112) cells/mm3) than for patients with HIV mono-infection, 694 (+/-140) cells/mm3 which is in agreement with this current study. Weldemhret *et al.,* (2016) conducted a similar study on HBV sero-prevalence and related risk factors in HIV-positive individuals attending ART clinic at Mekelle hospital, Tigray, Northern Ethiopia. The findings indicated that HIV/AIDS positive people with lower CD4 count, <200 cells/µl, demonstrated association with HBsAg seropositivity at p=0.05. Similar results were also found by Olawumi *et al.,* (2014) who noted that the mean CD4 count of HIV mono-infected patients is significantly higher than that of co-infected patients at p=0.014 which corroborates this study.

A study by Obeagu *et al.,* (2017) on the impact of HIV and Hepatitis B Virus reported contrary results. They noted a significant decrease (P<0.05) in CD4+ T cells of the HIV/HBV co-infected subjects compared to HIV mono-infected subjects. This shows that HBV may act as an accelerator of the pathogenesis of HIV. It has a suppressive effect on the CD4+ T cells as seen in the HIV/HBV co-infection. This increases the chances of morbidity and mortality rate of those infected if not treated well immediately. Those infected with HIV must avoid being infected with HBV to avert the debilitating danger to the immune system and hematologic system too. This could be due to different treatment guideline policies. Their patients were also not initiated on ARVS during the time of the study and this could cause a significant decrease in CD4 count among the co-infected, at baseline when we were recruiting our study participants the HIV/HBV co-infected had low mean CD4 count than the mono-infected but after initiation of ARVS, there was a significant increase in CD4 count among the co-infected. This explains why there was a significant increase of CD4 count among this group in this particular study as ARVS are known to improve the immune system of HIV-infected patients.

At baseline, the CD4 count for both HIV/HB co-infected and HIV mono-infected patients for males and females were similar. After initiation of ARVS, there was a

significant improvement of the CD4 count for both gender however females responded better after six months of evaluation of the CD4 count compared to their male counterparts. A study on susceptibility to hepatitis B infection, hepatitis B/ HIV co-infections, and hepatitis B immunity in HIV-positive patients starting HAART in Durban, South Africa by Chonco *et al.,* (2019) had similar results to our study The findings noted low CD4 count cells/ul at ART initiation, however, there was a marked improvement after starting the ARVS as they tend to suppress the virus and prevent it from multiplication and further damaging the patient's immune system. Similar results were obtained from a cross-sectional study conducted on HBV sero prevalence and their correlation with CD4 cells and liver enzymes among HIV-positive individuals in Northwest Ethiopia by Wondimeneh *et al.,* (2013). The study found that individuals with HIV/HBV, co-infection also had a lower mean CD4 level than HIV mono-infected study participants. The mean CD4 value in males was lower than in females.

A study by Anderson *et al.,* (2016) found that there was a reduced $CD4^+$ T-cell gain in HIV/HBV co-infected compared with HIV mono-infected patients. Hepatitis B virus surface antigen and HBeAg losses were 38% and 60%, respectively, at 24-months post-cART initiation. The HBV DNA suppression rates increased with time on cART from 54% to 75% in 6 and 24 months, respectively. This is consistent with this study where ARVS introduction has shown in the improvement of the immune system to HIV patients co-infected with Hepatitis B virus and with good adherence. This is because ARVS are known to suppress both the HIV and the Hepatitis B virus leading to strong immunity to an individual.

5.1.2 Influence of Hepatitis B virus co-infection on viral load among HIV clients

This study revealed reduced viral load at six months and significant improvement was noticed in HIV/HBV co-infected patients compared to those with HIV only. At six months some patients had achieved HIV viral suppression. This was associated with ART initiation of naive patients during recruitment to participate in the study and good drug adherence. ARTs are known to reduce or suppress the virus and with good

adherence, the patient is likely to achieve viral load suppression. A study by Kapiamba *et al.,* (2016) on Antiretroviral adherence and virological outcomes in HIV-positive patients in KwaZulu-Natal province found inconsistent results from our study where a statistically significant relationship between adherence and viral suppression was not demonstrated. This could be due to the method they used to assess drug adherence to their participants. They used pharmacy refill method and the study suggested pharmacy refill records cannot be recommended as an alternative method of monitoring response to antiretroviral therapy, but laboratory tests including CD4 cell count and or viral load must be combined with the pharmacy refill method for monitoring of antiretroviral therapy in HIV-positive patients**,** ARVS tend to reduce viral load and increase CD4 count among HIV patients.

Holstad *et al.,*(2011) in their study found similar results with this study that high levels of adherence were correlated with improved HIV viral load and low-risk behaviors (abstinence, consistent use of condoms, etc.). Those classified as high adherence and low-risk behavior (HALR), as well as those classified as high adherence and high-risk behavior (HAHR), had lower mean viral loads and higher CD4 counts than those in the other categories. Women in the low adherence and high-risk category (LAHR) had detectable viral loads and the lowest CD4 counts and are at higher risk for transmitting HIV to partners and unborn children.

The mean viral load at the beginning of the study and at six months was (30,385-21711copies/ml) respectively. Though there was significant drop in viral load among the HIV/HBV co-infected and HIV mono-infected there was no association between viral load and HIV/HBV co-infection (p=0.05). Participants who were taking alcohol or smoking had a high mean viral load compared to those who did not indulge. This may be due to poor adherence since most of people who take alcohol may forget to take their medication or take it at late hours. Others may have poor feeding habit despite their good adherence affecting nutritional issues and functioning of ART within the system. Baum *et al.,* (2010) found the effect of alcohol on $CD4^+$ cell decline appears to be independent of ART, through a direct action on CD4 cells, although alcohol and

substance abuse may lead to unmeasured behaviors that promote HIV disease progression. The effect of alcohol abuse on viral load, however, appears to be through reduced adherence to ART. In his main findings showed that frequent alcohol consumption is a predictor of $CD4^+$ cell decline, as evidenced by a significantly greater decline of CD4 cell counts in participants who used ART, as well as those who were ART naive. Moreover, the combination of frequent alcohol and crack-cocaine use also decreased $CD4^+$ cell count significantly over time, and the decrease appears to be independent of ART. In addition, frequent alcohol use increased plasma HIV viral load, although this relationship was statistically significant only in participants who were on ART. Thus, frequent alcohol consumption appears to affect HIV disease progression by accelerating the decline of $CD4^+$ cell count and increasing viral load only in those receiving ART (Marianna *et al.,* 2010). The study by Cook *et al.,* (2017) on alcohol consumption patterns and HIV viral suppression among persons receiving HIV care in Florida found similar results with this study where exceeding weekly recommended levels of alcohol consumption (heavy drinking) was significantly associated with poor HIV viral suppression and ART non-adherence, while binge drinking was associated with suboptimal ART adherence in this sample. He recommended clinicians should attempt to address heavy drinking in their patients with HIV as part of counseling.

Todd *et al.,* (2017) found Cigarette smoking to be associated with a high HIV viral load among adults presenting for antiretroviral therapy in Vietnam. Daily cigarette smoking in the last 30 days increased the odds of having a high viral load. The daily cigarette smoking in the last 30 days was associated with a 1.5 to 2-fold higher odds of having a VL >100,000 cp/ml. Tobacco use is increasingly recognized as a significant contributor to premature morbidity and mortality among HIV-infected patients. The findings provide further evidence of the negative effects of tobacco use among HIV-infected patients.

Otto-Knapp *et al.,* (2013) in the study on Hepatitis B prevalence and influence on HIV treatment outcome and mortality in the Chilean AIDS Cohort did not show significant differences between the groups HBV infected and not infected. Virological and

immunological responses to antiretroviral therapy (ART) were not influenced by HBsAg status, but in co-infected patients, initial ART was more frequently changed. Neither treatment outcome nor overall mortality was influenced by hepatitis B co-infection. Still, patients with hepatitis B co-infection had less stable ART regimens, which might be related to a higher risk of hepatotoxic drug effects. This is inconsistent with this study where great improvement on virological and immunological response was noticed among HIV/HBV co-infected individuals compared HIV mono-infected ones, this could be due to the several factors like treatment guidelines, adherence issues, and treatment support of clients by their caregivers.

More participants with one sexual partner had achieved viral load suppression compared to those with more than one sexual partner. This trend was also observed in those who used condoms during sexual intercourse compared to those who did not use condoms. It is advised to use condoms since you may have sex with an individual with a high viral load hence acquiring more of the virus in your system, other people may have a different strain of the virus which may be transmissible during sexual intercourse and may not respond well to their partners' immune system. Having many sexual partners may also expose one to other sexual transmitted diseases which may lower their immunity; hence, encouraging multiplication of the virus within the system leading to mortality. However, no mortalities were reported during our study period which the contradicts Nikolopoulo *et al.*,(2009) on the impact of Hepatitis B Virus infection on the progression of AIDS and mortality in HIV-Infected individuals which revealed no significant impact of concomitant HIV/HBV infection on progression to AIDS and all-cause mortality. The difference in our results may be due to the period of this study which was six months and it might not be enough to observe mortalities.

5.1.3 Influence of Hepatitis B virus co-infection on liver functions (ALT) among HIV clients

This study found serum ALT mean levels among mono-infected and co-infected were similar among male and female participants. This outcome is contrary to a study by

Olawumi *et al.,* (2014) which established that ALT and AST serum levels among mono and co-infected patients respectively were significantly higher among male patients compared to female patients. However, the difference was found to be more significant among the co-infected patients and his results confirm previous similar studies by (Puoti *et al.*, 2006; Thio *et al.*, 2009) that suggested that HIV infection accelerated the progression to hepatic complications in HBV infected men. Some traditional factors could have caused the difference in our current results with the other authors. HIV patients tend to seek treatment in all aspects to establish whether they can get a complete cure of the disease. Due to desperation and stigma some visit traditional healers even after receiving treatment at facility levels and being initiated ARVS, to receive traditional medicine which when combined with ARVS causes hepatotoxicity. Men are also found to be more in denial than women in our current society and this can affect their adherence issues; hence, the liver problems. The combination of TB drugs may also lead to high ALT levels since the liver is been overworked in the deamination process. The male lifestyle could also be a factor since men tend to take alcohol more than females and alcohol consumption is known to affect the liver hence elevated ALT level among the males.

This study affirms the one conducted on HBV sero-prevalence and their correlation with CD4 cells and liver enzymes among HIV positive individuals at the University of Gondar Teaching Hospital, Northwest Ethiopia by Wondimeneh *et al.*, (2013) which found that individuals with HIV/HBV, have relatively raised mean liver enzyme levels (ALT, AST, and ALP) than HIV mono-infected ones. This study also found a statistical significance between ALT Count at the beginning of the study and Hepatitis B virus co-infections contrary to the ALT at follow-up, p=0.233, and where HIV/HBV patients had high ALT level than HIV patients at the time of ARVS initiation. The initiation of ARVS has shown to lower ALT values to both participants however the high drop is observed in HIV/HBV positive participants. A study by Sarkar J *et al.,* (2016) on baseline characteristics of HIV & hepatitis B virus (HIV/HBV) co-infected patients from Kolkata, India found that HIV/HBV co-infected patients had proportionately more

advanced HIV disease (WHO clinical stage 3 and 4) than HIV mono-infected individuals (37.1 vs. 19.9%). The co-infected patients had significantly higher serum bilirubin, alanine aminotransferase (ALT), alkaline phosphatase, and ALT/platelet ratio index (APRI) which is consistent with this study which has shown that baseline ALT levels (an indicator of liver inflammation) were higher among HIV/HBsAg-positive patients (20.6u/l vs 18.49u/l), but the difference was not clinically significant. Another study in Nigeria and Sotu Tamil found similar results. This could be because of the virus attacks and damages the liver cells which in turn impair its enzyme production activities due to cirrhosis. While ALT levels are lower in HBV/HIV co-infection, liver damage quietly, yet rapidly, progresses. With improved control of HIV disease with HAART, liver disease has emerged as one of the leading causes of death in patients with HIV. This contradicts our study where there was no death reported due to HBV co-infection among the study participants despite initiating them on HAART when HBV was confirmed through diagnosis. However, mortality may happen in cases where one reports to the clinic in his late stages and co-infected with HBV or poor drug adherence, the drugs suppress the HBV virus preventing multiplication of the virus in the liver which is known to cause severe cirrhosis and later death to the client. Furthermore, a majority of our study participants did not report to the clinic at late stages as they looked healthy and started HAART immediately after enrollment to the clinic/study.

Hepatitis B virus is one of the major causes of chronic liver disease in the world. Hepatotoxicity can also occur due to treatment of HIV infection with HAART. It is, therefore important to assess the presence of Hepatitis B to enable the clinician to make prior therapeutic decisions (Thio C *et al.,* 2009). The World health organization (WHO) advocates HBsAg testing especially in areas of high HBV prevalence, but additional testing for HBV markers such as HBeAg and HBV DNA and tests to assess the stage of liver disease are not widely available in many resource-limited countries.

HIV also hastens the progression of HBV-related liver disease. Cirrhosis is more common despite lower ALT levels than in HBV mono-infection and is also more common with lower CD4 counts. HIV-HBV co-infected men are greater than 17 times

more likely to die of liver-related causes compared to those mono-infected with HBV (Hoffmann & Thio, 2007). The impact of co-infection is especially important in regions with the widespread use of ART. As the use of ART becomes more prevalent in parts of the world with high HBV endemicity and long-term survival increases, liver disease from chronic hepatitis B in HIV-infected population may likely emerge as a greater public health problem than before (Hoffman, 2007). It is unclear at present if the risk of hepatocellular carcinoma (HCC) is increased, but there is some evidence that HIV-infected individuals with lower CD4 counts are at greater risk of developing HCC.

Baseline ALT levels an indicator of liver inflammation were higher among HIV/HBV co-infected patients (27 vs 23 IU/L) and HIV mono-infected respectively, but the difference was not clinically significant. There was no evidence of severe liver disease among HBsAg-positive people during the first year on ART (Mbae *et al.,* 2016) this was consistent with our study, we did not notice abnormally high ALT levels among the HIV/HBV co-infected patients this could be because many of the study participants enrolled to the clinic were not in their late stages of the infection.

5.1.4 Risk factors for HIV/HBV co-infection among HIV patients

More females (63%) were co-infected with HBV than males (37%). However, this finding did not show any statistical significance (p=0.925). Furthermore, our findings were similar to another study which revealed that while more females (3.59%) than the males (3.27%) were seropositive, they were comparable (p = 0.91) in HBsAg seropositivity (Omatola *et al.,*2019). This finding also supports the report of (Sule *et al.,* 2010) from the same setting that both males and females were equal in exposure to HBV. The higher ratio of females to males in this study may be attributed to the fact that more females than males visit hospitals for medical attention in Nigeria, a reason previously reported (Uneke *et al.*, 2005).

Windowed, single, and divorced persons were less affected by HBV co-infection than married individuals. However, there was no statistical significance on marital status and

HIV/HBV co-infection. These results are not similar to others done were analysis by marital status showed that the widowed patients significantly had higher HBsAg prevalence (Omatola *et al.,* 2019). They also did not conform with other studies which found a significant association of marital status with HBV infection Sule *et al.,* (2011) in Kogi State, Sirisena *et al.,* (2002) in Plateau State, Nkiru *et al.,* (2004) in Anambra State and Mohammed *et al.,* (2015) in Kanu State. The possible explanation could be because windowed, single, and divorced persons may have one or fewer partners compared to married individuals where each of the partners may have several partners increasing their chances of been pre-disposed to HBV. Married people tend to trust each other more and their chances of using protective devices during sexual intercourse are low compared to the other group.

Educational related HBsAg sero-prevalence revealed higher HBV infection in HIV patients with secondary education compared to those with no formal education, primary and tertiary levels of education and there was no significant difference between patient's educational status and HBsAg seropositivity ($p=0.353$). This finding does not support the assertion by Nkiru *et al.,* (2004) that prevalence rates of infections such as HIV, HBV, and HIV/HBV co-infection were inversely associated with educational status. The possible explanation could be HBV is majorly transmitted through sexual contact without using protection and the majority of secondary students are at their adolescent's age where they may be tempted to do sex more compared to their counterparts. At the primary level children are young and may fear to do sex or have no knowledge of sex while those in the tertiary (college/university) are more enlightened and even if they will do sex majority have the knowledge and can use protective methods in fear of unwanted pregnancy and sexually transmitted diseases.

This study found a strong association between alcohol consumption and HIV/HBV co-infection ($p<0.001$). This study conforms with the previous report of Ndako *et al.,* (2012), but contradicts the finding of (Mbaawuaga *et al.,* 2014). People who consume a lot of alcohol are sometimes mentally impaired and are likely to be promiscuous. Furthermore, the fact that they may also fail to protect themselves through correct and

consistent condom use could be a possible explanation for the higher predisposition to concomitants HIV/HBV infection.

In this study, number of sexual partners was significantly associated with the Hepatitis B virus among HIV patients. Having multiple partners increased the chances of one contracting HBV. HBV is known to be commonly transmitted through sexual intercourse without using protection or contact with a person infected with HBV. The more partner's one has increases chances of contracting the disease. This does not corroborate the study that established an association between HIV status and high risk sexual behaviors' variables but did not find these variables to be associated with HBV. After adjustment, the number of sexual partners and age at first sex were associated with HIV status but lacked a significant association with HBV status. This study revealed condom use showed a statistically significant association with HBV infection. Condoms are known to protect individuals having sex with sexually transmitted diseases and this was consistent with other studies (Balew *et al.,* 2014).

Among the study participants who were vaccinated against Hepatitis B, no one had acquired the disease at the end of the study and there were no side effects reported by those who were vaccinated. The majority of the participants both HIV/HBV co-infected and HIV/HBV mono-infected had not taken the Hepatitis B vaccine before. Results from this study show that vaccination of Hepatitis B is a preventive measure of protecting one from acquiring the disease. None of the HIV/HBV positive participants had Hepatitis B vaccination before and this explains the importance of vaccination. These results are in agreement with a study which was done to determine whether a vaccine for hepatitis B virus is effective in protecting people who have HIV against hepatitis B virus infection and if the vaccine is safe in people living with HIV. The findings were the vaccine was safe and none of the patients vaccinated acquired Hepatitis B showed improved immunity against hepatitis B among people living with HIV and taking antiretroviral therapy at 12 months. This immunity was lost once they stopped taking antiretroviral therapy (Okwen *et al.,* 2014).

History of having blood transfusion before was not statistically significant with HBV co-infection this is consistent with other studies which found risk factors history of blood transfusion, unsafe injection, tooth extraction, history of surgery, catheterization, abortion, tattooing, and having a history of family liver disease did not show statistically significant association with HBV infection (Erena *et al.*,2014).

5.2 Conclusions

Following the results of this study, the following conclusions were made:

The study has shown that CD4 count is high among HIV mono-infected patients as compared to HIV patients co-infected with Hepatitis B virus while viral load levels are high among HIV co-infected compared HIV mono-infected patients. However, with the introduction of ARVS the CD4 count improves (increases) for the two groups while the viral load levels reduce.

Risk factors found to be associated with HBV co-infection among HIV patients are; having more than one sexual partner, not using condoms while having sexual intercourse, alcohol use, and smoking.

Introduction of ARVS has shown to improve the immune system among HIV/HBV co-infected and HIV mono-infected however improvement is more noticed to co-infected to indicate that HBV suppress the system in absence of drugs this is both for CD4 and Viral load

Participants who take alcohol have been shown to respond poorly when initiated ARVS than those who do not which can be attributed to adherence issues.

The mean ALT count was high in HIV/HBV co-infected than the HIV mono-infected. HBV is known to affect the liver and may cause toxicity leading to liver damage.

The majority of the study participants achieved HIV viral load suppression level after initiation of ARVS.

Neither of the study participants had nor have information of their respective family members having had Hepatitis B infection.

5.3 Recommendations

The government to consider the introduction of laboratory molecular technologies for the detection of HBV viral load methods as a way of determining the progression of the disease among HIV/HBV co-infected individuals as rapid methods only detect the presence of the virus.

Introduction of counseling services to patients on ARVS who also take alcohol as alcohol is known to affect the function of the liver and to cause liver cirrhosis. HBV infections in patients on ARVS and who take alcohol could lead to grievous outcomes.

All people need to be vaccinated against Hepatitis B but more emphasis should be on those screened positive for HIV since their immune system is weak.

Education should be done to the population on how Hepatitis B is acquired, treatment and prevention measures.

Proper and timely monitoring of all HIV patients co-infected with Hepatitis B on the liver since with the dual infection there are higher chances of liver cirrhosis

New guidelines should be considered for laboratory monitoring of HIV/HBV co-infected individuals as compared to mono-infected ones as it is important to understand the viral load of HBV regularly.

Regularly screened for Hepatitis B virus infections and seek immediate treatment once they test positive for the virus.

REFERENCES

Alexander LJ, Schoch AG, Mantooth WB(1949). Abortive treatment of syphilis; results obtained in the incubation, primary, and secondary stages of syphilis. *Am J Syph Gonorrhea Vener Dis. 33*(5), 429–436.

Alter, M. J. (2006). Epidemiology of viral hepatitis and HIV co-infection. *Journal of hepatology*, *44*, S6-S9.

Altfield, Marcus and Bruce D. Walker (2005). Acute HIV-1 Infection. *HIV Medicine*. Retrieved from http://www.hivmedicine.com/textbook/acuteinf.htm.

Anderson, M., Gaseitsiwe, S., Moyo, S., Thami, K. P., Mohammed, T., Setlhare, D., ... & Musonda, R. M. (2016, May). Slow CD4+ T-cell recovery in human immunodeficiency virus/hepatitis B virus co-infected patients initiating truvada-based combination antiretroviral therapy in Botswana. *Open forum infectious diseases*, *3*(3), 140.

Apidechkul, T., & Pongwiriyakul, S. (2016). Factors associated with HIV and HBV co-infection in Northern Thailand. *Asian Pacific Journal of Tropical Disease*, *6*(3), 174-178.

Baha, W., Foullous, A., Dersi, N., They-they, T. P., Nourichafi, N., Oukkache, B, & Bennani, A. (2013). Prevalence and risk factors of hepatitis B and C virus infections among the general population and blood donors in Morocco. *BMC public health*, *13*(1), 1-8.

Balabanian, K., Harriague, J., & Decrion, C. (2004). CXCR4-tropic HIV-1 envelope glycoprotein functions as a viral chemokine in unstimulated primary CD4+ T lymphocytes. *J Immunol*, *173*, 7150–60.

Balew, M., Moges, F., Yismaw, G., & Unakal, C. (2014). Assessment of hepatitis B virus

and hepatitis C virus infections and associated risk factors in HIV infected patients at Debretabor hospital, South Gondar, Northwest Ethiopia. *Asian Pacific Journal of Tropical Disease, 4*(1), 1-7.

Baum, M. K., Rafie, C., Lai, S., Sales, S., Page, J. B., & Campa, A. (2010). Alcohol use accelerates HIV disease progression. *AIDS research and human retroviruses, 26*(5), 511-518.

Bieniasz, P. D. (2006). Late budding domains and host proteins in enveloped virus release. *Virology*, *344*(1), 55-63.

Benhamou, Y., Bochet, M., Di Martino, V., Charlotte, F., Azria, F., Coutellier, A., ... & Multivirc Group. (1999). Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. *Hepatology*, *30*(4), 1054-1058.

Biggar, R. J., Goedert, J. J., & Hoofnagle, J. (1987). Accelerated loss of antibody to hepatitis B surface antigen among immunodeficient homosexual men infected with HIV. *The New England journal of medicine, 316*(10), 630-631.

Blocker, M. E., Levine, W. C., & Louis, M. E. S. (2000). HIV prevalence in patients with syphilis, United States. *Sexually transmitted diseases*, *27*(1), 53-59.

Bodsworth, N. J., Cooper, D. A., & Donovan, B. (1991). The influence of human immunodeficiency virus type 1 infection on the development of the hepatitis B virus carrier state. *Journal of Infectious Diseases, 163*(5), 1138-1140.

Caniglia, E. C., Sabin, C., Robins, J. M., Logan, R., Cain, L. E., Abgrall, S., & Hernán, M. A. (2016). When to monitor CD4 cell count and HIV RNA to reduce mortality and AIDS-defining illness in virologically suppressed HIV-positive persons on antiretroviral therapy in high-income countries: a prospective observational study. *Journal of acquired immune deficiency syndromes (1999), 72*(2), 214-221.

Cantin, R., Méthot, S., & Tremblay, M. J. (2005). Plunder and stowaways: incorporation of cellular proteins by enveloped viruses. *Journal of virology*, *79*(11), 6577-6587.

Casagrande J.T, M. C. Pike and P. G. Smith. An Improved Approximate Formula for Calculating Sample Sizes for Comparing Two Binomial Distributions.*Journal of International Biometric Society, 14*(3)*, 483-486*

Centers for Disease Control and Prevention (CDC. (2011). Discordant results from reverse sequence syphilis screening--five laboratories, United States, 2006-2010. *MMWR. Morbidity and mortality weekly report*, *60*(5), 133-137.

Chen, X., He, J. M., Ding, L. S., Zhang, G. Q., Zou, X. B., & Zheng, J. (2013). Prevalence of hepatitis B virus and hepatitis C virus in patients with human immunodeficiency virus infection in Central China. *Archives of virology*, *158*(9), 1889-1894.

Chonco, F. M., & Rangiah, S. (2019). Susceptibility to hepatitis B infection, hepatitis B/HIV co-infections and hepatitis B immunity in HIV-positive patients starting HAART in Durban, South Africa. *South African Family Practice*, *61*(2), 65-68.

Cicala, C., Arthos, J., Selig, S. M., Dennis, G., Hosack, D. A., Van Ryk, D., ... & Fauci, A. S. (2002). HIV envelope induces a cascade of cell signals in non-proliferating target cells that favor virus replication. *Proceedings of the National Academy of Sciences*, *99*(14), 9380-9385.

Ciuffi, A., Llano, M., Poeschla, E., Hoffmann, C., Leipzig, J., Shinn, P., ... & Bushman, F. (2005). A role for LEDGF/p75 in targeting HIV DNA integration. *Nature medicine*, *11*(12), 1287-1289.

Coffin, J.M., Hughes, S.H., & Varmus, H.E. (1997). Retroviruses. Plainview, NY USA: Cold Spring Harbor Laboratory Press.

Cook, R. L., Zhou, Z., Kelso-Chichetto, N. E., Janelle, J., Morano, J. P., Somboonwit, C.,

... & Bryant, K. (2017). Alcohol consumption patterns and HIV viral suppression among persons receiving HIV care in Florida: an observational study. *Addiction science & clinical practice*, *12*(1), 1-9.

Cornejo-Juárez, P., Volkow-Fernández, P., Escobedo-López, K., Vilar-Compte, D., Ruiz-Palacios, G., & Soto-Ramírez, L. E. (2006). Randomized controlled trial of Hepatitis B virus vaccine in HIV-1-infected patients comparing two different doses. *AIDS research and therapy*, *3*(1), 1-5.

Colin, J. F., Cazals-Hatem, D., Loriot, M. A., Martinot-Peignoux, M., Pham, B. N., Auperin, A., ... & Marcellin, P. (1999). Influence of human immunodeficiency virus infection on chronic hepatitis B in homosexual men. *Hepatology*, *29*(4), 1306-1310.

Cruz, A. R., Pillay, A., Zuluaga, A. V., Ramirez, L. G., Duque, J. E., Aristizabal, G. E., ... & Salazar, J. C. (2010). Secondary syphilis in Cali, Colombia: new concepts in disease pathogenesis. *PLoS neglected tropical diseases*, *4*(5), e690.

Day, S. L., Odem-Davis, K., Mandaliya, K. N., Jerome, K. R., Cook, L., Masese, L. N., ... & McClelland, R. S. (2013). Prevalence, clinical and virologic outcomes of hepatitis B virus co-infection in HIV-1 positive Kenyan women on antiretroviral therapy. *PloS one*, *8*(3), e59346.

Desalegn, Z., Wassie, L., Beyene, H. B., Mihret, A., & Ebstie, Y. A. (2016). Hepatitis B and human immunodeficiency virus co-infection among pregnant women in resource-limited high endemic setting, Addis Ababa, Ethiopia: implications for prevention and control measures. *European journal of medical research*, *21*(1), 1-7.

Diop-Ndiaye, H., Touré-Kane, C., Etard, J. F., Lo, G., Diaw, P. A., Ngom-Gueye, N. F., ... & Mboup, S. (2008). Hepatitis B, C seroprevalence and delta viruses in HIV-1

Senegalese patients at HAART initiation (retrospective study). *Journal of medical virology*, *80*(8), 1332-1336.

Dyer, J. R., Eron, J. J., Hoffman, I. F., Kazembe, P., Vernazza, P. L., Nkata, E., ... & Cohen, M. S. (1998). Association of CD4 cell depletion and elevated blood and seminal plasma human immunodeficiency virus type 1 (HIV-1) RNA concentrations with genital ulcer disease in HIV-1-infected men in Malawi. *Journal of Infectious Diseases*, *177*(1), 224-227.

Eckert, D. M., & Kim, P. S. (2001). Mechanisms of viral membrane fusion and its inhibition. *Annual review of biochemistry*, *70*(1), 777-810.

Erena, A. N., & Tefera, T. B. (2014). Prevalence of hepatitis B surface antigen (HBsAg) and its risk factors among individuals visiting Goba General Hospital, South East Ethiopia, 2012. *BMC research notes*, *7*(1), 1-5.

Erena, A. N., & Tefera, T. B. (2014). Prevalence of hepatitis B surface antigen (HBsAg) and its risk factors among individuals visiting Goba General Hospital, South East Ethiopia, 2012. *BMC research notes*, *7*(1), 1-5.

Fieldsteel, A. H., Cox, D. L., & Moeckli, R. A. (1981). Cultivation of virulent Treponema pallidum in tissue culture. *Infection and Immunity*, *32*(2), 908-915.

Fraser, C. M., Norris, S. J., Weinstock, G. M., White, O., Sutton, G. G., Dodson, R., ... & Venter, J. C. (1998). Complete genome sequence of Treponema pallidum, the syphilis spirochete. *Science*, *281*(5375), 375-388.

Freitas, S. Z., Soares, C. C., Tanaka, T. S. O., Lindenberg, A. S. C., Teles, S. A., Torres, M. S., ... & Motta-Castro, A. R. C. (2014). Prevalence, risk factors and genotypes of hepatitis B infection among HIV-infected patients in the State of MS, Central Brazil. *Brazilian journal of infectious diseases*, *18*(5), 473-480.

Garrus, J. E., von Schwedler, U. K., Pornillos, O. W., Morham, S. G., Zavitz, K. H., Wang, H. E., ... & Sundquist, W. I. (2001). Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *cell*, *107*(1), 55-65.

Gatanaga, H., Yasuoka, A., Kikuchi, Y., Tachikawa, N., & Oka, S. (2000). Influence of prior HIV-1 infection on the development of chronic hepatitis B infection. *European Journal of Clinical Microbiology and Infectious Diseases*, *19*(3), 237-239.

Gilson, R. J., Hawkins, A. E., Beecham, M. R., Ross, E., Waite, J., Briggs, M., ... & Weller, I. V. (1997). Interactions between HIV and hepatitis B virus in homosexual men: effects on the natural history of infection. *Aids*, *11*(5), 597-606.

Godornes, C., Leader, B. T., Molini, B. J., Centurion-Lara, A., & Lukehart, S. A. (2007). Quantitation of rabbit cytokine mRNA by real-time RT-PCR. *Cytokine*, *38*(1), 1-7.

Griemberg, G, Ravelli, M.R., Etcheves, P.C., Orfus, G., & Pizzimenti, M.C.(2000).Syphilis and pregnancy. Prenatal control, sero-prevalence and false biological positives. *Medicina (B Aires)*,60: 343-347.

Gupta, R., Hill, A., Sawyer, A. W., & Pillay, D. (2008). Emergence of drug resistance in HIV type 1-infected patients after receipt of first-line highly active antiretroviral therapy: a systematic review of clinical trials. *Clinical infectious diseases*, *47*(5), 712-722.

Hadler, S. C., Judson, F. N., O'Malley, P. M., Altman, N. L., Penley, K., Buchbinder, S., ... & Francis, D. R. (1991). Outcome of hepatitis B virus infection in homosexual men and its relation to prior human immunodeficiency virus infection. *Journal of Infectious Diseases*, *163*(3), 454-457.

Olawumi, H. O., Olanrewaju, D. O., Shittu, A. O., Durotoye, I. A., Akande, A. A., & Nyamngee, A. (2014). Effect of hepatitis-B virus co-infection on CD4 cell count

and liver function of HIV infected patients. *Ghana medical journal*, *48*(2), 96-100.

Hayes, R. J., Schulz, K. F., & Plummer, F. A. (1995). The cofactor effect of genital ulcers on the per-exposure risk of HIV transmission in sub-Saharan Africa. *The Journal of tropical medicine and hygiene*, *98*(1), 1-8.

Hoffmann, C. J., & Thio, C. L. (2007). Clinical implications of HIV and hepatitis B co-infection in Asia and Africa. *The Lancet infectious diseases*, *7*(6), 402-409.

Holstad, M. M., DiIorio, C., & McCarty, F. (2011). Adherence, sexual risk, and viral load in HIV-infected women prescribed antiretroviral therapy. *AIDS Patient Care and STDs*, *25*(7), 431-438.

Integrated Biological and Behavioural Surveillance Survey.(2007). HIV/STI Integrated Biological and Behavioural Surveillance Survey. *Federal Republic of Nigeria*. Retrieved from https://catalog.ihsn.org/index.php/catalog/3909.

Introduction to HIV/AIDS November 2005. <www. avert.org>

Iroezindu, M.O.,, Agbaji, O.O., Daniyam, C.A., Isiguzo, G.C., Isichei, C., Akanbi, & M.O. (2013). Liver function test abnormalities in Nigerian patients with human immunodeficiency virus and hepatitis B virus co-infection. I*nt J STD AIDS*. 24(6), 461-7.

Juanpere-Rodero, N., Martin-Ezquerra, G., Fernandez-Casado, A., Magan-Perea, L., Garcia-Alguacil, M. A., Barranco-Sanz, C., ... & Lloreta-Trull, J. (2013). Cell and tissue interactions of Treponema pallidum in primary and secondary syphilitic skin lesions: an ultrastructural study of serial sections. *Ultrastructural pathology*, *37*(1), 36-42.

Whitaker, J. A., Rouphael, N. G., Edupuganti, S., Lai, L., & Mulligan, M. J. (2012). Strategies to increase responsiveness to hepatitis B vaccination in adults with HIV-

1. *The Lancet infectious diseases*, *12*(12), 966-976.

Idoko, J., Meloni, S., Muazu, M., Nimzing, L., Badung, B., Hawkins, C., ... & Thio, C. L. (2009). Impact of hepatitis B virus infection on human immunodeficiency virus response to antiretroviral therapy in Nigeria. *Clinical infectious diseases*, *49*(8), 1268-1273.

Kapiamba, G., Masango, T., & Mphuthi, D. (2016). Antiretroviral adherence and virological outcomes in HIV-positive patients in Ugu district, KwaZulu-Natal province. *African Journal of AIDS Research*, *15*(3), 195-201.

Keeffe, E. B., Dieterich, D. T., Han, S. H. B., Jacobson, I. M., Martin, P., Schiff, E. R., ... & Wright, T. L. (2006). A treatment algorithm for the management of chronic hepatitis B virus infection in the United States: an update. *Clinical Gastroenterology and Hepatology*, *4*(8), 936-962.

Kerubo, G., Khamadi, S., Okoth, V., Madise, N., Ezeh, A., Abdalla, Z., & Mwau, M. (2015). Hepatitis B, hepatitis C and HIV-1 coinfection in two informal urban settlements in Nairobi, Kenya. *PloS one*, *10*(6), e0129247.

Khan, F., Shams, S., Qureshi, I. D., Israr, M., Khan, H., Sarwar, M. T., & Ilyas, M. (2011). Hepatitis B virus infection among different sex and age groups in Pakistani Punjab. *Virology journal*, *8*(1), 1-5.

Kottilil, S., Jackson, J.O., Polis, M.A. (2005). Hepatitis B and hepatitis C in HIV-infection. *Indian J Med Res*, 121, 424-50.

Krogsgaard, K., Lindhardt, B. Ö., Nielsen, J. O., Andersson, P., Kryger, P., Aldershvile, J., ... & Pedersen, C. (1987). The influence of HTLV-III infection on the natural history of hepatitis B virus infection in male homosexual HBsAg carriers. *Hepatology*, *7*(1), 37-41.

Laukamm-Josten, U., Müller, O., Bienzle, U., Feldmeier, H., Uy, A., & Guggenmoos-

Holzmann, I. (1988). Sir, Decline of naturally acquired antibodies to hepatitis B surface antigen in HIV-1 infected homosexual men. *Aids*, *2*(5), 400-401.

Leader, B. T., Godornes, C., VanVoorhis, W. C., & Lukehart, S. A. (2007). CD4+ lymphocytes and gamma interferon predominate in local immune responses in early experimental syphilis. *Infection and immunity*, *75*(6), 3021-3026.

Lee, H. C., Ko, N. Y., Lee, N. Y., Chang, C. M., & Ko, W. C. (2008). Seroprevalence of viral hepatitis and sexually transmitted disease among adults with recently diagnosed HIV infection in Southern Taiwan, 2000–2005: upsurge in hepatitis C virus infections among injection drug users. *Journal of the Formosan Medical Association*, *107*(5), 404-411.

Lee, K. H., Choi, H. J., Lee, M. G., & Lee, J. B. (2000). Virulent Treponema pallidum 47 kDa antigen regulates the expression of cell adhesion molecules and binding of T-lymphocytes to cultured human dermal microvascular endothelial cells. *Yonsei medical journal*, *41*(5), 623-633.

Lok, A. S., & McMahon, B. J. (2009). Chronic hepatitis B: update 2009. *Hepatology*, *50*(3), 661-662.

Magnuson, H. J., Thomas, E. W., Olansky, S., Kaplan, B. I., de Mello, L., & Cutler, J. C. (1956). Inoculation syphilis in human volunteers. *Medicine*, *35*(1), 33-82.

Martin-Serrano, J., Zang, T., & Bieniasz, P. D. (2001). HIV-1 and Ebola virus encode small peptide motifs that recruit Tsg101 to sites of particle assembly to facilitate egress. *Nature medicine*, *7*(12), 1313-1319.

Martin-Serrano, J., Zang, T., & Bieniasz, P. D. (2003). Role of ESCRT-I in retroviral budding. *Journal of virology*, *77*(8), 4794-4804.

Mbaawuaga, E. M., Iroegbu, C. U., Ike, A. C., & Jombo, G. T. A. (2014). Studies on prevalence, co-infection and associated risk factors of hepatitis B virus (HBV) and Human immunodeficiency virus (HIV) in Benue State, Nigeria. *Sci J Public Health*, *2*(6), 569-576.

Mbae M et al. (2016). *Early mortality risk of HIV/hepatitis B virus co-infected patients initiating ART in Kenya.* Conference on Retroviruses and Opportunistic Infections (CROI), Boston, abstract 562.

McBroom, R. L., Styles, A. R., Chiu, M. J., Clegg, C., Cockerell, C. J., & Radolf, J. D. (1999). Secondary syphilis in persons infected with and not infected with HIV-1: a comparative immunohistologic study. *The American journal of dermatopathology*, *21*(5), 432-441.

Mendes-Corrêa, M. C. J., Barone, A. A., Cavalheiro, N. D. P., Tengan, F. M., & Guastini, C. (2000). Prevalence of hepatitis B and C in the sera of patients with HIV infection in São Paulo, Brazil. *Revista do Instituto de Medicina Tropical de São Paulo*, *42*, 81-85.

Mitchell, R. S., Beitzel, B. F., Schroder, A. R. W., Shinn, P., Chen, H., Berry, C. C., ... & Emerman, M. (2004). Retroviral DNA integration: ASLV, HIV, and MLV show distinct target site preferences. *PLoS biology*, *2*(8), e234.

Morris, L., & Cilliers, T. (2005). Viral structure, replication, tropism, pathogenesis and natural history. *HIV/AIDS in South Africa*, 79-142.

Mohammed, M. U., Abdulhamid, A., Badamasi, M. M., & Ahmed, M. (2015). Rainfall dynamics and climate change in Kano, Nigeria. *Journal of Scientific Research and Reports*, 386-395.

Mugomeri, E., Senauoane, M. B., Ruhanya, V., Chin'ombe, N., & Nyandoro, G. (2015). Occurrence of HBV/HIV coinfection by laboratory values in Roma, Lesotho. *Germs*, *5*(1), 8.-11.

Muriuki, B. M., Gicheru, M. M., Wachira, D., Nyamache, A. K., & Khamadi, S. A. (2013). Prevalence of hepatitis B and C viral co-infections among HIV-1 infected individuals in Nairobi, Kenya. *BMC research notes*, *6*(1), 1-6.

National AIDS Control Organization, Ministry of Health and Family Welfare, Govt. of India, New Delhi. (2000). Clinical case definition for AIDS. Specialist training and reference module. p. 33.

National Institute of Health. HIV Infection and AIDS. (2005). An Overview. US department of Health and Human Services. Retrieved from http://www.niaid.nih.gov/factsheets/hivinf.htm

Ndako, J. A., Nwankiti, O. O., Echeonwu, G. O., Junaid, S. A., Anaele, O., & Anthony, T. J. (2011). Studies on prevalence and risk factors for Hepatitis B Surface Antigen among secondary school students in north-central, Nigeria. *Sierra Leone Journal of Biomedical Research*, *3*(3), 163-168.

Nikolopoulo, G. K., Paraskevis, D., Hatzitheodorou, E., Moschidis, Z., Sypsa, V., Zavitsanos, X., ... & Hatzakis, A. (2009). Impact of hepatitis B virus infection on the progression of AIDS and mortality in HIV-infected individuals: a cohort study and meta-analysis. *Clinical Infectious Diseases*, *48*(12), 1763-1771.

Nkiru, E.C., Edo, A.D., Obiora, N.G., Igwe, C.U. (2004) The seroprevalence of hepatitis B surface antigen and human immunodeficiency virus among pregnant women in Anambra State, Nigeria, *The Nigerian postgraduate medical journal*, 9(1), 7-10.

Nyirenda, M., Beadsworth, M. B. J., Stephany, P., Hart, C. A., Hart, I. J., Munthali, C., ... & Zijlstra, E. E. (2008). Prevalence of infection with hepatitis B and C virus

and coinfection with HIV in medical inpatients in Malawi. *Journal of Infection*, *57*(1), 72-77.

Obeagu, E. I., & Obeagu, G. U. (2017). Occult Hepatitis B infection and immunity. *Int. J. Curr. Res. Med. Sci*, *3*(8), 89-100.

Omatola, C. A., Idofe, J., Okolo, M. L. O., Adejo, P. O., Maina, M. M., & Oyiguh, J. A. (2019). Seroprevalence of HBV among people living with HIV in Anyigba, Kogi State, Nigeria. *African health sciences*, *19*(2), 1938-1946.

Okwen, M. P., Reid, S., Njei, B., & Mbuagbaw, L. (2014). Hepatitis B vaccination for reducing morbidity and mortality in persons with HIV infection. *Cochrane Database of Systematic Reviews*, (10).

Olawumi, H. O., Olanrewaju, D. O., Shittu, A. O., Durotoye, I. A., Akande, A. A., & Nyamngee, A. (2014). Effect of hepatitis-B virus co-infection on CD4 cell count and liver function of HIV infected patients. *Ghana medical journal*, *48*(2), 96-100.

Omatola, C. A., Idofe, J., Okolo, M. L. O., Adejo, P. O., Maina, M. M., & Oyiguh, J. A. (2019). Seroprevalence of HBV among people living with HIV in Anyigba, Kogi State, Nigeria. *African health sciences*, *19*(2), 1938-1946.

Otedo, A. E. O. (2004). HBV, HIV co-infection at Kisumu district hospital, Kenya. *East African medical journal*, *81*(12), 626-630.

Otto-Knapp, R., Cortes, C. P., Saavedra, F., Wolff, M., & Weitzel, T. (2013). Hepatitis B prevalence and influence on HIV treatment outcome and mortality in the Chilean AIDS Cohort. *International Journal of Infectious Diseases*, *17*(10), e919-e924.

Platt, E. J., Durnin, J. P., & Kabat, D. (2005). Kinetic factors control efficiencies of cell entry, efficacies of entry inhibitors, and mechanisms of adaptation of human immunodeficiency virus. *Journal of virology*, *79*(7), 4347-4356.

Pollack, T. M., Ngo, L., Thuy, P. T., & Colby, D. J. (2016). Response to hepatitis B vaccination among HIV-infected adults in Vietnam. *Journal of virus eradication*, *2*(2), 102-106.

Previsani N, Lavanchy D. (2002). Hepatitis B. World Health Organization. (WHO/CDS/CSR/LYO/2002.2).

Puoti, M., Airoldi, M., Bruno, R., Zanini, B., Spinetti, A., Pezzoli, C., ... & Carosi, G. (2002). Hepatitis B virus co-infection in human immunodeficiency virus-infected subjects. *AIDS rev*, *4*(1), 27-35.

Puoti, M., Cozzi-Lepri, A., Paraninfo, G., Arici, C., Moller, N. F., Lundgren, J. D., ... & Monforte, A. D. A. (2006). Erratum: Impact of lamivudine on the risk of liver-related death in 2,041 HBsAg-and HIV-positive individuals: Results from an inter-cohort analysis (Antiviral Therapy (2006) 11 (657-674)). *Antiviral Therapy*, *11*(6), 831.

Ray, N., & Doms, R.W. (2006). HIV-1 coreceptors and their inhibitors. *Curr Top Microbiol Immunol. 303*, 97–120.

Ranjbar, R., Davari, A., Izadi, M., Jonaidi, N., & Alavian, S. M. (2011). HIV/HBV co-infections: epidemiology, natural history, and treatment: a review article. *Iranian Red Crescent Medical Journal*, *13*(12), 855-862.

Rewari, B.B., & Joshi, P.L. (2003). Epidemiology of HIV/AIDS - reference to India. In: Das S, editor. Medicine Update Vol.13. Association of Physicians of India: Mumbai, 79-82.

Risbud, A. (2005). Human immunodeficiency virus (HIV) & sexually transmitted diseases (STDs). *Indian J Med Res*, *121*(4), 369-76.

Rockstroh, J. K. (2006). Influence of viral hepatitis on HIV infection. *Journal of Hepatology*, *44*, S25-S27.

Rodrigue, K. W., Aimé, K. S. L., Serges, T., Nguwoh, P. S., Gaelle, P. T., Ibrahim, M. M. K., ... & Josep, F. (2019). Prevalence of HIV and HBV and associated risk factors in communal areas: programmatic implications in the peripheral areas of Yaounde. *International Journal of Health and Clinical Research*, *2*(3), 14-20.

Sarkar, J., Saha, D., Bandyopadhyay, B., Saha, B., Kedia, D., Mazumder, D. G., ... & Guha, S. K. (2016). Baseline characteristics of HIV & hepatitis B virus (HIV/HBV) co-infected patients from Kolkata, India. *The Indian journal of medical research*, *143*(5), 636.

Schacker, T., Ryncarz, A. J., Goddard, J., Diem, K., Shaughnessy, M., & Corey, L. (1998). Frequent recovery of HIV-1 from genital herpes simplex virus lesions in HIV-1–infected men. *Jama*, *280*(1), 61-66.

Scherdin, U. L. R. I. C. H., Rhodes, K., & Breindl, M. I. C. H. A. E. L. (1990). Transcriptionally active genome regions are preferred targets for retrovirus integration. *Journal of virology*, *64*(2), 907-912.

Schroder A. (2012). HIV-1 integration in the human genome favors active genes and local hotspots. Cell.;110:521–29. I*nt J STD AIDS.*,23(6), 435-8.

Sellati, T. J., Bouis, D. A., Kitchens, R. L., Darveau, R. P., Pugin, J., Ulevitch, R. J., ... & Radolf, J. D. (1998). Treponema pallidum and Borrelia burgdorferi lipoproteins and synthetic lipopeptides activate monocytic cells via a CD14-dependent pathway distinct from that used by lipopolysaccharide. *The journal of*

Immunology, *160*(11), 5455-5464.

Shaw, T., & Locarnini, S. (2004). Entecavir for the treatment of chronic hepatitis B. *Expert review of anti-infective therapy*, *2*(6), 853-871.

Sharma, S. K., Mohan, A., & Kadhiravan, T. (2005). HIV-TB co-infection: epidemiology, diagnosis & management. *Indian Journal of Medical Research*, *121*(4), 550-567.

Sirisena ND, Njoku MO, Idoko JA, Isamade E, Barau C, Jelpe D, Zamani A, Otowo S. (2002). Carriage rate of hepatitis-B surface antigen (HBsAg) in an urban community in Jos, Plateau State, Nigeria. *The Nigerian Postgraduate Medical Journal.; 9*(1):7-10.

Sorrell, M. F., Belongia, E. A., Costa, J., Gareen, I. F., Grem, J. L., Inadomi, J. M., ... & Trotter, H. T. (2009). National Institutes of Health consensus development conference statement: management of hepatitis B. *Hepatology*, *49*(S5), S4-S12.

Stary, G., Klein, I., Brüggen, M. C., Kohlhofer, S., Brunner, P. M., Spazierer, D., ... & Stingl, G. (2010). Host Defense Mechanisms in Secondary Syphilitic Lesions: A Role for IFN-γ-/IL-17–Producing CD8+ T Cells?. *The American journal of pathology*, *177*(5), 2421-2432.

Sule, W. F., Okonko, I. O., Yumusa, I. P., Odu, N. N., & Frank-Peterside, N. (2011). Hepatitis B surface antigen (HBsAg) and risk factors of transmission among patients attending hospital in Ankpa, Kogi State, Nigeria. *Nature and science*, *9*, 37-41.

Thio, C. L. (2009). Hepatitis B and human immunodeficiency virus coinfection. *Hepatology*, *49*(S5), S138-S145.

Tedaldi, E. M., Baker, R. K., Moorman, A. C., Wood, K. C., Fuhrer, J., McCabe, R. E., ... & HIV Outpatient Study (HOPS) Investigators. (2004). Hepatitis A and B vaccination practices for ambulatory patients infected with HIV. *Clinical Infectious Diseases*, *38*(10), 1478-1484.

Tipple C.(2011) Getting the measure of syphilis: qPCR to better understand early infection. *Sex Transm Infect.*;87(6):479–485.

Todd, J. V., Cole, S. R., Pence, B. W., Lesko, C. R., Bacchetti, P., Cohen, M. H., ... & Adimora, A. A. (2017). Effects of antiretroviral therapy and depressive symptoms on all-cause mortality among HIV-infected women. *American journal of epidemiology*, *185*(10), 869-878.

Tosca, A., Lehou, J., Hatjivasiliou, M., Varelzidis, A., & Stratigos, J. D. (1988). Infiltrate of syphilitic lesions before and after treatment. *Sexually Transmitted Infections*, *64*(5), 289-293.

Turlure, F., Maertens, G., Rahman, S., Cherepanov, P., & Engelman, A. (2006). A tripartite DNA-binding element, comprised of the nuclear localization signal and two AT-hook motifs, mediates the association of LEDGF/p75 with chromatin in vivo. *Nucleic acids research*, *34*(5), 1653-1665.

Mukherjee, T., Nanda, S., Barve, S., & Gupta, R. (2013). Interpreting liver function test in HIV-HBV coinfection. *National Journal of Medical Research*, *3*(4).

Uneke, C. J., Ogbu, O., Inyama, P. U., Anyanwu, G. I., Njoku, M. O., & Idoko, J. H. (2005). Prevalence of hepatitis-B surface antigen among blood donors and human immunodeficiency virus-infected patients in Jos, Nigeria. *Memórias do Instituto Oswaldo Cruz*, *100*, 13-16.

Van Voorhis, W. C., Barrett, L. K., Koelle, D. M., Nasio, J. M., Plummer, F. A., & Lukehart, S. A. (1996). Primary and secondary syphilis lesions contain mRNA for Thl cytokines. *Journal of Infectious Diseases*, *173*(2), 491-495.

van Voorhis, W. C., Barrett, L. K., Nasio, J. M., Plummer, F. A., & Lukehart, S. A. (1996). Lesions of primary and secondary syphilis contain activated cytolytic T cells. *Infection and immunity*, *64*(3), 1048-1050.

Verma, S., Mahajan, A., Sharma, M., Gupta, V., Tandon, V.R. (2006). Clinical Profile of HIV/AIDS patients in Jammu (JandK)-One-year prospective study. Paper presented in HIV CONGRESS 20 10th -12 th March atMumbai, India. Abstract published HIV Congress 2006 proceedings, abstract book.pp.73.

Wasley, A., Grytdal, S., & Gallagher, K., (2006). Surveillance for acute viral hepatitis--United States. *MMWR Surveill Summ*. 2008, 57(2), 1-24.

Wambani, J. R., Ogola, P. E., Arika, W. M., Rachuonyo, H. O., Kemboi, N. G., Lihana, R., & Burugu, M. W. (2015). Anti retroviral drug hepatotoxicity and risk factors in HIV patients with or without hepatitis B and C: a review. *J Infect Dis Ther*, *3*(6), 1-5.

Wambani, J. R., Ogola, P. E., Arika, W. M., Rachuonyo, H. O., Kemboi, N. G., Lihana, R., & Burugu, M. W. (2015). Anti retroviral drug hepatotoxicity and risk factors in HIV patients with or without hepatitis B and C: a review. *J Infect Dis Ther*, *3*(6), 1-5.

Weldemhret, L., Asmelash, T., Belodu, R., & Gebreegziabiher, D. (2016). Sero-prevalence of HBV and associated risk factors among HIV positive individuals attending ART clinic at Mekelle hospital, Tigray, Northern Ethiopia. *AIDS research and therapy*, *13*(1), 1-7.

WHO, (2021). Clinical Guidelines: Antiretroviral therapy. Retrieved from https://www.who.int/hiv/pub/arv/chapter4.pdf

Wondimeneh, Y., Alem, M., Asfaw, F., & Belyhun, Y. (2013). HBV and HCV seroprevalence and their correlation with CD4 cells and liver enzymes among HIV positive individuals at University of Gondar Teaching Hospital, Northwest Ethiopia. *Virology journal*, *10*(1), 1-8.

Zhu, T., Mo, H., Wang, N., Nam, D. S., Cao, Y., Koup, R. A., & Ho, D. D. (1993). Genotypic and phenotypic characterization of HIV-1 patients with primary infection. *Science*, *261*(5125), 1179-1181.

Zoufaly, A., Onyoh, E. F., Tih, P. M., Awasom, C. N., & Feldt, T. (2012). High prevalence of hepatitis B and syphilis co-infections among HIV patients initiating antiretroviral therapy in the north-west region of Cameroon. *International journal of STD & AIDS*, *23*(6), 435-438.

APPENDICES

Appendix 1: Questionnaire

SECTION 1: DEMOGRAPHIC CHARACTERISTICS

1.Gender of the study participant

1.Male

2.Female

2.Age of the study participant

3.Marital status of the study participant

1.Married

2.Single

3.Divorced

4.Windowed

4. What type of marriage Are you in?

1. Monogamous

2. Polygamous

3. None

Others specify…….

5. Education levels of the study participants

1. Primary level

2. Secondary level

3. Tertiary level(college/University)

4. No formal education

6. Employment status of the study participant

1. Self employed
2. Employed
3. 3.Not employed

7. Where do you live urban or rural area?

1. Urban area
2. Rural area

SECTION II: RISK FACTORS OF HIV CO-INFECTION WITH HEPATITIS B

8. Do you use condoms while having sex?

1. Yes
2. 2.No

9. How many sexual partners do you have?

1. One
2. Two
3. More than two

10. Have you ever been screened before for Hepatitis B

1. Yes

1. 2.No

11. Do you smoke and take alcohol

1. Yes
2. No

12. Have any of your family members had Hepatitis B infection?

1. Yes
2. No

3. I do not know

13. Have you been done blood transfusion before?

1. Yes
2. No

14. Have you been vaccinated against Hepatitis B before?

1. Yes
2. No

SECTION III: LABORATORY RESULTS

Viral load	CD4 COUNT	ALT

15. Presence of Hepatitis B virus co-infection in the Blood specimen?

1. Yes
2. No

16. Current status of viral load results

1. Viral load suppressed
2. Un detectable levels
3. High

Appendix II: Informed consent

Title: Effects of Hepatitis B co- infection on immune progression markers among HIV patients attending comprehensive care clinics in Makueni County.

Introduction

Human immunodeficiency virus (HIV) is the virus that causes acquired immunodeficiency syndrome, also known as AIDS. HIV kills or damages the cells of the body's immune system, destroying CD4 positive (CD4+) T cells (soldiers of the body against virus), a type of white blood cell vital to fighting off infection. HIV is a virus spread through certain body fluids.Hepatitis B is a virus that infects the liver .Most adults who get it have it for a short time and then get better.It is caused by the hepatitis B virus. It is spread through contact with the blood and body fluids of an infected person.You may get hepatitis B if you:Have sex with an infected person without using a condom, Share needles (used for injecting drugs) with an infected person,Share personal items like razors or with an infected person

We are conducting a study to investigate the effects Hepatitis B co-infection on immune progression markers among HIV patients attending this facility. In order to be sure that you are informed about being in this research, we are asking you to read (or will read to you) this consent form. The purpose of this consent form is to give you the information you will need to help you decide whether or not to participate in the study. Please read the form carefully. You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. Before you decide if you wish to be in this study, you need to know about any good or bad things that may arise if you decide to join. This form tells you about the study. This consent form may contain some words that are unfamiliar to you. Please ask us to explain anything you may not understand.

Being in the study is your choice

When we have answered all your questions, you can decide if you want to participate in the study or not. This process is called ‘informed consent. This consent form gives you Information about the study and the risks will be explained to you. Once you understand the Study, and if you agree to take part, you will be asked to sign your name or make your mark on this form in the presence of a witness. We will give you a copy of this form for your records. Before you learn about the study, it is important that you know the following:

□□Your participation in this study is entirely out of your choice (voluntary)

□□You may decide not to answer questions, give any specimens or even withdraw from the study at any time.

Purpose for the Research

We are asking you to participate in this study to help us determine the effects Hepatitis B co-infection on immune bio-markers among HIV patients attending comprehensive care clinics in Makueni County. We would like to know if Hepatitis B co-infection among HIV patients leads to disease progression. Hepatitis B vaccination effectiveness will also be determined. This will help to give information on planning for proper management of the patients in future so that we stop their spread and improve the immune status of our patients hence protecting them from morbidities and mortalities due to co-infection

Study groups

The study groups will comprise of HIV Patients seeking services in the comprehensive care clinics in Makueni County who are selected to participate in the study aged above 18 years of age. All groups of people mentioned here are very important to this study.

Procedures

If you agree to participate in this study by signing at the end of this form, you will participate in the following activities: You will be asked questions related to this study such as your education background, income and sources of income. You will also be asked questions to assess your knowledge on risk factors for Hepatitis B co-infection among HIV patients as well as questions regarding your lifestyle issues that maybe potentially put you to risk for Hepatitis B.This questionnaire will take approximately 30 – 45 minutes of your time. This will also be done again after six months when you will be coming for a follow up. We will draw blood for Hepatitis B testing, amount of virus in your body (viral load) the soldiers of the immune system(CD4) and the functioning of your liver(Alanine aminotransferase) testing after getting informed consent from you. This selection is done in a way that ensures that every patient has equal chance of being included into the study and you will receive the medical services as usual regardless of whether you were selected or not.

Precautions

You might feel a little pain when withdrawing blood from the veins, however, there are no other expected complications associated with this exercise. The team is well trained and experienced staff will guide you through this exercise and will take necessary precaution to ensure minimum discomfort.

Possible Risks/discomfort

There are no disturbing procedures that will be carried out on you. You may feel uncomfortable during the interview due to the sensitive nature of the some questions including loss of privacy, but safeguards will be implemented to minimize this risk. We will minimize risk and discomfort from the interview by using a trained staff to place you at ease during the interview. You may skip any questions that you do not want to

answer and may terminate the interview at any time without consequence. You will also be free to withdraw from the study any time you feel like.

Data security and Confidentiality

All the information gathered by the research team will be used in confidence for the sole purpose of this research only. Any records relating to your identity and test results will remain confidential. Your name will not be put (divulged) in any report of the results, and you will receive a copy of this consent form. No one will have access to the interviews except the researchers and supervisors. The study team will provide you with examination results immediately they have been released by our laboratory technologists performing the tests. Strict data management procedures are intended to ensure confidentiality of the study subjects.

New findings

Results will be distributed/ disseminated to the relevant health ministries in Kenya, the county and other stakeholders in the country.

Benefits

Results obtained will help in making recommendations of on employing new public health approaches/methods to reduce the burden of co-infections with Hepatitis B among HIV patients in Makueni County and other areas of the country.

Costs to you

There is no cost to you for participating in the study.

Reimbursement

As participant in this study you will be reimbursed for out of pocket expenses spent to visit the study site. The compensation will include return bus ticket at a rate of Kshs 250 per visit related to the study.

If You Decide Not to Be in the Research

You are free to decide if you want to be in this research. Your decision will not affect the health care/service you would normally receive. You will therefore receive the usual treatment you deserve within the health facility.

Leaving the Research

If you choose to be in the study, you can still decide not to complete the interview. If you leave the study, please tell the interviewer why you are leaving so that this information can be used to improve our work and provide more support if possible. It is your right to withdraw from the study at any time

Problems and questions

If you ever have questions about this study, you should contact: Geoffrey Maitha, Study

Principal Investigator, (Mobile: 0723461116) or Prof.Gidion Kikuvi from Jommo Kenyatta University or Dr.Peter Wanzala-0721624374 and Dr Fredrick Kirui-0723543111 both from Kenya Medical Research Institute.

Your rights as a Participant

This research has been reviewed and approved by the Ethical Review Committee of the

Kenyan Medical Research Institute (KEMRI), if you have any questions about your rights

as a research participant you may contact the secretary of the KEMRI ERC at 020-272-2541, or 020-272-6781.

Your statement of consent and signature

If you have read the informed consent, or had it read and explained to you, and you understand the information and voluntarily agree to join this study, please carefully read the statements below and think about your choice before signing your name or making your mark below. No matter what you decide, it will not affect your rights in anyway:

□□The risks and benefits involved in this study have been read and explained to me.

□□I have been given the chance to ask any questions I may have and I am content with the answers to all of my questions.

□□I know that my records will be kept confidential and that I may leave this study at any time

□□The name, phone number and address of whom to contact in case of an emergency has been told to me, and has also been given to me in writing.

□□I agree to take part in this study as a volunteer, and were given a copy of this

Informed consent form to keep.

_____

Participant's Name (printed)

_____ _____

Signature or Participant or thumb print (for those who cannot sign) Date

If volunteers cannot read the form themselves, a witness must sign here:

I was present throughout the entire informed consent process with the participant. All questions from the subject were answered and the participant has agreed to take part in the research.

_____

Printed Name of Witness

_____ _____

Signature of Witness Date

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_____

Printed Name of Person Who Obtained Consent (Study staff)

_____ _____

Signature of Person Who Obtained Consent Date

NOTE: You are not giving up any of your legal rights by signing this informed consent document.

Appendix III: Approval letter ethics and scientific review

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713349, 0722-205901, 0733-400003, Fax: (254) (020) 2720030
E-mail: director@kemri.org, info@kemri.org, Website. www.kemri.org

KEMRI/RES/7/3/1 **October 09, 2017**

TO: **GEOFFREY MUTISYA MAITHA,**
PRINCIPAL INVESTIGATOR

THROUGH: **DIRECTOR, CPHR,**
NAIROBI

Forwarded 16/10/17

Dear Sir,

RE: **KEMRI/SERU/CPHR/003/3524 *(RESUBMITTED INITIAL SUBMISSION)*: EFFECTS OF HEPATITIS B CO-INFECTION ON IMMUNE BIO-MARKERS AMONG HIV PATIENTS ATTENDING COMPREHENSIVE CARE CLINICS IN MAKUENI COUNTY**

Reference is made to your undated letter. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised documents on the same day (October 02, 2017).

This is to inform you that the Committee determines that the issues raised at the 266th Joint Committee A, B, C and ERC meeting held on **August 31, 2017** have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **October 09, 2017** for a period of one year. Please note that authorization to conduct this study will automatically expire on **October 08, 2018**. If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval by **August 27, 2018**

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until a written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advice SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

DR. MERCY KARIMI NJERU,
ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT

Appendix IV: Approval letter from county department of health

REPUBLIC OF KENYA

GOVERNMENT OF MAKUENI COUNTY
DEPARTMENT OF HEALTH
OFFICE OF THE COUNTY DIRECTOR OF HEALTH
P.O. BOX 89-90300 MAKUENI
Website: makueni.go.ke Email: countyhealthmkn@gmail.com

GOMC/DOH/CDH/GEN.III/(135) 23rd November 2017

To: Geoffrey Mutisya Maitha

Thro'
The Head
KEMRI Scientific & Ethics Review Unit
Kenya Medical Research Institute
P.O. Box 54840-00200
NAIROBI

Dear Sir/Madam,

RE: RESEARCH COLLABORATION TO UNDERTAKE EFFECTS OF HEPATITIS B CO-INFECTION ON IMMUNE BIO-MAKERS AMONG HIV PATIENTS ATTENDING COMPREHENSIVE CARE CLINICS IN MAKUENI COUNTY.

Reference is made to your letter dated 9th October 2017 on the above subject matter.

By way of this letter, I wish to acknowledge receipt of your request and permission is granted to undertake this research. This office will give the necessary support to ensure the research is successful

COUNTY DIRECTOR OF HEALTH
MAKUENI COUNTY
23 NOV 2017
P. O. Box 89 - 90300, MAKUENI.
Email: countyhealthmkn@gmail.com

Yours faithfully,

Dr. Kiio S. Ndolo
Director of Medical Services
Makueni County

cc
ECM Health Services - Makueni
Chief Officer Health Services - Makueni

Appendix V: Approval Letter from Board of Postgraduate Studies Jomo Kenyatta University

Geffmaitha@yahoo.com
0723-461116

OFFICE OF THE GRADUATE
RECEIVED
04 MAY 2018
PROGRAM COORDINATOR
ITROMID

JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

P.O. BOX 62000
NAIROBI – 00200
KENYA
Email: director@bps.jkuat.ac.ke

TEL: 254-067-52711/52181(6114)
FAX: 254-067-52164/52030
Mobile:0708-602225

REF JKU/2/11/ TM406-0745/2016 **22nd February 2018**

GEOFFREY MUTISYA MAITHA
C/o SOPH
JKUAT

Dear Mr. Maitha

RE: APPROVAL OF RESEARCH PROPOSAL AND SUPERVISORS

Kindly note that your PhD. research proposal entitled: **"Effects of Hepatitis B co-infection on immune bio-markers among HIV patients attending comprehensive care clinics in Makueni county"** has been approved. The following are your approved supervisors:-

3 Prof. Gideon Kikuvi
4 Prof. Peter Wanzala
3 Fredrick Kirui

PROF. (ENG) GEOFREY MANG'URIU
AG. DIRECTOR, BOARD OF POSTGRADUATE STUDIES

Copy to: Dean , SOPH

JKUAT is ISO 9001:2008 certified
Setting Trends in Higher Education, Research and Innovation