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SEROPREVALENCE OF HEPATITIS B VIRUS AMONG PEOPLE LIVING WITH HIV WITH UNSUPPRESSED VIRAL LOADS IN ETHIOPIA

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This is to certify that the thesis prepared by Zewude Birhanu entitled **"SEROPREVALENCE OF HEPATITIS B VIRUS AMONG PEOPLE LIVING WITH HIV WHO UNSUPPRESSED VIRAL LOADS IN ETHIOPIA.",** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Science Specialty in Diagnostic and Public Health Microbiology complies with the regulations of University and meets the accepted standards with respect to originality and quality.

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LIST OF ABBREVIATIONS

Ab	Antibody
Ag	Antigen
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
CADRE	Cyclic Acquired HIV drug resistance
CHB	Chronic hepatitis B infection
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
ECLIA	Electrochemiluminescence immunoassay
EPHI	Ethiopian Public Health Institute
HBSA	Hepatitis B surface Antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HIV	Human immunodeficiency virus
HRP	Horseradish peroxidase
NHIVMRL	National Human Immunodeficiency Virus Molecular-Referral Laboratory
PLHIV	People living with Human Immunodeficiency Virus
RCT	Randomized controlled trial
SDGS	Sustainable Development Goals
SOP	Standard Operating Procedure
UNAIDS	Joint United Nations Programme
VL	Viral load
WHO	World Health Organization

ABSTRACT

Background: The rates of Hepatitis B virus (HBV) - Human immunodeficiency virus (HIV) co-infection worldwide is estimated at 7.6%. People with HIV and infected with HBV are at increased risk for liver-related morbidity as indicated by elevated plasma CRP level. There is a need to establish the burden of HBV-HIV co-infection, to characterize the most affected populations, and to inform national and regional screening programs and clinical management. However, there is limited national representative data on HIV-HBV co-infection rate and factors influencing infection in Ethiopia.

Objective: To determine the sero-prevalence of HBV and its associated factors among people living with HIV (PLHIV) and unsuppressed HIV viral loads and, assess virological status by polymerase chain reaction (PCR) in Ethiopia, 2026.

Methods: A cross-sectional investigation comprised 900 PLHIV with unsuppressed viral loads using national preserved-sample data was conducted from Jan 1, 2025 and February 15, 2026. Sociodemographic data were retrieved from the national Viral Load database registered during Cyclic acquired HIV drug resistance survey. HBsAg testing was performed using rapid test kits (RDT kit). About 200 samples were rechecked by ECLIA and those found positive by RDT were subjected for virological test by using Real-time PCR. CRP level was determined for 120 samples, including 20 HBsAg positive. Data was analyzed using IBM SPSS version 22.0. Descriptive summary and chi-square test were performed. Logistic regression analysis was employed to identify factors associated with the co-infection of HBV and HIV. The level of significance was set at 0.05.

Results: The median (IQR) age of participants was 35 (26-44) years with age range from 12 to 75 years. The majority of the participants were female, 496/900 (55.1%). HBsAg positive was found in 46 out of 900 PLHIV (5.1 %); 95% CI: 3.7- 6.5%, the 46 positive samples were re-tested by PCR where 45 (97.8%) had detectable HBV DNA. Being male (AOR = 4.60; 95% CI: 2.28-9.29, $p < 0.001$) were found significantly associated with HBsAg positivity. The majority 13/20(65%) of HBsAg positive participants had CRP result of 5 mg/L and above.

Conclusion: The findings showed that moderate seroprevalence of HBV infection among PLHIV. Almost all RDT positive also had detectable HBV DNA that validates the reliability of rapid serologic screening. The findings highlight that males were more likely predictors of infection. Hence, screening for HBV infection among high-risk groups such as PLHIV patients using RDT is advisable.

Keywords: HBV prevalence, HIV, Unsuppressed viral load, National data.

1. INTRODUCTION

1.1.Background

Hepatitis is an inflammation of the liver that affects millions of people each year. It is mainly caused by viral hepatitis. HBV is found among the most five common viral hepatitis; (A, B, C, D, and E). It is a Deoxyribonucleic acid (DNA) virus that replicates in liver cells, resulting in acute or chronic infection (1). Commonly, it causes chronic hepatitis, cirrhosis, and Hepatocellular carcinoma (HCC) (2). Most people are unaware of their viral hepatitis infection and unknowingly transmit the infection to other people, making it a silent epidemic due to its highly asymptomatic nature (3).

Viral Hepatitis infections are common among People living with HIV (PLHIV), due to their shared modes of transmission (parenteral, mother-to-child, and sexual), especially in areas where HBV infection is endemic, leading to co-infection(4). This co-infection poses a significant global public health concern because it changes the natural features of HBV, such as its genome replication status, the prevalence of chronic infection, and the development of liver disease that leads to cirrhosis, liver failure, and HCC (5). Although the precise processes by which HBV interacts with Human Immunodeficiency virus (HIV) are still unclear, the impact of HIV on HBV is critical. A large number of co-infected people are unaware of their serological status, which increases the chance of silently developing severe liver illnesses (6).

HBV is reported to be 50–100 times more infectious than HIV; it can survive for several weeks even in dried blood. Blood is the most important vehicle for its transmission. Semen, tears, and saliva are also possible vehicles for its transmission. Percutaneous contact with infected blood, unprotected sexual contact, mother-to-infant, unsafe blood transfusion, sharing razors, toothbrushes, needles, and syringes, tattooing, and body piercing are some modes of HBV transmission (7).

C-reactive protein (CRP) is an acute-phase reactant produced by the liver cells in response to inflammation, infection, or tissue injury. Elevated CRP levels are commonly observed in chronic viral hepatitis and HIV co-infection, reflecting systemic immune activation and hepatic inflammation (8). In HIV-positive individuals, CRP has been used as a surrogate marker for immune-virological discordance and disease progression, especially when CD4 counts are low or

viral suppression is incomplete (9). High-sensitivity CRP (hs-CRP) assays allow detection of low-grade inflammation and are increasingly applied in liver disease monitoring and cardiovascular risk assessment. Although population-level CRP data are limited in Ethiopia, emerging evidence from urban HIV clinics suggests its utility in identifying patients at risk of poor treatment outcomes and liver-related complications (10).

Early detection of HBV infection is imperative to facilitate timely therapeutic intervention and interrupt subsequent transmission pathways. Increasing access to HBV testing while balancing sensitivity, specificity, and affordability is crucial to meet the World Health Organization (WHO) HBV elimination targets. WHO guidelines recommend that serological screening is performed using either a laboratory-based immunoassay or a rapid diagnostic test. While Rapid diagnostic tests (RDTs) are convenient and simple to use, quality control of the results can be challenging, and they typically make it difficult to acquire data in national surveillance systems. To resolve these challenges by testing in centralized settings using quality-assured enzyme immunoassays (11).

The sustainable development goals include eliminating viral hepatitis as a major public health problem by 2030. In 2016, the WHO announced a global plan to reduce new infections of chronic HBV by 90% and deaths from both diseases by 65% by 2030. Testing, treating, and virological suppression are critical components of this method (12). Ethiopia's antiretroviral treatment program follows national standards that were taken from the WHO guidelines announced in 2016. The guideline suggests screening all HIV-positives for both HBV and HCV at the time of ART initiation (13,14).

1.2. Statements of the problem

HBV infection is a major public health problem culminating in an estimated 1.1 million fatalities globally in 2022. Globally, 254 million people were living with chronic HBV infection in 2022, with 1.2 million new infections each year. An estimated 40.8 million people were living with HIV, 630 000 deaths from HIV-related causes, while the rates of HBV-HIV co-infection were estimated 7.6% worldwide (15). HIV-HBV co-infection causes exacerbating patient morbidity and mortality rates. It is also associated with an increased risk of liver-related disease progression and continuous non-acquired immunodeficiency syndrome (AIDS)-defining morbidity in HIV-infected patients. Moreover, many PLHIV are unaware that they are infected with HBV and they are at high risk of developing liver-related disease (16).

Individuals who have unsuppressed HIV viral load despite taking antiretroviral therapy (ART) are at an increased risk of HBV reactivation and progression of liver disease. Individuals with HIV and HBV co-infection and unsuppressed HIV can lead to higher HBV viral loads, exacerbating liver-related complications and increasing mortality rates (17). However, many HIV-positive individuals lack access to routine screening for HBV, leading to under-diagnosis and under-treatment of co-infection. This is particularly concerning in low-income countries where healthcare infrastructure is inadequate (18).

Currently, ART regimens may not adequately address HBV co-infection, especially in patients who have unsuppressed HIV viral load. In addition, the presence of HBV can hinder the effectiveness of ART, leading to poorer health outcomes and increased risk of liver disease (19). The high prevalence of HBV among unsuppressed HIV-positive individuals poses a significant burden on healthcare systems, necessitating integrated approaches to manage both infections effectively. Failure to address this issue could lead to increased morbidity and mortality in this vulnerable population (17). Ethiopia has a notable prevalence of both HIV and HBV. Studies indicate that approximately 5.2% of HIV-positive individuals in Ethiopia are co-infected with HBV, which is considered a hyper endemic level according to WHO standards (19).

Despite the availability of ART, many HIV-positive individuals in Ethiopia are not achieving viral suppression, which is crucial for effective management of both infections. Research

indicates that unsuppressed HIV can lead to increased HBV replication and liver disease progression. Yet, screening and treatment protocols for HBV among HIV-positive patients are often inadequate, resulting in a significant number of undiagnosed and untreated cases. According to the aforementioned claims, there is no noticeable national serological or virological prevalence of HBV among HIV-positive clients who are on ART medications. That means there is limited evidence of the prevalence of HBV among HIV patients who are unsuppressed to ART. Although population-level CRP data are limited locally, emerging evidence from urban HIV clinics shows its utility in identifying immune-virological discordance and chronic comorbidities (20). In Ethiopia HBV prevalence is mainly done by using rapid tests usually HBsAg and this may not necessarily indicate an active infection. Especially in HIV patients with HAART, additional evidence such as polymerase chain reaction is crucial. Therefore, this study aims to estimate the prevalence of HIV-HBV co-infection, CRP rate and associated factors among people living HIV and unsuppressed viral load.

1.3. Significance of the study

The determination of the rate of HBV co-infection within the HIV-positive cohort using serological and virological testing is critically important for controlling HBV among HIV patients. The results of this study will assist policymakers and other responsible bodies in planning necessary training programs related with early detection of HBV among PLWHIV. Additionally, the findings will provide insights to healthcare providers for the early detection and management of HBV-HIV co-infection. Co-infected clients will be notified of their diagnosis, which enables them to take measures to limit HBV and HIV co-infection from viral detection point of view, thereby reducing disease progression and minimizing the risk of transmitting HBV to others. Furthermore, the outcomes of this investigation are intended to establish a critical baseline reference, informing subsequent public health interventions and policy evaluations.

2. LITERATURE REVIEW

2.1. Global burden of HBV and HIV

Viral hepatitis causes 1.3 million annual deaths globally, HBV affects approximately 257 million people, with a global prevalence of 3.2 (16, 22). An estimated 37.7 million PLHIV (with the vast majority (exceeding two-thirds) located in sub-Saharan Africa, including a subpopulation of 2.7 million individuals living with HBV co-infection (22). In Ethiopia, HBV prevalence is notably higher than global averages, reaching 6.9% nationally and up to 12.6% among clinically vulnerable subpopulations such as waste handlers and HIV-positive individuals (23).

2.2. HBV-HIV co-infections

Viral hepatitis remains a critical public health challenge globally, accounting for an estimated 1.34 million fatalities annually. This annual mortality toll exceeds the single-year global mortality attributed to HIV (1 million) and approaches that of tuberculosis (1.67 million) (12). On a global scale, the burden of Hepatitis B virus (HBV) co-infection among people living with HIV (PLHIV) is estimated at approximately 4 million individuals (24). Consequently, HBV-associated chronic liver disease is emerging as a primary driver of elevated morbidity and mortality within the PLHIV cohort (25).

The global landscape of HIV-HBV co-infection exhibits significant geographical variation, approximating 5% in Western Europe and North America, yet peaking at 30.6% within certain low-income regions (12,26). Another worldwide study estimated 5%–10% of PLHIV are co-infected with HBV (27). The highest burden 69% of HIV/HBV co-infected individuals lives in the WHO-African Region mainly in Sub-Saharan area where higher rates of co-infection reach up to 40% (28).

The prevalence of HBV among HIV-positive individuals is notably high, with estimates suggesting that from 5% to 20% of HIV-infected individuals are also co-infected with HBV, depending on the region and population studied. This co-infection complicates the clinical management of both diseases (29) Various studies conducted in different part of the world indicated that the prevalence of HBV among HIV patients were 8.4% in Vietnam (30), 8.7% in Myanmar (25) and 7.8% in Taiwan (31). the seroprevalence of HBV within the HIV-positive

cohort were 2.5% in Brazil (32), and 5.3% in East democratic republic of Congo (33) . Epidemiological data indicates that the burden of HBV co-infection among the PLHIV population in Africa approximates 15%, reflecting a profound public health challenge (19). In a study carried out in South Africa, the prevalence of HBV infection was found 6.7% among HIV-positive individuals (34). Data from a cross-sectional investigation involving a cohort of people living with HIV in the eastern DRC demonstrated that the rate of concurrent HBV infection stood at 8.0% (33). Another study from Cameroon showed that the proportion of HBV-tested patients ranged from 0.8 to 72.5% (35). A study from Sierra Leone also indicated the overall prevalence of HBsAg positivity was 12.0% (36).

According to the study done at Northern Tanzania the seroprevalence of Hepatitis B surface antigen (HBsAg) within the PLHIV cohort was documented at 2.3%. Notably, a history of blood transfusion emerged as the sole independent predictor significantly associated with HBV infection, whereas all other evaluated sociodemographic and clinical variables demonstrated no statistically significant association (37).

Study from different parts of Ethiopia showed that the prevalence of Hepatitis B in HIV patients were 6.7% (38), 10% (39), 5.9 % (26), and 5.96% (40). Study from Wolaita Sodo of Ethiopia showed that overall prevalence of HBsAg among HIV-positive study participants was (8.4%) (41).

2.3. C-reactive protein

C-reactive protein is a sensitive acute-phase reactant which is a key biomarker of systemic inflammation (acute or chronic) produced by human liver and is widely used to assess cardiovascular and infectious disease risk. In healthy individuals, C-reactive protein (CRP) functions as a trace plasma protein, maintaining baseline concentrations below 5 mg/L. However, upon initiation of an acute-phase response, serum CRP levels undergo a rapid and profound escalation. This systemic increase is detectable within 6 to 12 hours of the initial insult, culminating in a peak concentration within 24 to 48 hours. Notably, concentrations exceeding 100 mg/L are characteristically driven by severe inflammatory stimuli, including major physical trauma and systemic sepsis (42).

In addition to viral co-infections, systemic inflammation plays a critical role in HIV disease progression. CRP has been widely studied as a biomarker of inflammation in HIV-infected individuals. Elevated CRP levels are associated with unsuppressed HIV viral load, increased risk of liver disease, and higher mortality rates (43). It was also shown that CRP, along with other inflammatory markers, was predictive of all-cause mortality in HIV patients, independent of cluster differentiation (CD4) count and viral load (44). In resource-limited settings like Ethiopia, CRP testing is not routinely performed, but emerging evidence suggests that elevated CRP may correlate with liver enzyme abnormalities and poor treatment outcomes in HIV patients co-infected with HBV (45).

2.4. Factors associated with HBV-HIV co-infection

2.4.1. Sociodemographic related factors

In study carried out in Central Italy, out of 56 patients with HBV-HIV co-infection; 48 (85.7%) were male and 8 (14.3%) were female (46). Additionally, in Addis Ababa, Ethiopia, multivariable logistic regression analysis identified male sex as the strongest independent predictor of HBV co-infection among people living with HIV, with an odds ratio of 2.42 (47). Similarly, a study conducted in northeastern Ethiopia found that females had a lower prevalence of HIV-HBV co-infection compared to males (48). This disproportionate rate of HBV-HIV co-infection between opposite sexes could be explained by sex hormones difference which may play a substantial role. Estrogen in women helps regulate stronger immune responses, which aids in fighting off HBV. Conversely, testosterone in men can suppress parts of the immune response, making it easier for HBV to establish chronic, long-term infection (87). Furthermore, HBV/HIV co-infection was found higher among age groups between 18 and 35 years (46,49).

2.4.2. Individual related factors

In study from Mekele of Ethiopia showed that a history of multiple sexual partners was associated with a profoundly elevated risk of HBV infection; individuals reporting multiple partners exhibited an nearly thirtyfold increased likelihood of testing positive compared to those who did not (AOR = 29.90; 95% CI: 7.82–114.80) (39). Consistently, a study conducted in Wolaita Sodo, Ethiopia, demonstrated a significantly elevated HBsAg prevalence among patients reporting a history of multiple sexual partners compared to those without this risk factor (13.2% versus 2.9%; AOR = 7.08; 95% CI: 2.29–21.90; p = 0.001) (41).

Behavioural risk factors such as multiple sexual partnerships are associated with elevated CRP levels, reflecting on going immune activation and increased susceptibility to sexually transmitted infections (50). CRP serves as a biomarker for both acute and chronic inflammation, and its elevation may signal underlying risk in high-exposure populations.

2.4.3. Clinical related factors

A study from Ethiopia revealed that, Importantly, HBV co-infection status was significantly associated with a reduced rate of HIV viral load suppression [AOR] = 7.14; 95% CI: 2.18–23.30). Co-infected individuals were over seven times more likely to exhibit unsuppressed HIV viral loads compared to those with HIV mono-infection, whereas other clinical parameters showed no such association (51).

Having a history of sexually transmitted infections was also strongly associated with HIV-HBV co-infection. The fact that HIV and hepatitis B viruses share the same routes of transmission supports this elucidation (AOR=4.44; 95% CI 1.31–15.0) (39). CRP has been proposed as a supplementary marker for monitoring treatment response in HIV patients, especially in cases of immune-virological discordance (52). In Addis Ababa, patients with baseline CD4 >350 cells/mm³ and viral load <1000 copies/ml were less likely to show discordant responses, and CRP positivity was more common among those with poor immunologic recovery despite virological suppression (53).

History of sexually transmitted infections was also strongly associated with HBV infection (AOR=4.44; 95% CI 1.31–15.0) (54). CRP levels are elevated in individuals with concurrent STIs due to shared inflammatory pathways and immune activation (55).

Data from an Addis Ababa cohort indicates that HIV-positive individuals with severely compromised immune systems (CD4 counts under 200 cells/μL) face a significantly elevated risk of Hepatitis B co-infection compared to patients with healthier CD4 counts over 500 cells/μL (AOR=15.34, 95% CI=4.77–49.3) (40). CRP correlates inversely with CD4 count and serves as a surrogate marker for immune deterioration (56).

In Northeast Ethiopia, active viral replication closely correlated with dual infection. Compared to patients with undetectable viral loads, the risk of HBV/HIV co-infection was 16.53 times higher

in patients with viral loads under 1,000 copies/mL and 6.53 times higher in those exceeding 1,000 copies/mL (57). CRP may help differentiate between virological suppression and residual immune activation in patients with low-level viremia (58).

On the other hand, an investigation in northern Ethiopia identified a history of sexually transmitted infections as a prominent predictor for Hepatitis B. Participants with a prior STI diagnosis faced more than four times the odds of HBV infection compared to those without a history of STIs (AOR=5.21, 95% CI 1.39–19.5) (39). These comorbidities are independently associated with elevated CRP levels, reflecting systemic inflammation and increased risk of liver disease progression (59).

2.5. Conceptual framework

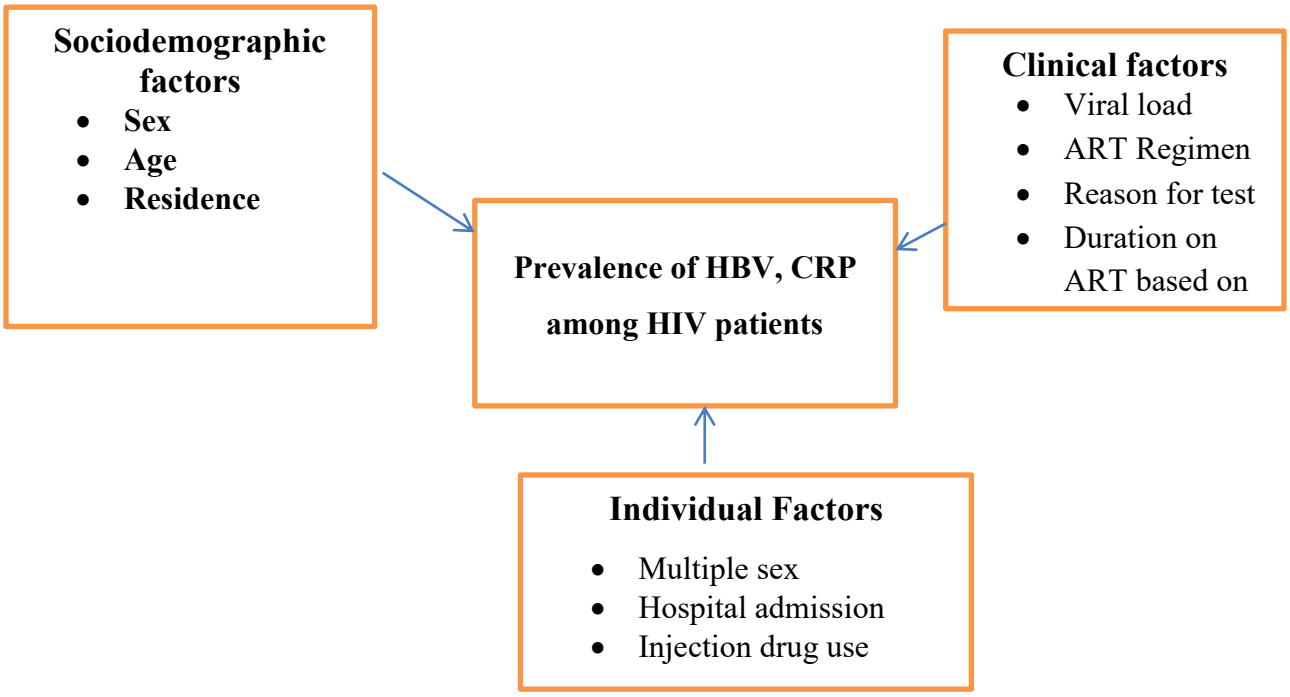


Figure 1 Conceptual framework developed on association of factors on prevalence of HBV among HIV patients from national data, Ethiopia, 2026 (60–63)

3. OBJECTIVE OF THE STUDY

3.1. General objective

To determine the serological prevalence of HBV among PLHIV with unsuppressed HIV viral loads by PCR confirmation rate among seropositive cases in Ethiopia, 2025.

3.2. Specific Objectives

- ✓ To determine the serological prevalence of HBV infection among PLHIV with unsuppressed HIV Viral load, Ethiopia, 2025.
- ✓ To determine factor associated with serological prevalence of HBV infection among PLHIV with unsuppressed HIV Viral load
- ✓ To assess virological status among HBV seropositive cases
- ✓ To identify CRP level in a subset of PLHIV with unsuppressed viral load.

4. MATERIALS AND METHODS

4.1. Study Design

A Cross-Sectional Study was conducted nationally to assess prevalence of HBV among PLHIV and with unsuppressed viral load.

4.2. Study Area and period

The study was conducted using well characterized and stored national sample data obtained during national Cyclical Acquired HIV Drug Resistance Patient Monitoring Survey carried out between June 2022 and February 2023 from 15 viral load testing laboratories. The 15 testing laboratories were distributed across the nation and collects samples from all corners of Ethiopia. The number of samples collected from each laboratory was in proportion to that laboratory's contribution to the total number of high viral load specimens. All the 15 viral load testing sites locally store all high viral load remnant plasma specimens from routinely collected samples as part of standard of care. A web-based database, "VL Database" that collects information from all viral load tests in all regional labs is maintained at Ethiopian Public Health Institute (EPHI). This database was used to allocate the number of samples to be collected from each lab.

The preserved sample data were analyzed between Jan 1, 2025 and February 15, 2026 for this present study.

4.3.Population

4.3.1. Source population

The source population was PLHIV and has access to viral load testing in Ethiopia.

4.3.2. Study population

The study population was PLHIV and have unsuppressed viral load during Cyclic Acquired HIV drug resistance Survey. The samples (Plasma) for the survey were collected between the months of June 2022 and February 2023.

4.4. Inclusion Criteria and Exclusion criteria

4.4.1. Inclusion Criteria

All high VL ART clients and had their remnant plasma sample was shipped to Ethiopian public health Institute National HIV Referral Laboratory (EPHI NHIVRL).

4.4.2. Exclusion Criteria

ART clients with incomplete information (Age, sex and Unique ART number), and insufficient sample volume were excluded.

4.5. Study variables

4.5.1 Dependent variable

- Serological prevalence of HBV
- CRP level (Sub analysis)
- Virological status of HBV

4.5.2 Independent Variables

- ✓ Sex
- ✓ Age
- ✓ ART regiment
- ✓ HIV viral load result
- ✓ Duration on ART (in month)

4.6. Sample Size Determination and Sampling Technique.

4.6.1. Sample Size Determination

This study used remnant specimens collected from a national HIV drug resistance (HIV DR) survey conducted among PLHIV with unsuppressed viral loads in Ethiopia, 2025. The sample size for the parent HIV DR survey was calculated using PASS 22 software, applying the Exact (Clopper-Pearson) formula for a two-sided 95% confidence interval for a single proportion. Based on an assumed HBV infection among PLWHIV HIV of 5.96 % (47), a design effect of 1.3 to account for clustering by health facility, and an anticipated 20% loss during sample titration, the required effective sample size was 880. Since the expected HBV prevalence is lower, a total

of 900 specimens were collected and enrolled for the parent survey, all of which were available for the present study.

Fifteen VL testing laboratories contributed specimens for this project. A probability proportional to size sampling approach was employed, with the sample size for each laboratory determined in proportion to its contribution to the total high VL testing volume, as presented in Table 1.

The total sample size available for HBV testing was 900 specimens. The allocation for each laboratory, was based on the total sample size outlined (Table 1). An adjustment was made accordingly to the expected number of specimens per laboratory based on these evaluations.

Table 1 Proportional allocation of sample size for each regional laboratory, Addis Ababa, Ethiopia, 2025.

No	Labelled sample from	% adjusted children and adult	Sample size	
1	Adama regional Lab	0.158	900*.158	142
2	Addis Ababa Regional Lab	0.114	900*0.114	103
3	ALERT Centre Clinical Laboratory	0.036	0.036*900	32
4	Amhara Public Health Institute (Bahidar)	0.079	0.079*900	71
5	Debra Berhan Hospital	0.024	0.024*900	22
6	Debre Markos Referral Hospital	0.025	900*0.025	23
7	Dessie Regional Lab	0.087	0.087*900	78
8	EPHI HIV Molecular Lab	0.13	0.13*900	117
9	Gondar University Hospital	0.025	0.025*900	23
10	Harare Regional Lab	0.021	0.021*900	19
11	Hawassa Public Health Laboratory	0.077	0.077*900	69

12	Jimma University Specialized Hospital Laboratory	0.05	0.05*900	45
13	Nekemte Regional Lab	0.036	0.036*900	32
14	Tigray Health Research Institute Lab	0.111	0.111*900	100
15	Tikur Anbessa Specialized Hospital Laboratory	0.027	0.027*900	24
	Grand total	1		900

For virological confirmation, all RDT-positive samples underwent PCR testing. Following the principle of complete enumeration of all eligible seropositive cases, no further sample size calculation was required (64).

Sample size required for RDT performance evaluation using ECLIA

To confirm the accuracy of the rapid diagnostic test (RDT) used for HBsAg screening, a subset of 200 samples was tested using the Elecsys as a reference standard. Due to cost constraints, confirmatory testing could not be performed on all 900 samples. All 20 available RDT-positive samples (26 of the original 46 were depleted) and a simple random sample of 180 RDT-negative samples were selected for confirmatory testing. A subset of 150–200 samples is adequate for validating a diagnostic test against a reference standard when the target condition is relatively rare. This sample size provides acceptable precision for estimating sensitivity and specificity (65).

Sample Size for CRP evaluation

For CRP analysis, testing was performed on a subset of 120 specimens. Among the 46 HBsAg-positive samples, 26 were depleted (insufficient residual volume after prior testing), leaving 20 HBsAg-positive samples available for CRP testing. All 20 were included and 100 HBsAg-negative samples were selected by systematic random sampling (the first one with lottery method and continue with kith interval). The sample size for CRP was therefore determined by sample availability and resource constraints. It was suggested that 12–30 participants per group are

adequate. The justifications for this sample size are based on rationale about feasibility; precision about the mean and variance; and regulatory considerations (66). Thus, a sample of 20 HBsAg-positive and 100 HBsAg-negative participants were analyzed based on this recommendation for descriptive analysis.

4.6.2. Sampling Methods

The stratified proportional sampling strategy was used to select adult (age >15) and children (age <15) remnant VL specimens with High Viral load (HVL) during the larger survey. Data from the national VL database was used to identify each specimen and retain only those that met all inclusion criteria.

4.7. Measurement and Data Collection

4.7.1. Data Collection Procedure

Sample Collection, Handling and Processing

The sampling strategy described above used to select 900 left over VL specimens with high VL over a nine-months sampling period. The identified specimens were stored under appropriate conditions (at -80^{o}C temperature). For this study, all high VL samples were collected from selected sites, and HBV co-infection testing was performed on the same samples.

Data collection procedure

Socio-demographic characteristics and other variables of the study participants were collected by ART focal person during recording of the standardized VL request form. In addition, a web-based database, “VL Database” that collected information from all VL tests in all regional labs were maintained at EPHI and information from that database was used. All data those are collected by the ART focal person at the site and collected from the national database at EPHI are crosschecked and entered into a new format that was prepared to collect all data.

4.7.2. Laboratory Analysis

Rapid diagnostic tests principle (Bio line™ HBsAg)

Abbott Bio line HBsAg was used to determine the HIV-HBV co-infection status. Hepatitis B rapid diagnostic tests (RDTs) operate as lateral flow immunochromatographic systems. When a patient sample is applied, capillary action pulls the liquid upward through a pre-treated strip, where it first encounters mobile mouse anti-HBsAg antibodies tagged with gold colloid nanoparticles. If the surface antigen is present in the specimen, it binds to these colored conjugates to create mobile immune complexes. As the fluid front migrates across the nitrocellulose membrane, these complexes are trapped by fixed, unlabeled anti-HBsAg antibodies at the test line, assembling a visible sandwich-like structure that shows up as a distinct purple line to indicate a reactive result. Unbound gold particles and built-in chicken IgY markers bypass this zone entirely and proceed to a secondary control line embedded with anti-chicken IgY antibodies, validating that fluid migration was successful and that sufficient sample volume was introduced.

HBV Viral Load Assay

HBV DNA was tested using the Abbott m2000sp/rt Real Time Polymerase Chain Reaction (RT-PCR) platform following manufactures instruction. The Abbott m2000sp/rt operates with two integrated systems: sample preparation and simultaneous amplification and detection. During sample preparation, nucleic acids were extracted and concentrated. Amplification is then performed by DNA polymerase in the presence of an internal control (IC). The IC is a DNA sequence unrelated to the HBV target, added to each specimen at the beginning of sample preparation. This sequence is co-amplified during PCR, which serves as an internal control. Furthermore, control samples at negative, low-positive, and high-positive were tested and yielded acceptable result.

Electrochemiluminescence Immunoassay (Elecsys HBsAg II)

The Electro-chemiluminescence immunoassay (ECLIA) is a highly sensitive, fully automated technology used for the quantitative or qualitative detection of Hepatitis B virus (HBV) markers, particularly on Roche cobas e411 analyzer. ECLIA offers superior sensitivity and specificity compared to enzyme-linked immunosorbent assay (ELISA) and is often used to detect HBsAg, anti-HBs, HBsAg, anti-HBe, and anti-HBc.

Test principle

This automated Elecsys HBsAg II assay utilizes a sandwich-principle electrochemiluminescence framework completed within an 18-minute runtime. Initially, a 50 µL patient sample is incubated with a cocktail of biotinylated monoclonal anti-HBsAg antibodies alongside ruthenium complex-labeled monoclonal and polyclonal anti-HBsAg antibodies. These components bind to any target viral antigens present in the serum, assembling an antibody-antigen-antibody sandwich structure.

Solid-phase separation is achieved by introducing streptavidin-coated microparticles, which secure the immunocomplexes via high-affinity biotin-streptavidin binding. This total reaction fluid is then drawn into a specialized measuring chamber, where internal magnets immobilize the microparticles onto an electrode surface, allowing a dedicated washing solution (ProCell/ProCell M) to flush away unattached matrices. When an electrical voltage is applied directly to the electrode, it triggers a chemiluminescent reaction. A photomultiplier tube measures the resulting light emission, and the system software automatically computes the sample concentration by contrasting this signal against a pre-calibrated cutoff baseline.

Interpretation of the results

Samples with a cutoff index < 0.90 are non-reactive in the Elecsys HBsAg II assay. These samples are considered negative for HBsAg and do not need further testing.

Samples having a cutoff index in the range ≥ 0.90 to < 1.0 are considered borderline in the Elecsys HBsAg II assay.

Samples with a cutoff index ≥ 1.0 are considered reactive.

All initially reactive or borderline samples should be re determined in duplicate using the Elecsys HBsAg II assay. If cutoff index values < 0.90 are found in both cases, the sample is considered negative for HBsAg.

Initially reactive or borderline samples giving cutoff index values of ≥ 0.90 in either of the redeterminations are considered repeatedly reactive.

Repeatedly reactive samples must be investigated using an independent neutralization test (Elecsys HBsAg Confirmatory Test). Samples confirmed by neutralization with anti-HBs are regarded as positive for HBsAg.

C-reactive protein

Quantitative CRP test is working on the principle of particle-enhanced turbidimetric immunoassay technique. Synthetic latex particles coated with monoclonal anti-CRP antibodies aggregate in the presence of C - reactive protein in the sample. An increase in turbidity is directly proportional to the concentration of CRP in the sample. If the CRP concentration greater than or equal to 5 mg/L indicates a high infection status, whereas if the CRP concentration less than 5 mg/L, indicates normal (42,67)

Data Quality Assurance

The reliability of the study findings was ensured through the implementation of quality control measures throughout the research activities. Training was provided to data collectors before the start of data collection, along with intensive supervision during the collection process.

4.7.3. Pre-Analytical

The collected plasma was checked for proper labeling with participant identification (Medical Record Number), the volume of serum, content, and validity of stored sera at the EPHI national HIV viral load (NHIVR) laboratory.

4.7.4. Analytical

All laboratory tests were performed by well-trained laboratory personnel following standard operational procedures to ensure the reliability and validity of test results. Refrigerated samples

and reagents were allowed to come to room temperature before testing. Serum/plasma samples, including positive and negative controls, were run simultaneously.

4.7.5. Post-Analytical

Standard operational procedures were continued to be utilized to ensure the reliability and validity of test results. Reports were recorded as soon as results are available, and any remaining serum samples were stored appropriately.

4.8. Data Analysis and Interpretation

The collected data was edited, cleaned, entered, and analyzed using the Statistical Package for Social Sciences (SPSS) software, version 22. Descriptive statistics such as frequency, mean, and percentage were calculated to describe the characteristics of the study population. Chi-square/cross-tabulation and binary logistic regression analysis were employed to determine association between variables. Compiled results were presented in the form of text, tables, or graphs.

4.9. Operational Definitions

Serological Prevalence: is the number or percentage of people in a population who test positive for a specific disease based on the presence of antibodies in their blood serum (68).

A moderate HBV prevalence: HBsAg prevalence between 2% and 7% (88).

Virological Prevalence: a specific virus in a population, often measured as the percentage of people with detectable virus (DNA HBV) or, more commonly in HIV treatment, the rate of virological failure, when viral load remains high despite treatment, indicating poor adherence or drug resistance (69).

Viral Load Levels: Viral load refers to the amount of HIV copies per milliliter of blood.

Normal CRP: Plasma CRP concentration less than 5mg/L (42)

High CRP: Plasma CRP concentration greater than or equal to 5 mg/L

Unsuppressed viral load: HIV viral load result greater than 1000 copies/mL(70)

Undetectable: An even lower level, where the virus cannot be detected by standard lab tests (71).

4.10. Ethical Considerations

The study was approved by Departmental Research and Ethics Review committee (DRERC/820/25/MLS) of the Department of Medical Laboratory Sciences, College of Health Sciences, and Addis Ababa University. Additionally, a formal letter of cooperation was issued by the Department of Medical Laboratory Sciences to EPHI.

Confidentiality/Privacy Protections

Confidentiality Agreement: All staff involved in the research was required to sign a confidentiality agreement (Appendix 2) prior to handling any records or accessing data systems. Anonymization of Samples: All samples tested were anonym zed to protect participant identities. Confidentiality was maintained throughout the collection, storage, entry, and analysis of data.

Data Collection: Patient-specific data, including hospital/clinic and bar code numbers, was collected to track back HBV test results. However, all potentially identifying information was excluded when entering data into the protocol database.

Data Security: Electronic data files, computers, and other storage devices containing data were password-protected. Each specimen will be identified by a study ID.

Record Keeping: All records were stored securely under lock and key, and any electronic files were password-protected. Data were stored without any patient-identifying information to further ensure confidentiality.

Access Restrictions: Only the principal investigators had access to all of the data, ensuring that participant confidentiality is maintained throughout the research process.

Waiver of Informed Consent

This protocol utilizes remnant VL plasma specimens and information collected for routine diagnostic purposes to assess the prevalence of HBV among individuals with high HIV VL.

Justification for Waiver of Consent

During the specimen collection for routine HIV VL testing, no written consent is obtained from patients. This activity was part of routine program monitoring, intended to inform optimal ART regimen selection in Ethiopia.

A request for a non-research determination was submitted to all Institutional Review Boards (IRBs), in line with the standards set by the WHO in its 2017 Guidelines on Ethical Issues in Public Health Surveillance.

Given that the samples had already been collected and stored, obtaining retroactive informed consent from the over 460,000 patients on ART in Ethiopia would not be feasible or practical.

A waiver was sought from all IRBs to enable HIV drug resistance testing on these remnant specimens without obtaining written consent from patients. As the protocol team was not interacting with patients, obtaining consent is not viable.

Data Handling and Result Reporting

Since there was a unique Identification number of clients HBV infection status was communicated to the respective patient clinics. Testing clinics was making every effort to link results to ART numbers and report findings to attending clinicians. This information was documented in the participants' medical records, ensuring that all relevant data is maintained securely and confidentially.

4.11. Dissemination of Results

Upon completion of the study, the results will be shared with the Department of Medical Laboratory Sciences at Addis Ababa University and relevant stakeholders. Key dissemination strategies include:

Availability in the University Library: The findings will be made available in the university library to serve as a reference material for students, researchers, experts, and policymakers for future interventions.

Publication in Peer-Reviewed Journals: The study results will be prepared for publication in both local and international peer-reviewed journals to reach a wider audience within the scientific community.

Presentation at Conferences: The findings will also be presented at related conferences, facilitating discussions and feedback from other professionals in the field.

This comprehensive approach aims to ensure that the results contribute to the existing body of knowledge and inform future public health strategies in Ethiopia and beyond.

5. RESULTS

5.1. Socio-demographic characteristics of study participants

In this study, 900 PLHIV who had unsuppressed viral load were tested to determine HIV and HBV co-infection rates. Table 2 depicts the Sociodemographic and clinical characteristics of the participants. The median (IQR) age of participants was 35 (26-44) year. The minimum age was 12 years whereas a 75-year-old participant was reported to be the eldest in the study. The majority of the participants were female, 496(55.1%). The majority of the participants were also in the age grouping 18-35, 416 (46.2%). Half of the participants were from the Oromia region 456, (50.7%) and only one participant was from Gam Bella (Table 2).

5.2. Clinical characteristics of study participants

The majority of patients in this dataset have been on antiretroviral therapy (ART) for 24-28 months, with 628 (69%) falling in this range. Only a negligible proportion (0.2–0.3%) had been on ART for more than four years. Regarding HIV viral load range of the participants, the largest group 384(42.7%) had viral loads between 1,000–10,000 copies/ml, while a notable proportion of 165 patients (18.3%) presented with very high viral loads exceeding 100,000 copies/ml. With respect to ART regimens, the majority of participants 768(85.3%) were initiated on Dolutegravir (DTG)-based regimens. Similarly, most participants 784 (87.1%) were on first-line regimen, with a very small proportion (0.6%) requiring third-line treatment. The primary reason for viral load testing was routine viral load monitoring 70.6% were annual tests. Only 1.4% was specifically ordered due to suspected ART failure (Table 2).

Table 2: Sociodemographic characteristics and clinical details of study participants

Variable	Categories	Frequency	Percentage
Sex	Female	496	55.1
	Male	404	44.9
Age group (Year)	12-17	57	6.3
	18-35	416	46.2
	36-59	406	45.1
	≥60	21	2.3
Region	ADDIS ABABA	172	19.1
	OROMIA	456	50.7
	AMHARA	192	21.3

	AFAR	7	0.8
	GAMBELLA	1	0.1
	BENISHANGUL GUMUZ	3	0.3
	HARARI	35	3.9)
	SNNP	34	3.8
Duration on ART stay in	less than 24	269	29.9
	24-48	628	69.8
	greater than 48	3	0.3
Viral Load Results	1000-10000	384	42.7
	10001-100000	351	39
	greater than 100000	165	18.3
ART Regimen	DTG	768	85.3
	Non-DTG	132	14.7
ART Regimen category	first line	784	87.1
	second line	111	12.3
	third line	5	0.6
Reasons of test	Annual test	635	70.6
	Confirmatory Test	252	28
	Suspected ART failure test	13	1.4
Total participant (sample Size)		**900**	**100**

5.3. Serological Prevalence of HBV among PLHIV with unsuppressed viral load

In this study, a total of 900 PLHIV were screened for HBV infection by using rapid diagnostic testing to detect the presence of HBsAg. Of the 900 PLHIV with unsuppressed viral load, 46 (5.1%; 95% CI: 3.7% to 6.5%) tested positive (Figure 2).

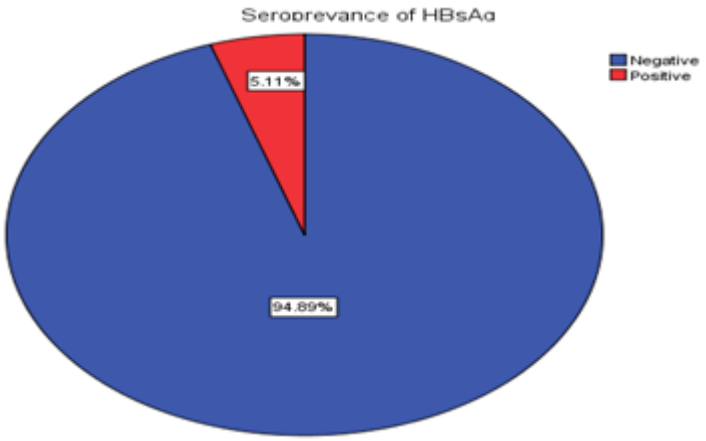


Figure 2: Serological prevalence of HBV infection among patients with unsuppressed HIV viral infection from national sample data, 2025/26.

When stratified by age, the highest HBV and HIV co-infection was observed among age group 18–35 years, where 27 out of 416 (6.5%) were positive. The lowest rate of co-infection was seen in the ≥60 age group where all 21 individuals were negative for HBsAg, with only 7 out of 286 (2.5%) tested positive. HIV-HBV co-infection is more prevalent on males 35 (8.7%) than females 11/496(2.2%). The highest positivity rate was recorded in Amhara Region, where 13/192 (6.8%) participants tested positive (Table 3). Based on bivariate analysis Sex, age group, and Duration on ART were eligible for multivariate analysis (Table 3).

Table 3: Result of HBsAg test among PLHIV with unsuppressed viral load, Ethiopia 2025

Variable	Categories	HBsAg RDT test result		Total, N (%)	P-value
		Negative,	Positive,		
Sex	Female	485(97.8)	11(2.2)	496(55.1)	0.001
	Male	369(91.3)	35(8.7)	404(44.9)	
Age group (Year)	12-17	54(94.7)	3(5.3)	57(6.3)	0.271
	18-35	389(93.5)	27(6.5)	416(46.2)	
	36-59	390(96.1)	16(3.9)	406(45.1)	
	≥60	21(100)	0(0)	21(2.3)	
Region	ADDIS ABABA	164(95.3)	8(4.7)	172(19.1)	0.930
	OROMIA	433(95/)	23(5)	456(50.7)	
	AMHARA	179(93.2)	13(6.8)	192(21.3)	
	AFAR	7(100)	0(0)	7(0.8)	
	GAMBELLA	1(100)	0(0)	1(0.1)	
	BENISHANGUL	3(100)	0(0)	3(0.3)	
	HARARI	34(97.1)	1(2.9)	35(3.9)	
	SNNP	33(97.1)	1(2.9)	34(3.8)	
Duration on ART stay in Months	less than 24	255(94.8)	14(5.2)	269(29.9)	0.083
	24-48	597(95.1)	31(4.9)	628(69.8)	
	greater than 48	2(66.7)	1(33.3)	3(0.3)	
Viral Load Results Copies/ml	1000-10000	365(95.1)	19(4.9)	384(42.7)	0.827
	10001-100000	334(95.2)	17(4.8)	351(39)	
	Greaterthan100000	155(93.9)	10(6.1)	165(18.3)	
ART Regimen	DTG	731(95.2)	37(4.8)	768(85.3)	0.335
	Non-DTG	123(93.2)	9(6.8)	132(14.7)	
ART Regimen category	first line	745(95)	39(5)	784(87.1)	0.731
	second line	104(93.7)	7(6.3)	111(12.3)	
	third line	5(100)	0(0)	5(0.6)	
Reasons of test	Annual test	600(94.5)	35(5.5)	635(70.6)	0.549
	Confirmatory Test	241(95.6)	11(4.4)	252(28)	
	Suspected ART failure test	13(10*0)	0(0)	13(1.4)	

5.4 Molecular evidence of HBV DNA among HBsAg positive study participants

HBV DNA levels were evaluated by real time PCR in all clients who were positive for HBsAg. Out of the 46 participants who initially tested positive for HBsAg by rapid diagnostic test, 97.83% had detectable HBV DNA viral load while in 2.17% it was not detectable DNA level.



Figure 3: DNA viral load of HBV by real time PCR among HBsAg positive study participants, Ethiopia 2025/26.

5.5. CRP Test results of study participants

Out of 900 PLHIV, who were tested for HBsAg by RDT only 120 of them were tested for CRP level. Out of 120 who were underwent CRP 20 participants were HBsAg positive and 100 were negative for HBsAg testing. The majority of HBsAg positives, 13(65%) had high infection status based on CRP. While among the 100 HBsAg negative participants 36(36%) had relatively low percentage of high infection status based on CRP assessment (Table 4).

Table 4: CRP test result of study participants Ethiopia, 2025.

Variable	Category	HBsAg RDT positive, N (%)	HBsAg RDT Negative, N (%)	Total
Infection status as per CRP test	High	13(65%)	36(36%)	49(40.83.2%)
	Normal	7(35%)	64(64%)	71(59.17%)
	Total	20	100	120

5.6. ECLIA/ROCH test for study participants

Confirmation of HBsAg test result was done by Elecsys test which uses the ECLIA. Two hundred samples (20 positive and 180 negative) were used to confirm the rapid test kit result. The comparison of HBsAg results showed that 175 of the rapid test kit negative sample were also negative with ECLIA and out of 20 kit positive samples 19 were also positive by ECLIA. Using ECLIA as a standard it is indicated that 1 false positive and 19 true positives, out of 20 RDT positive samples. It was also indicated that 175 samples were truly negative and 5 were falsely negative out of 180 negative RDT results. This revealed that RDT has specificity of 175/176 (99.4% (**95% CI: 98.3%-100%)**) and sensitivity of 19/24(79.2% (**95% CI: 63.0%-95.4%)**) compared to ECLIA. The positive predictive value and negative predictive value of RDT was 95.0% and 97.2% respectively. The overall accuracy of RDT compared to ECLIA is 97%. Furthermore, the RDT HBSAg test results were in an excellent agreement beyond chance with ECLIA test results (Cohens kappa coefficient= +0.85) (Table 5).

Table 5: HBSAg /ECLIA/ROCH test result of study participants from national data, Ethiopia, 2025.

HBsAg test (RDT) result	**Confirmatory HBSA /ECLIA/ROCH test result**		
	Positive	Negative	Total
Positive	19	1	20
Negative	5	175	180
Total	24	176	200

5.7. Factors associated with HBV infection among PLHIV with unsuppressed Viral load.

In the multivariable analysis, sex was found to be a strong predictor of HBSAg positivity. Male participants had significantly higher odds of having HBV infection than females, with an AOR of 4.60(95% CI: 2.28-9.30, $p < 0.001$). This indicates that being male was independently associated with more likelihood of HBsAg positivity (Table 6).

Table 6: Factors associated with prevalence of HBV among study participants from national data, Ethiopia, 2025.

Variable	Groups	HBsAg (RDT)		P-value	COR (95%CI)	P-value	AOR (95% C.I)
		Positive	Negative				
Sex	Male	35(8.66%)	369(91.3%)	0.001	4.18(2.1-8.35)	0.001	4.60(2.28-9.30)
	Female	11(2.22%)	485(97.8%)		1		1
Age in Years	12-17	54(94.7)	3(5.3)		1		1
	18-35	389(93.5)	27(6.5)	0.722	1.25(0.37-4.26)	0.433	1.64(0.474-5.70)
	36-59	279(97.6)	7(2.4)	0.639	0.42(0.145-1.23)	0.684	0.766(0.21-2.77)
	≥60	254(94.8)	14(5.2)	0.998	0.93(0.365-2.36)	-	-
Duration on ART in Month	Less than 24	14(5.2%)	255(94.8%)		1		1
	24-48	31(4.9%)	597(95.1%)	0.866	0.94(0.495-1.8)	0.641	0.85(0.44-1.66)
	Greater than 48	1(33.3%)	2(66.7%)	0.078	9.12(0.8-106.6)	-	-

6. Discussion

In the present study, serologic testing among 900 PLHIV and with unsuppressed viral load 46(5.1%) clients tested positive for HBsAg. This prevalence reflects a moderate burden of HBV infection in the study population. Among the 46 HBsAg positives 97.8% were with detectable HBV DNA. RDT had 99.4% specificity and 79.2% sensitivity in comparison to ECLIA. Furthermore, male sex had significantly higher odds of HIV-HBV co-infections. When compared with findings from other countries and regions, our result shows both consistencies and differences.

Our finding of 5.1% HBV-HIV co-infection is comparable to the prevalence reported in East democratic republic of Congo (5.3%) (72) and with some Ethiopian studies, such as 5.9% seroprevalence reported from Mekelle hospital, North Ethiopia in 2014 (60) and 5.96% from selected hospitals in Addis Ababa, Ethiopia between September 2012 to December 2018 (73). These similarities suggest that HBV prevalence in our setting aligns with the sub-Saharan Africa, where endemicity is moderate and vaccination programs are gradually expanding. The consistency across these studies may reflect similar epidemiological patterns, healthcare access, and risk exposures.

Several studies reported higher HBV prevalence compared to our finding. In South Africa, 6.7% among HIV-positive individuals (29). Similarly, studies in Vietnam (8.4%) (74), Myanmar (8.7%) (75), Taiwan (7.8%) (76), and Ethiopia (6.7% (77), 8.4% in Wolaita Sodo (78), and 10% (79) all reported higher prevalence rates. These differences may be explained by regional endemicity, differences in population composition (HIV-positive vs. mixed populations), and variations in vaccination coverage or healthcare infrastructure. In particular, Asian countries such as Vietnam, Myanmar, and Taiwan are historically HBV-endemic regions, which may account for their higher prevalence. Conversely, some studies reported lower HBV-HIV co-infection rate compared to our finding. In Brazil, 2.5% (32) and Northern Tanzania, 2.3% (80) were documented.

In the present study, among the 46 participants who initially tested positive for HBsAg by rapid diagnostic test, confirmatory virological testing revealed that 45 individuals (97.8%) were truly

infected with HBV DNA, while only one case (2.2%) was not confirmed. This very high confirmation rate demonstrates the reliability of the rapid diagnostic test in detecting HBV infection and underscores the importance of molecular assays for definitive diagnosis. A study from South Africa reported similar finding where 6.7% of HBsAg-positive cases represented true chronic infections (29). This consistency highlights those rapid diagnostic tests, when followed by DNA confirmation; provide robust evidence of HBV infection status across different populations. While, studies conducted in different part of Ethiopia have documented from 5.9% to 10%, confirmatory results (60,63,73,77,78). This reinforces the accuracy of serological screening. Our finding of a 97.8% DNA confirmation rate, suggests that false positives are rare and that rapid tests remain a valuable first-line screening tool in resource-limited settings. In contrast a slight discrepancy observed in one case where (2.2%) confirmatory result reported (81). This may be attributed to transient antigenemia, resolved infection, or technical variation in testing.

The present study employed the Elecsys electro-chemiluminescence immunoassay (ECLIA) as the reference standard for confirming HBsAg results. ECLIA demonstrated excellent diagnostic performance, identifying both true positives and true negatives with high accuracy. Its ability to detect low-level antigenemia and minimize false results underscores its suitability as a confirmatory assay in hepatitis B virus (HBV) diagnostics.

These findings are consistent with previous reports that highlight the superior sensitivity and specificity of ECLIA compared to conventional immunoassays. Tang et al. reported that ECLIA achieves sensitivity and specificity above 95% for infectious disease markers, including HBsAg, making it more reliable than ELISA in clinical practice (82). Garlic emphasized that ECLIA is particularly effective in detecting HBV markers at low concentrations, thereby reducing the risk of missed infections (83). Scheiblauer et al. also demonstrated that ECLIA outperforms rapid diagnostic tests, which often show variability in sensitivity despite high specificity (84). Furthermore, Candiotti and Allain noted that confirmatory assays such as ECLIA are critical in transfusion medicine to prevent HBV transmission (85).

In the present study, infection status based on C-reactive protein (CRP) testing was categorized into two levels; 49 individuals (40.83%), were classified as having a high infection status,

whereas 71(59.17%) had normal results. Majority of HBsAg positives, 13(65%) had high infection status based on CRP. While among the 100 HBsAg negative participants 36(36%) had relatively low percentage of high infection status based on CRP assessment. Altogether, 120 individuals were tested, representing the full sample. These findings highlight that systemic inflammation, as reflected by elevated CRP, is common among the HBV-HIV co-infected subjects. This result is consistent with previous evidence that CRP serves as a reliable biomarker of inflammation in HIV-HBV co-infected individuals (43). Elevated CRP levels have been associated with unsuppressed HIV viral load, increased risk of liver disease, and higher mortality rates (43). In particular, Kuller et al. demonstrated that CRP, along with other inflammatory markers, was predictive of all-cause mortality in HIV patients with HBV, independent of CD4 count and viral load (44). Even tough, in resource-limited settings such as Ethiopia, CRP testing is not routinely performed. Emerging evidence indicates that elevated CRP may correlate with liver enzyme abnormalities and poor treatment outcomes in HIV patients co-infected with HBV (45). In this study it is observed that a substantial proportion of participants demonstrated high infection status, which may because of underlying viral co-infections. The integration of CRP testing into HIV care particularly when they are co-infected with HBV could provide an accessible tool for identifying patients at higher risk of adverse outcomes, guiding closer monitoring and timely interventions.

In the present study, sex was found to be a strong predictor of HBsAg positivity. Male participants had significantly higher odds compared to females, with an adjusted odds ratio (AOR) of 4.34(95% CI: 2.14-8.80, $p < 0.001$). This indicates that being female was independently associated with a reduced likelihood of HBV infection, suggesting that male sex is a key risk factor in HBV transmission and persistence. This finding is consistent with results from Addis Ababa, and north East Ethiopia, where multivariable logistic regression analysis showed that male sex was the most independent predictor for viral hepatitis B co-infection, with an odds ratio of 2.42(60,86). This similarity reinforces the evidence that men are disproportionately affected by HBV infection in Ethiopian settings.

7. Strength and limitation of study

7.1 Strength of the study

- ✓ Study assesses HBVSAg using both serological and molecular test at Advanced Laboratory (EPHI).
- ✓ Study conducted at national level and includes all region of the country.
- ✓ Stud concern a public health issues/ community health issues.

7.2 Limitation of the study

- ✓ Cross-sectional study design was made impossible this study to proof causation.
- ✓ Large sample size made the study not feasible to carryout laboratory test.

8. Conclusion and Recommendation

8.1. Conclusion

This study demonstrates a moderate HBV seroprevalence with a very high DNA confirmation rate that validates the reliability of rapid serologic screening. The findings highlight that male sex are strong predictors of HIV-HBV co-infection, reflecting behavioural risk factors. Together, these results underscore the importance of confirmatory testing, demographic risk assessment, and contextual health system factors in shaping effective HBV and HIV prevention and control strategies.

8.2. Recommendation

The considerable rate of HIV-HBV co-infection observed in this study suggests that routine HBV testing should be considered for all PLHIV, particularly those with unsuppressed viral loads. In resource-limited settings such as Ethiopia, RDTs for HBV screening yields results comparable to PCR and ECLIA methods. HIV positive patient with unsuppressed viral load should have routine screening for HBsAg

Concerned health sectors and professionals should create awareness (provide health education) about those diseases co-infection, they should enhance patient follow up. Future researcher should conduct longitudinal study with larger sample size in order to determine causation and by modifying the sample strategy. PCR and ECLIA method should be used instead of RDTs for HBV screening for all study participants since it yields best result.

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Annex I: Study tool for extraction of data

Section	Variable	Response Options / Format
1. Participant Info	Unique Participant ID	
	Date of data extraction	DD/MM/YYYY
	Facility Name / Code	----------
	Region / Zone / Woreda	----------
2. Sociodemographic Factors	Age	----------years
	Sex	Male Female
	Residence	
3. Clinical Factors	Duration on ART	------- in months
	Most recent HIV viral load	------copies/Ml
	Date of viral load test	----------DD/MM/YYYY
4. Laboratory Testing	HBsAg	Positive Negative
	CRP level	---------- (mg/L)
	RDTs test result	
	PCR result	
	Date of sample collection	----------DD/MM/YYYY
	Sample type	Plasma
	Sample storage documented	Yes /No

Annex II: Laboratory Protocol

A. HBsAg Rapid diagnostic Test Procedure

Specimen collection and handling

1. Plasma using venipuncture, draw whole blood and insert it into the collection tube (containing anticoagulants including heparin, EDTA and sodium citrate) and centrifuge the blood to generate a plasma specimen.
2. Serum using venipuncture, draw whole blood and insert it into the collection tube (NOT containing anticoagulants) then leave for 30 minutes to allow blood coagulation to occur. Centrifuge the tube to generate a serum specimen.
3. If plasma or serum specimens are not tested immediately, they should be refrigerated at 2 - 8 °C. For storage period longer than 2 weeks, freezing (below -20 °C) is recommended. Bring plasma or serum specimens to room temperature (15 - 40 °C) prior to use.
4. Plasma or serum specimens containing a precipitate may yield inconsistent test results. Such specimens must be clarified by centrifugation prior to assaying.

Test procedure

1. Bring the test device and specimen to a temperature between 15 - 40 °C prior to testing.

2. Remove the test device from the foil pouch, and place it on a flat, dry surface. Label the test device
with a patient identifier.

3. Using a micropipette, dispense 100µl of serum, plasma or whole blood specimen into the specimen well “S”.

4. As the test begins to work, you will see a purple color move across the result window in the center
of the test device.

5. Interpret test results at 20 minutes.

Test interpretation

1. A colored control line will appear at “C” in the result window to show that the test is working properly.

2. The “T” section of the result window indicates the test result.

• Non-reactive result: The presence of only the control line (C) within the result window indicates a non-reactive result.

B. Abbott RealTime HBV assay

Biological principles of the procedure

The Abbott RealTime HBV assay consists of three reagent kits:

• Abbott Real-time HBV Amplification Reagent Kit

• Abbott Real-time HBV Control Kit

• Abbott Real-time HBV Calibrator Kit

The Abbott Real-time HBV assay uses PCR to generate amplified product from the DNA genome of HBV in clinical specimens. A DNA sequence that is unrelated to the HBV target sequence is introduced into each specimen at the beginning of sample preparation. This unrelated DNA sequence is simultaneously amplified by PCR and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample. The amount of HBV target sequence that is present at each amplification cycle is measured through the use of fluorescent-labeled oligonucleotide specifically bound to the amplified product. The amplification cycle at which fluorescent signal is detected by the Abbott m2000rt is inversely proportional to the log of the HBV DNA concentration present in the original sample.

Sample Preparation

The purpose of sample preparation is to extract and concentrate nucleic acid, to make the target accessible for amplification, and to remove potential inhibitors of amplification from the extract. This process is accomplished by the m2000sp, an automated sample preparation system designed to use magnetic micro particle processes for the purification of nucleic acids from samples. The assay is suitable for use with both 0.5 mL and 0.2 mL sample input volumes.

The Abbott mSample Preparation SystemDNA (4 x 24 Preps) reagents lyse the virion, capture the nucleic acids, and wash the particles to remove unbound sample components. Proteinase K is included in the lysis step to digest proteins associated with the nucleic acids.12,13 The bound nucleic acids are eluted and transferred to a 96-deep well plate. The nucleic acids are then ready for amplification. The IC is introduced into the sample preparation procedure and is processed along with the calibrators, controls, and specimens.

Reagent Preparation and Reaction Plate Assembly

The Abbott m2000sp combines the Abbott Real-time HBV amplification reagent components (HBV Oligonucleotide Reagent, DNA Polymerase, and Activation Reagent). The Abbott m2000sp dispenses the resulting master mix to the Abbott 96-Well Optical Reaction Plate along

with aliquots of the nucleic acid samples prepared by the Abbott m2000sp. After manual application of the Abbott Optical Adhesive Cover, the plate is ready for transfer to the Abbott m2000rt.

Amplification

During the amplification/detection reaction on the m2000rt instrument, the target DNA is amplified by the DNA Polymerase in the presence of deoxynucleotide triphosphates (dNTPs) and magnesium. First, the HBV and IC primers anneal to their respective targets and are extended by the polymerase. After a denaturation step in which the temperature of the reaction is raised above the melting point of the double-stranded DNA product, the newly created DNA strand is denatured from the target DNA.

During each round of thermal cycling, amplification products dissociate to single strands at high temperature, allowing primer annealing and extension as the temperature is lowered. Exponential amplification of the target is achieved through repeated cycling between high and lower temperatures. Amplification of both targets (HBV and IC) takes place simultaneously in the same reaction.

The target sequence for the Abbott Real-time HBV assay is in the Surface gene in the HBV genome. This region is specific for HBV and is highly conserved. The primers are designed to hybridize to this region with the fewest possible mismatches among HBV genotypes A through H. The IC target sequence is derived from the hydroxypyruvate reeducates gene from the Pumpkin plant Cucurbit a pepo, and is provided as a DNA plasmid in a buffer solution.

Detection

The presence of HBV amplification products is detected during the extension/anneal step by measuring the fluorescence of the HBV probe that binds to the target during the extension/anneal step. Similarly, the presence of IC amplification is detected during the extension/anneal step by measuring the fluorescence of the IC probe.

The HBV and IC probes are single-stranded DNA oligonucleotides consisting of a probe sequence with a fluorescent moiety that is covalently linked to the 5' end of the probe and a quenching moiety that is covalently linked to the 3' end of the probe.

In the absence of the HBV or IC target sequences, probe fluorescence is quenched. In the presence of HBV or IC target, the HBV or IC probes specifically bind to their target. During the extension/anneal step, the DNA polymerase cleaves, or nucleolytically digests, the bound probe

as it moves along the template strand. This separates fluorophore from the quencher, allowing fluorescent emission and detection.

The HBV and IC probes are each labeled with a different fluorophore, thus allowing for simultaneous detection of both amplified products at each cycle. The amplification cycle at which fluorescent signal is detected by the Abbott m2000rt is inversely proportional to the log of the HBV DNA concentration present in the original sample.

C. Electrochemiluminescence immunoassay (Elecsys HBsAg II)

Specimen collection and preparation

Serum collected using standard sampling tubes or tubes containing separating gel.

D. CRP test principle

Quantitative CRP test is working on the principle of particle-enhanced turbid metric immunoassay technique. Synthetic latex particles coated with monoclonal anti-CRP antibodies aggregate in the presence of C - reactive protein in the sample. An increase in turbidity is directly proportional to the concentration of CRP in the sample. If the CRP concentration greater than or equal to 5 mg/L indicates a high infection status, whereas if the CRP concentration less than 5 mg/L, indicates normal

Test principle: Particle enhanced immunoturbidimetric assay.

Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbid metrically.

Reagents - working solutions

R1: TRIS buffer with bovine serum albumin and immunoglobulin (mouse); preservative; stabilizers.

R2: Latex particles coated with anti-CRP (mouse) in glycine buffer; preservative; stabilizers R1 is in position B and R2 is in position C.

Reagent handling

Ready for use Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

Storage and stability: Shelf life at 2-8 °C: On-board in use and refrigerated on the analyzer (12 weeks). See expiration date on cobas pack label.

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers. Only the specimens listed below were tested and found acceptable.

Serum or Plasma (Li-heparin and K2-EDTA plasma)

Centrifuge samples containing precipitates before performing the assay. See the limitations and interferences section for details about possible sample interferences. Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Stability: 11 days at 15-25 °C; 2 months at 2-8 °C and 3 years at -15 to -25 °C

Declaration

I, the undersigned agree to accept responsibility for the scientific ethical, and technical conduct of the research project and for the provision of required progress reports as per the terms and conditions of the research publications office.

M.Sc. Candidate: Zewude Birhanu (B.Sc.)

Signature: _____

Date of submission: _____

This thesis paper has been submitted with our approval as advisors.

Advisor: Dr. Kassu Desta (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Mr. Biruk zerfu (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia