RESEARCH ARTICLE

Hepatitis B virus seromarkers among HIV infected adults on ART: An unmet need for HBV screening in eastern Ethiopia

Desalegn Admassu Ayana1,2*, Andargachew Mulu1,2, Adane Mihret1,2, Berhanu Seyoum1,2, Abraham Aseffa1,2, Rawleigh Howe1,2

1 College of Health and Medical Sciences, Haramaya University, Oromia, Ethiopia, 2 Armauer Hansen Research Institute, Addis Ababa, Ethiopia

* desadmassu@gmail.com

Abstract

Progression of chronic HBV to cirrhosis, end-stage liver disease (ESLD), and hepatocellular carcinoma (HCC) is more rapid in HIV positive individuals than those with HBV alone; however, the distribution of HBV seromarkers in HIV infected individuals on antiretroviral therapy (ART) is not well described. To address this problem, we assessed the distribution of hepatitis B surface antigen (HBsAg), hepatitis B core antibody (anti-HBc) and hepatitis B surface antibody (anti-HBs) among HIV infected adults on ART in Eastern Ethiopia. A cross-sectional study was conducted from September 2017 to February 2018. Socio-demographic, behavioral and health related factors, and clinical data were collected using questionnaire and checklist. Plasma samples were tested for HBsAg, anti-HBc and anti-HBs seromarkers using ELISA. Data were double entered into EpiData 3.1, cleaned, exported to and analyzed using STATA 13. Descriptive and logistic regression analysis were conducted and statistical significance was decided at p < 0.05. A total of 901 participants were included and the prevalence of HBsAg was found to be 11.7% [95%CI (10, 14)]. Among the co-infected, 47.6% were also positive for anti-HBc, of which 58% were on an ART containing tenofovir (TDF). Among those screened for the three seromarkers, 38.1% were negative for all and 21% were positive only for anti-HBc (IHBc). Being single, history of genital discharge and taking ART with TDF combination were significantly associated with HBV co-infection (p < 0.05). There is high burden HBV co-infection among individuals on ART. The unmet need of HBV screening prior to ART initiation leaves many co-infected individuals without appropriate management including therapy, close monitoring or vaccination when indicated, impacting disease prevention.

Introduction

Globally, 37.9 million (32.7 million-44.0 million) people were living with HIV in 2018, of which 20.6 million (18.2 million-23.2 million) were from the eastern and southern Africa region [1]. The proportion and risk factors of HBV co-infection vary widely with geographical location: 5–10% in North America, Europe and Australia, and 20–30% in sub-Saharan Africa (SSA) and Asia [2, 3].
HIV positive individuals are more likely to be infected with HBV than HIV-negative individuals, possibly as a result of shared risk factors [4]. Progression of chronic HBV to cirrhosis, end-stage liver disease (ESLD), and hepatocellular carcinoma (HCC) is more rapid in HIV co-infected individuals [5, 6].

HBV replication markers appear to be influenced by HIV infection [7]. HIV co-infection prevented HBsAg secretion with significantly elevated HBsAg quantity present in cell lysates in co-infected hepatic cell lines [8]. In HBV co-infection, HBsAg may hence be too low to be detected using serological tests and anti-HBc may be the only serological evidence of exposure to the virus [9]. In HBsAg negative individuals, anti-HBc positivity may be associated with occult hepatitis infection (OBI), characterized by low but detectable viral replication using PCR based approaches. [10]. HIV infection is also a risk factor for HBsAg negative HBV infection and the development of OBI, since it occurs more frequently in HIV-infected patients [11].

Testing and diagnosis of hepatitis infection is critical to both prevention and treatment services, and provides an opportunity to reduce transmission, through counselling on risk behaviors and vaccination [12]. HBV co-infection status is not commonly tested in ART clinics despite the recommendation for hepatitis B and C routine screening and vice versa [13]. Lack of HBV screening may lead to treatment without the use of the recommended tenofovir (TDF) in the regimen, which may be further associated with flares of hepatitis B due to ART-associated immune reconstitution [14]. In addition, the use of lamivudine (3TC) as the single agent in an ART regimen with activity against hepatitis B is contraindicated due to high likelihood of resistance development to YMDD (tyrosine-methionine-aspartate-aspartate), the highly conserved motif in HBV [15, 16].

In Ethiopia, reports indicate that HBsAg prevalence ranges from 2.7% [17] to 14% [18] among HIV infected individuals. Nearly all reports are based on HBsAg testing with less emphasis on anti-HBc and anti-HBs seromarkers despite their clinical importance. Screening for these seromarkers is also important to identify non-HBV exposed individuals who would benefit from HBV vaccination [13] or to identify those with chronic disease requiring follow-up monitoring or treatment.

This study therefore, assessed HBsAg, anti-HBc and anti-HBs seromarkers among HIV infected adults on ART to determine their magnitude, exposure related factors and reveal the unmet need of HBV screening.

Materials and methods

Study setting and period

The study was conducted in ART clinics of three selected public hospitals (Hiwot Fana Specialized University Hospital, Dilchora General Hospital, and Karamara General Hospital located in Harar, Dire Dawa and Jigjiga towns, respectively), in Eastern Ethiopia.

The hospitals provide specialist services to both the urban trade hubs and the pastoral and agro-pastoral communities inhabiting the borders with Somalia and Djibouti, each catering to an estimated five million populations. The study was conducted from September 2017 to February 2018.

Study population

Consenting ART experienced HIV infected adults who attended ART clinic during the study period were recruited. The study included participants which were not HBV vaccinated and not screened for HBV seromarkers.
Sample size determination
The sample size was calculated using OPEN Epi 3.1 assuming HBV co-infection of 6.9% [19] among HIV infected individuals on ART, and 3.7% among blood donors [20], and adding 10% for assumed non-response. The final calculated sample size was 920.

Sampling procedure
A sample size of 385, 325 and 210 was allocated proportionally to Hiwot Fana, Dilchora and Karamara hospitals, respectively. Participants who were not HBV vaccinated and not previously screened for HBV were recruited consecutively at each site until the allocated sample size was attained.

Data collection
Based on the routine follow-up of HIV infected individuals, an ART nurse screened eligible subjects, conducted interview and sent participants to the laboratory for sample collection with a unique identifier. History of opportunistic infection, history of tuberculosis (TB), baseline and current CD4+T cell count, duration on ART, initial and current ART regimen, and if changed, reason for regimen change, WHO clinical stages, medication adherence and other clinical and demographic information were captured from participants’ ART follow-up form using a checklist. A senior Internal Medicine specialist was involved for patient consultations as necessary at each site.

Sample processing and serology
In the laboratory, 10 ml blood was collected in sterile anticoagulated tube, plasma was separated, labelled and stored at -20˚C by medical technologists until transferred to Haramaya University. The plasma samples were screened and interpreted for HBsAg, ant-HBc and ant-HBs using ELISA (Monolisa HBsAg ULTRA, Monolisa Anti-HBc PLUS, Monolisa Anti-HBs PLUS, BIORAD, France) in the Medical Laboratory Science Department, College of Health and Medical Sciences, Haramaya University, following the manufacturer’s instruction.

Quality control
To maintain data quality, data collectors were trained and questionnaire was pre-tested in different sites other than those selected for the study before the actual data collection. Samples were kept at -20˚C until processed. Standard operating procedures (SOP) and pre-analytical, analytical and post analytical quality control measures were applied. ELISA test results were determined based on the cut-off values following the manufacturer’s instruction.

Data management and analysis
Data were cleaned, coded and entered to EPI Data version 3.1 and analysed using Stata version 11(Stata Corp, USA). Descriptive analysis was used to calculate prevalence, and summarize sociodemographic and associated factors. A binomial logistic regression model was used and associated variables with p≤0.25 were entered to multiple regression analysis to control for potential confounders. Adjusted odds ratio (AOR) and 95% confidence interval (CI) were used to assess the strength of association between dependent and independent variables. Finally, statistical significance was decided at p<0.05.
Ethical considerations
This study was reviewed and approved by the Institutional Health Research Ethics Review Committee (IHRERC) of the College of Health and Medical Sciences, Haramaya University (Ref. No. IHRERC/137/2017) and AHRI/ALERT Ethics Review Committee, Addis Ababa (Ref. No. P019/17). Written signed informed consent was obtained before data collection. Laboratory results of HBV seromarkers were reported to the respective attending clinician for the necessary intervention. To maintain confidentiality, participants' information was coded and names and personal identifiers were not used.

Results
Socio-demographic characteristics of study participants
A total of 901 (98%) HIV infected individuals on ART were included in this study. Of the total participants, 817 (90.7%) were urban residents, and 622 (69%) were female of which 539 (86.6%) were within the reproductive age group. The median age of the respondents was 40 years (IQR 32, 45) with an average family size of 3.12. Among the study participants, 291 (32.3%) did not attend formal education, and 71 (7.9%), had attended tertiary education. “Table 1”

Behavioral and health related characteristics of study participants
Of the screened HIV infected individuals on ART, 173 (19.2%) reported current alcohol consumption, 118 (13.1%) khat chewing, 544 (60.4%) a history of body piercing, 293 (32.5%) had tattoos and 314 (34.9%) shared sharp tools such as needles and razors. Three hundred sixty-two (40.2%) had history of hospital admission, 59 (6.5%) genital discharge, 186 (20.6%) history of dental extraction, and 119 (13.8%) had multiple sexual contacts.

Distribution of HBsAg, anti-HBc and anti-HBs seromarkers among study participants
Based on the three HBV seromarkers assessed, 334 (38.1%) were negative for all, 176 (20.1%) were positive for both anti-HBc and anti-HBs and 53 (6.0%) were positive only for HBsAg. Those positive for “anti-HBc only” (IAHBc) totaled 184 (21.0%), of which 113 (61.4%) were females and 93% of these females were urban residents. “Table 2”

Clinical characteristics of HBV co-infected and HIV mono-infected individuals
Nearly all, 99.8%, of the study participants were on ART, and had been receiving treatment for a median duration of 86.0 months (IQR 51,118). A total of 850 (95.6%) were taking first line and 39 (4.4%) were taking second line ART regimen currently. From a total of 313 (35.2%) who had ART regimen changed, 144 (46.0%) were due to undesirable side effects and 39 (12.5%) were due to apparent treatment failure. Among participants taking second line drugs due to treatment failure, 3 (7.5%) were HBsAg positive, and one was in WHO clinical stage III category.

Based on the clinical data record of the study participants, 244 (27.2%) had history of tuberculosis (TB) and 476 (53.1%) had a history of opportunistic infections (OI). The vast majority of the participants, 835 (93.8%) had good ART adherence, and 844 (94.8%) were in WHO stage I category. “Table 3”
The median baseline and current CD4+ T cells counts of the participants were 180 and 519 cells/μl (IQR: 104.5, 274.5; 353.75, 691), respectively. Among the total participants, 481 (58.0%) had baseline CD4+ T cells count ≥200 cells/μL. Among these, 57 (11.9%) were HBsAg positive, of which 23 (40.4%) were taking ART combination with TDF and 3TC. Currently, 63 (7.9%) had CD4+ T cells count ≥200 cells/μL.

From HBV co-infected individuals, 31 (29.5%) had a history of tuberculosis, 81 (77.1%) had history of opportunistic infections and 101 (96.2%) had good adherence and were in the WHO clinical stage I category.

A total of 567 (63.8%) participants were currently taking ART containing TDF and 3TC combinations. From those who were both HBsAg and anti-HBc positive, 29 (58%) were currently taking an ART containing both TDF and 3TC, however, 21 (42%) were taking ART regimens comprising 3TC only.

Prevalence of Hepatitis B virus surface antigen and associated factors

Among the study participants, 105 [11.7%, 95%CI (10–14)] were positive for HBsAg. The distribution was 11.9% among females and 11.1% among males. The majority, 92 (87.6%), of the HBsAg positives were urban residents and 74 (70.5%) were female. Nearly half, 50 (47.6%) of the HBsAg positives were also positive for anti-HBc, among which, 42 (84%) were urban
residents, and 31 (62.0%) were females, of which 27 (87.1%) were within the reproductive age group.

Binary logistic regression analysis was conducted including sociodemographic, behavioral, health related and clinical variables. Marital status, tattooing, hospital admission, sharing sharp tools, discharge from genitalia, genital mutilation, history of opportunistic infections and presence of TDF and 3TC in the ART regimen were considered for multivariable logistic regression analysis \( (p \leq 0.25) \). In the final model, marital status, history of genital discharge and ART with TDF and 3TC combination remained statistically significant \( (p < 0.05) \). Besides, sharing sharp tools and genital mutilation remained strong predictors though they were marginally significant \( (p = 0.051) \). HIV infected individuals who were single were 2 times more likely to be HBsAg positive compared to married ones \( [\text{AOR} \, 2.10; \, 95\%\text{CI} \, (1.03, \, 4.28) \, p = 0.041] \). History of genital discharge increased the chance of being HBsAg positive by 2.9 times \( [\text{AOR} \, 2.90; \, 95\%\text{CI} \, (1.18, \, 7.09) \, p = 0.020] \). Those who were currently treated with ART without TDF were 1.9 times more likely to be HBsAg positive compared to those treated with TDF combinations \( [\text{AOR} \, 1.89; \, 95\%\text{CI} \, (1.10, \, 3.23) \, p = 0.020] \). Likewise, those who shared sharp tools were 1.9 times more likely and females who had genital mutilation were 1.8 times more likely to be HBsAg positive compared to their counterparts. However, the \( p \) value was marginally significant \( [\text{AOR} \, 1.97; \, 95\%\text{CI} \, (0.99, \, 3.93) \, p = 0.051; \, \text{AOR} \, 1.81; \, 95\%\text{CI} \, (0.99, \, 3.28) \, p = 0.052] \). “Table 4”

### Prevalence of Anti-HBc and associated factors among study participants

A total of 419 [(46.5%, 95%CI (43, 50)] HIV infected individuals on ART were positive for anti-HBc seromarkers, among which the majority, 272 (64.9%) were female and 383

---

**Table 2. Distribution of HBV seromarkers among HIV infected individuals on ART in selected public Hospitals, Eastern Ethiopia, 2017/18 (n = 876).**

| HBV seromarkers | Results | Interpretation | Frequency n (%) |
|-----------------|---------|----------------|-----------------|
| HBsAg           | Negative| Susceptible/no evidence of prior infection | 334 (38.13) |
| Anti-HBc        | Negative|                |                 |
| Anti-HBs        | Negative|                |                 |
| HBsAg           | Negative| Immune due to natural infection | 176 (20.09) |
| Anti-HBc        | Positive|                |                 |
| Anti-HBs        | Positive|                |                 |
| HBsAg           | Positive| Chronically infected based on Anti HBC total | 42 (4.79) |
| Anti-HBc        | Positive|                |                 |
| Anti-HBs        | Negative|                |                 |
| HBsAg           | Positive| “Unclear”       | 53 (6.05%) |
| Anti-HBc        | Negative| 1. Early infection/low Anti-HBc level |                 |
| Anti-HBs        | Negative| 2. False positive HBsAg, thus susceptible |                 |
| HBsAg           | Negative| Unclear         | 184 (21.0) |
| Anti-HBc        | Positive| 1. Resolved infection (most common) |                 |
| Anti-HBs        | Negative| 2. False positive Anti Hbc, thus susceptible |                 |
| |            | 3. “Low level” chronic infection |                 |
| |            | 4. Resolving acute infection |                 |
| |            | 5. Isolated anti-Hbc (IAHBC) |                 |

* HBsAg only positive was not included in the advisory committee recommendations
** not included in the advisory committee recommendations

Adapted from CDC, Recommendations of the Advisory Committee on Immunization Practices (ACIP) 2006.

[https://doi.org/10.1371/journal.pone.0226922.t002](https://doi.org/10.1371/journal.pone.0226922.t002)
(91.4%) were urban residents. The distribution was 43.7% among female and 52.7% among male.

To assess independent predictors of anti-HBc positivity, factors that were significant in bivariate analysis (p ≤ 0.25) were entered into multivariable logistic regression analysis. In the final model gender, level of education, occupation and multiple sexual contact remained significant predictors (p ≤ 0.05). Males were 1.6 times more likely to be anti-HBc positive [AOR; 1.59 95%CI (1.11, 2.26) p = 0.010]. Unemployed participants were 2 times and daily laborers were 1.9 times more likely to be anti-HBc positive compared to government employees [AOR; 2.17 95%CI (1.28, 3.67) p = 0.004; AOR; 1.90 95%CI (1.11, 3.26) p = 0.020]. Those who had multiple sexual contacts were 2 times more likely to be anti-HBc positive compared to their counterparts [AOR; 2.21 95%CI (1.44, 3.38) p = 0.001]. High school students were 63% less
likely of being anti-HBc positive compared to college or university graduates [AOR; 0.37 95% CI (0.19, 0.69) p = 0.002]. “Table 5”

Discussion

The overall prevalence of HBsAg and anti-HBc among HIV infected individuals on ART was 11.7% and 46.5%, respectively. Among the HBsAg positives, 47.6% were also positive for anti-HBc and (29/50) 58% of these were currently on an ART regimen containing TDF and 3TC. Among the total participants screened for the three seromarkers, 38.1% were negative for all, and 21% were positive only for anti-HBc (IAHBc), and 96% of the participants were on ART for more than 6 months. Being single, history of genital discharge and taking ART with TDF were significant predictors of HBV co-infection. Male gender, unemployment, daily laborer, history of multiple sexual contacts and level of education were significant predictors of HBV exposure.

The HBV co-infection prevalence in this study is higher compared to HBV prevalence of 4.7% in Addis Ababa [21], 5.9% in Mekelle hospital [22], 5.5% in University of Gondar Hospital [23], 6.3% in Southern Ethiopia [24], 6.9% in Hawassa Referral Hospital [25], a pooled prevalence of 5.2% in meta-analysis regardless of ART status [26] and a 7.4% global prevalence report [27]. However, it is less than the 14% HBV prevalence reported from Shashemene town in Southern Ethiopia [18] and the overall prevalence of 15% in sub-Saharan Africa [28]. A similar finding of 11.7% was reported among HIV infected HAART naïve individuals in north-west Gondar [29]. A recent population-based HIV impact assessment (EPHIA 2017–2018) report in Ethiopia indicated a 4.8% HBV co-infection among adults of 15–64 years of age.
(3.6% in women to 7.4% in men) in urban Ethiopia using rapid diagnostic test [30]. The variations in prevalence reports may be attributed to ART exposure that could significantly reduce the level of HBV DNA [31], geographical variation of HBsAg carriage that range from 1.9% to over 40% [32] and mutations in the S region of HBV [6]. The difference in sample size and the diagnostic tools may also affect to the prevalence reports in Ethiopia.

The high HIV/HBV co-infection in this report is an evidence for the serious health burden that demands immediate intervention considering the recent HIV increase from 1.14% in 2014 [15] to 3% in 2018 in urban population in Ethiopia [30] due to the shared risk factors. Additionally, 87.1% of those females who were positive for both HBsAg and anti-HBc were within the reproductive age group. This increases the risk of vertical and horizontal transmission, as well as progression to chronic liver disease unless the necessary precautions are implemented.

Studies reported that one-third of the world’s population has serologic evidence of past or present HBV infection [33]. Though anti-HBc is widely used in HBV screening as an epidemiological marker [34], data on the magnitude of anti-HBc is limited in Ethiopia. The anti-HBc seropositivity in this study is higher than the 22.5% anti-HBc prevalence reported in southern Ethiopia [24], and slightly lower compared to the 52.4% reported among ART experienced patients in Addis Ababa [35] and the 55.1% reported from sub-Saharan Africa [28]. About 70–
90% of all HIV patients show evidence of past or active HBV infection in Kenya [32]. The possible explanation for such variations could be the simultaneous infection with HIV that reduces immune control of previous HBV infection facilitating HBV reactivation and HBV DNA replication without presence of detectable HBSAg [36]. Moreover, immune status of the study participants and stages of HBV disease [37] may affect likelihood of HBSAg detection. The higher occurrence of occult HBV infection in HIV-positive people may also be attributed to the lower rate of HBSAg [4]. The anti-HBc carriage among HIV infected individuals on ART may indicate a high rate of HBV transmission as anti-HBc typically persists for life, regardless of whether the infection resolves or remains chronic [38]. However, the anti-HBc test should be supported by testing for HBSAg and anti-HBs in order to decide whether it indicates HBV immunity through natural infection, chronic HBV infection, IAHBc [39] or OBI [40].

In our study, based on the seromarkers assessed, 37.3% of the study participants were negative for HBSAg, anti-HBc and anti-HBs, and therefore susceptible to HBV infection [39]. These group could be protected from HBV infection through vaccination if pre-ART HBV screening had been implemented in Ethiopia. The unmet need of pre-ART HBV screening and subsequent appropriate vaccination hampered the HBV infection control effort [13], and as well as contributing to progression of untreated chronic HBV to cirrhosis, ESLD, and HCC [5, 6]. Similarly, 20.1% were positive for both anti-HBc and anti-HBs indicating protection due to natural infection, and 4.8% were positive for both HBSAg and anti-HBc indicating the requirement for further follow up, to prevent adverse consequences [39]. Though it was not defined in the advisory committee recommendations [39], we found 6.1% of HBSAg only positive cases. This might have occurred due to low anti-HBc levels depending on the immune tolerant and inactive phases of HBV infection which may affect its detection [41].

Furthermore, 21% of the total screened were positive for anti-HBc and negative for both HBSAg and anti-HBs, hence categorized as “isolated anti-HBc” (IAHBc), or “anti-HBc alone” [42]. In Ethiopia, we have not observed any data reporting IAHBc to date and therefore, the burden is unknown despite its clinical and public health implications. IAHBc may represent several clinical entities including the window phase of acute HBV when anti-HBs is not yet detected, the late stage of prior infection after anti-HBs has fallen to undetectable levels, OBI, or false positive anti-HBc [40, 43]. HIV co-infection was demonstrated to be a risk factor for HBSAg negative infection [11, 44], and pre-S1 [45] and ‘a’ determinant mutations also prevent HBSAg secretion, ultimately affecting virus detection [46, 47]. High anti-HBc levels in the immune active and immune reactivation phases of chronic HBV infection [41] and the higher occurrence of OBI (2% to 10%) in HIV-positive people may also affect detection rate of HBSAg [48, 49], leading to IAHBc. Patients who have undergone viral clearance may also lose the ability to produce anti-HBs after long periods of time due to a waning T-cell response [40]. OBI has much impact on different clinical and public health aspects, including transmission, risk of reactivation and enhancing liver disease progression that can lead to HCC [50]. This further emphasizes the critical need of HBV screening among HIV infected individuals before ART initiation to prevent the potential risk of HBV transmission.

In our study, nearly all study participants were on ART with different combinations for a median of 86 months. For HBV co-infected individuals if treatment is indicated, the national guideline recommends TDF + 3TC (or FTC) + EFV as a preferred regimen [51]. We found that 70% of the participants had been treated with an ART regimen containing TDF compared to the 51.2% report among HBSAg and anti-HBc positive individuals on ART [52]. The rest were on ART with 3TC as the only anti-HBV-active agent. This may cause unnecessary consequences on the patients due to continued HBV viremia and progression [53], resulting from high rates of drug resistance mutations (DRMs) and virologic breakthrough [54].
Furthermore, 3TC resistance confers partial or complete cross-resistance to other HBV inhibitors such as emtricitabine (FTC), telbivudine and entecavir (ETV), thus limiting treatment options [15]. In Ethiopia, the unmet need of HBV screening, might have affected the clinical outcome of the patients who were eligible for TDF but remain taking 3TC only leading to circulation of drug resistant strains among the community.

Being single, history of genital discharge, and ART without TDF [31] were statistically significant predictors of HBsAg positivity. This study, did not find a statistically significant association between HBsAg positivity and many of the sociodemographic characteristics such age, sex, residence and level of education like other similar studies [18, 21, 22, 24, 55]. History of hospital admission, surgery, dental extraction [22, 55] and sharing of sharp materials [25, 55] were not significantly associated with HBsAg.

Male gender [35], occupation (being unemployed and daily laborer), level of education and multiple sexual partner were significant predictors for HBV exposure. The risk difference by gender may be due to differences in risk behavior over years of life [56]. However, exposure to HBV infection was not significantly associated with body piercing and tattooing [24], and age and history of genital discharge. No significance difference was observed by residence, marital status and history of dental extraction as a risk for HBV exposure [24, 35]. Those who shared sharp tools were 1.9 times more likely and females who had genital mutilation were 1.8 times more likely to be HBsAg positive compared to their counterparts. However, the p value remained statistically insignificant (p > 0.05).

**Limitations**

This study did not conduct HBeAg and anti-HBc IgM tests to differentiate between active viral replication and acute infection, respectively among the study participants. HIV viral load, liver function tests and related data were incomplete to assess other important clinical parameters.

**Conclusion and recommendations**

This study showed a relatively high prevalence of HIV/HBV coinfection, HBV co-infected participants taking 3TC as the only anti-HBV agent, susceptible HIV infected adults that require HBV vaccine and IAHBc group. In general, the unmet need for HBV screening prior to ART initiation may affect the quality of care given to the patients, increase the risk of life-threatening complications among the co-infected, but untreated ones and hamper the prevention effort towards HIV and HBV as they share transmission routes. In addition, it brings into attention the importance of integrating anti-HBc screening due to its public health implication in HBV transmission as well as drug resistant mutations.

**Acknowledgments**

We would like to express our gratitude to HIV infected individuals on ART in the study areas. We also would like to thank ART clinic and Medical Laboratory staff, and administration of Dilchora Hospital in Dire Dawa, Hiwot Fana Specialized University Hospital in Harar and Karamara Hospital in Jigjiga, for their unreserved support and collaboration during data collection.

**Author Contributions**

**Conceptualization:** Desalegn Admassu Ayana.

**Data curation:** Desalegn Admassu Ayana.

**Formal analysis:** Desalegn Admassu Ayana.
Funding acquisition: Abraham Aseffa.

Methodology: Desalegn Admassu Ayana, Andargachew Mulu, Adane Mihret, Berhanu Seyoum, Abraham Aseffa, Rawleigh Howe.

Project administration: Desalegn Admassu Ayana, Adane Mihret.

Resources: Andargachew Mulu, Adane Mihret, Berhanu Seyoum, Abraham Aseffa.

Supervision: Andargachew Mulu, Adane Mihret, Berhanu Seyoum, Abraham Aseffa, Rawleigh Howe.

Writing – original draft: Desalegn Admassu Ayana.

Writing – review & editing: Desalegn Admassu Ayana, Andargachew Mulu, Adane Mihret, Berhanu Seyoum, Abraham Aseffa, Rawleigh Howe.

References
1. UNAIDS. Global AIDS update. 2019 Fact Sheet. 2019.
2. Singh AE, Wong T. Background document: HIV and hepatitis C co-infection. Department of HIV/AIDS, World Health Organization. 2009.
3. Luetkemeyer A. Current issues in the diagnosis and management of tuberculosis and HIV coinfection in the United States. Topics in HIV medicine: a publication of the International AIDS Society, USA. 2010; 18(4):143–8.
4. Burnett R, Francois G, Kew M, Leroux-Roels G, Meheus A, Hoosen A, et al. Hepatitis B virus and human immunodeficiency virus co-infection in sub-Saharan Africa: a call for further investigation. Liver international. 2005; 25(2):201–13. https://doi.org/10.1111/j.1478-3231.2005.01054.x PMID: 15780040
5. Chung RT. Hepatitis C and B viruses: the new opportunists in HIV infection. Top HIV Med. 2006; 14(2):78–83. PMID: 16835462
6. Chun HM, Roediger MP, Hullsiekh KH, Thio CL, Agan BK, Bradley WP, et al. Hepatitis B virus coinfection negatively impacts HIV outcomes in HIV seroconverters. Journal of Infectious Diseases. 2011; 205(2):185–93. https://doi.org/10.1093/infdis/jir720 PMID: 22147794
7. Konopnicki D, Mocroft A, De Wit S, Antunes F, Ledergerber B, Katlama C, et al. Hepatitis B and HIV: prevalence, AIDS progression, response to highly active antiretroviral therapy and increased mortality in the EuroSIDA cohort. Aids. 2005; 19(6):593–601. https://doi.org/10.1097/01.aids.0000163936.99410.1 PMID: 15802978
8. Iser DM, Warner N, Revill PA, Solomon A, Wightman F, Saleh S, et al. Co-infection of hepatic cell lines with HIV-hepatitis B virus (HBV) leads to an increase in intracellular hepatitis B surface antigen (HBsAg). Journal of Virology. 2010.
9. Altain J-P. Occult hepatitis B virus infection. Transfusion clinique et biologique. 2004; 11(1):18–25. https://doi.org/10.1016/j.tracci.2003.11.007 PMID: 14980545
10. Coffin CS, Mulrooney-Cousins PM, Osiowy C, van der Meer F, Nishikawa S, Michalak TI, et al. Virological characteristics of occult hepatitis B virus in a North American cohort of human immunodeficiency virus type 1-positive patients on dual active anti-HBV/HIV therapy. Journal of clinical virology. 2014; 60(4):347–53. https://doi.org/10.1016/j.jcv.2014.04.021 PMID: 24881491
11. Mphahlele MJ, Lukhwareni A, Burnett RJ, Moropeng LM, Ngobeni JM. High risk of occult hepatitis B virus infection in HIV-positive patients from South Africa. Journal of clinical virology. 2006; 35(1):14–20. https://doi.org/10.1016/j.jcv.2005.04.003 PMID: 15916918
12. WHO. Guidelines on hepatitis B and C testing: World Health Organization; 2017.
13. FMOH. 2016_FMOH_Guideline for prevention and Control of Viral Hepatitis in Ethiopia. Guideline. 2016.
14. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach: World Health Organization; 2016.
15. FMOH. National guidelines for comprehensive HIV prevention, care and treatment. Addis Ababa: Ministry of Health. 2017.
16. Bozdaiy A, Uzunaliagle O, Turkylmez A, Aslan N, Sezgin O, Sahin T, et al. YSDD: a novel mutation in HBV DNA polymerase confers clinical resistance to lamivudine. Journal of viral hepatitis. 2003; 10(4):256–65. https://doi.org/10.1046/j.1365-2893.2003.00435.x PMID: 12823591
17. Manyazewal T, Sisay Z, Biadgilign S, Abegaz WE. Hepatitis B and hepatitis C virus infections among antiretroviral-naive and -experienced HIV co-infected adults. J Med Microbiol. 2014; 63(Pt 5):742–7. https://doi.org/10.1099/jmm.0.063321-0 PMID: 24757219.

18. Negero A, Sisay Z, Medhin G. Prevalence of Hepatitis B surface antigen (HBsAg) among visitors of Shashemene General Hospital voluntary counseling and testing center. BMC research notes. 2011; 4 (1):35.

19. Belayneh F. Prevalence of Hepatitis B Virus Infection and Associated Factors among HIV Positive Adults Attending ART Clinic at Hawassa Referral Hospital, SNNPR, Ethiopia. OALib. 2015; 02(05):1–7. https://doi.org/10.4236/oalib.1001490

20. Haftle Y, Seyoum B, Alemayehu T. Hepatitis B Virus Infection and Associated Factors among Blood Donors at Dire Dawa, Eastern Ethiopia. Journal of Antivirals & Antiretrovirals. 2016; 08(04). https://doi.org/10.4172/2155-6113.1000702

21. Yemanebrhane N, Addise D, Abebe F, Shewaameare A, Tsegaye A. Magnitude of Hepatitis B Virus and Hepatitis C Virus among HAART Taking Patients and Association with Liver and Renal Function and CD4+ T Cells Level. Journal of AIDS & Clinical Research. 2017; 08(06). https://doi.org/10.4172/2157-2689.1000144

22. Weldemhret L, Asmelash T, Belodu R, Gebreegziabiher D. 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. 2016; 13(1):6.

23. Deressa T, Damtie D, Fonseca K, Gao S, Abate E, Alemu S, et al. The burden of hepatitis B virus (HBV) infection, genotypes and drug resistance mutations in human immunodeficiency virus-positive patients in Northwest Ethiopia. PLoS One. 2017; 12(12):e0190149. https://doi.org/10.1371/journal.pone.0190149 PMID: 29281718; PubMed Central PMCID: PMC5744989.

24. Belayneh F. Prevalence of Hepatitis B Virus Infection and Associated Factors among HIV Positive Adults Attending ART Clinic at Hawassa Referral Hospital, SNNPR, Ethiopia OALib. 2015; 2(05):1–7.

25. Belyhun Y, Maier M, Mulu A, Diro E, Liebert UG. Hepatitis viruses in Ethiopia: a systematic review and meta-analysis. BMC Infect Dis. 2016; 16(1):761. https://doi.org/10.1186/s12879-016-2090-1 PMID: 27993129; PubMed Central PMCID: PMC5168848.

26. WHO. Global hepatitis report 2017: World Health Organization; 2017.

27. EPHIA2017-2018. Ethiopia Population-Based HIV Impact Assessment. Summary sheet. 2018.

28. Soriano V, Puoti M, Peters M, Benhamou Y, Sulkowski M, Zoulim F, et al. Care of HIV patients with chronic hepatitis B and C Viral Infections in Kenya. Nairobi: Gastroenterology Society of Kenya; 2012.

29. Ocana S, Casas ML, Buhigas I, Lledo JL. Diagnostic strategy for occult hepatitis B virus infection. World journal of gastroenterology: WJG. 2011; 17(12):1553. https://doi.org/10.3748/wjg.v17.i12.1553 PMID: 21472120

30. Lule N, Nyawira B. Guidelines for the Treatment of Chronic Hepatitis B and C Viral Infections in Kenya. Nairobi: Gastroenterology Society of Kenya; 2012.

31. Ocana S, Casas ML, Buhigas I, Lledo JL. Diagnostic strategy for occult hepatitis B virus infection. World journal of gastroenterology: WJG. 2011; 17(12):1553. https://doi.org/10.3748/wjg.v17.i12.1553 PMID: 21472120

32. Busch MP. Should HBV DNA NAT replace HBsAg and/or anti-HBc screening of blood donors? Transfusion clinique et biologique. 2004; 11(1):26–32. https://doi.org/10.1016/j.traccl.2003.12.003 PMID: 14980546

33. Shimelis T, Torben W, Medhin G, Tebeje M, Andualem A, Demessie F, et al. Hepatitis B virus infection among people attending the voluntary counselling and testing centre and anti-retroviral therapy clinic of St Paul’s General Specialised Hospital, Addis Ababa, Ethiopia. Sex Transm Infect. 2008; 84(1):37–41. https://doi.org/10.1136/sti.2007.027362 PMID: 17804606.

34. Soriano V, Puoti M, Peters M, Benhamou Y, Sułkowski M, Zoulim F, et al. Care of HIV patients with chronic hepatitis B: updated recommendations from the HIV-Heptatitis B Virus International Panel. Aids. 2008; 22(12):1399–410. https://doi.org/10.1097/QAD.0b013e3282f8b46f PMID: 18614862
37. Webster GJ, Reignat S, Maini MK, Whalley SA, Ogg GS, King A, et al. Incubation phase of acute hepatitis B in man: dynamic of cellular immune mechanisms. Hepatology. 2000; 32(5):1117–24. https://doi.org/10.1053/jhep.2000.19324 PMID: 11050064

38. Hollinger FB. Hepatitis B virus infection and transfusion medicine: science and the occult. Transfusion. 2008; 48(5):1001–26. https://doi.org/10.1111/j.1537-2995.2008.01701.x PMID: 18454738

39. CDC. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States. Recommendations of the Advisory Committee on Immunization Practices (ACIP) Part II: Immunization of Adults. MMWR 2006; 55((No. RR-16)):1–33.

40. Ponde RA, Cardoso DD, Ferro MO. The underlying mechanisms for the ‘anti-HBc alone’ serological profile. Arch Virol. 2010; 155(2):149–58. https://doi.org/10.1007/s00705-009-0559-6 PMID: 20091193.

41. Wu J-F, Ni Y-H, Chen H-L, Hsu H-Y, Chang M-H. The impact of hepatitis B virus precore/core gene carboxyl terminal mutations on viral biosynthesis and the host immune response. Journal of Infectious Diseases. 2014; 209(9):1374–81. https://doi.org/10.1093/infdis/jit638 PMID: 24273041

42. Mast E, Weinbaum C, Fiore A, Alter M, Bell B, Finelli L, et al. Centers for Disease Control and Prevention (CDC) A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) Part II: immunization of adults. MMWR Recomm Rep. 2006; 55:1–33.

43. Kang SY, Kim M-Ha, Lee WJ. The prevalence of “anti-HBc alone” and HBV DNA detection among anti-HBc alone in Korea. Journal of medical virology. 2010; 82(9):1508–14. https://doi.org/10.1002/jmv.21862 PMID: 20648604

44. Iser DM, Avihingsanon A, Wisedopas N, Thompson AJ, Boyd A, Matthews GV, et al. Increased intrahepatic apoptosis but reduced immune activation in HIV-HBV co-infected patients with advanced immunosuppression. AIDS. 2011; 25(2):197–205. https://doi.org/10.1097/QAD.0b013e3283410ccb PMID: 21076271

45. Melegari M, Bruno S, Wands JR. Properties of hepatitis B virus pre-S1 deletion mutants. Virology. 1994; 199(2):292–300. https://doi.org/10.1006/viro.1994.1127 PMID: 8122362

46. Makondo E, Bell TG, Kramvis A. Genotyping and molecular characterization of hepatitis B virus from human immunodeficiency virus-infected individuals in southern Africa. PLoS One. 2012; 7(9):e46345. https://doi.org/10.1371/journal.pone.0046345 PMID: 23029487

47. Salisse J, Sureau C. A function essential to viral entry underlies the hepatitis B virus “a” determinant. Journal of virology. 2009; 83(18):9321–8. https://doi.org/10.1128/JVI.00678-09 PMID: 19570861

48. Shire NJ, Rouster SD, Stanford SD, Blackard JT, Martin CM, Fichtenbaum CJ, et al. The prevalence and significance of occult hepatitis B virus in a prospective cohort of HIV-infected patients. J AIDS Journal of Acquired Immune Deficiency Syndromes. 2007; 44(3):309–14. https://doi.org/10.1097/QAI.0b013e31802e2a9 PMID: 17159656

49. Tsui JJ, French AL, Seaberg EC, Augenbraun M, Nowicki M, Peters M, et al. Prevalence and long-term effects of occult hepatitis B virus infection in HIV-infected women. Clinical Infectious Diseases. 2007; 45(6):736–40. https://doi.org/10.1086/520989 PMID: 17712758

50. Raimondo G, Pollicino T, Cacciola I, Squadrito G. Occult hepatitis B virus infection. Journal of hepatology. 2007; 46(1):160–70. https://doi.org/10.1016/j.jhep.2006.10.007 PMID: 17112622

51. FMOH. Guideline for prevention and Control of Viral Hepatitis in Ethiopia. 2016.

52. Yared HB, Muluken DM, Solomon GS, Helmut K. Higher prevalence of Hepatitis B virus infection among ARV- exposed than naive HIV-infected individuals in North Shewa Zone, Ethiopia. Journal of AIDS and HIV Research. 2015; 7(1):10–7. https://doi.org/10.5897/jahr2014.0313

53. Saha D, Pal A, Biswas A, Panigrahi R, Sarkar N, Sarkar J, et al. Characterization of treatment-naive HIV/HBV co-infected patients attending ART clinic of a tertiary healthcare centre in eastern India. PLoS One. 2013; 8(8):e73613. https://doi.org/10.1371/journal.pone.0073613 PMID: 24023688

54. Rusine J, Onda P, Asimwe-Kateera B, Boer KR, Uwimana JM, Mukababyire O, et al. High seroprevalence of HBV and HCV infection in HIV-infected adults in Kigali, Rwanda. PLoS one. 2013; 8(5):e63303. https://doi.org/10.1371/journal.pone.0063303 PMID: 23717409

55. Balew M, Moges F, Yismaw G, Boer KR, Uwimana JM, M. Asian Pacific Journal of Tropical Disease. 2014; 4(1):1–7. https://doi.org/10.1615/s2222-1808(14)60304-2

56. Abebe A, Nokes D, Dejene A, Enquelaslassie F, Messele T, Cutts F. Seroepidemiology of hepatitis B virus in Addis Ababa, Ethiopia: transmission patterns and vaccine control. Epidemiology & Infection. 2003; 131(1):757–70.