Hepatitis B and hepatitis C virus infections among antiretroviral-naive and -experienced HIV co-infected adults

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Most HIV positive people have not been tested for viral hepatitis and their treatments have not been optimized for possible co-infections. The aim of this study was to investigate the serological pattern of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections among antiretroviral (ARV)-naive and -experienced HIV co-infected adults in Addis Ababa, Ethiopia. A total of 500 frozen HIV positive serum and plasma samples collected from ARV-naive (n = 250) and -experienced (n = 250) adults were randomly selected and screened for HBsAg, anti-HBs, HBeAg and anti-HCV using rapid two-site sandwich immunochromatographic assay. The test was performed at Aklilu Lemma Institute of Pathobiology, Addis Ababa University. Positive specimens for HBsAg and anti-HCV markers were further confirmed using third generation ELISA. Of the 500 specimens tested, 15 (3%), 58 (11.6%), 3 (0.6%), 18 (3.6%), 3 (0.6%) and 1 (0.2%) were positive for HBsAg, anti-HBs, HBeAg, anti-HCV, HBsAg and HBeAg, and HBsAg and anti-HBs markers, respectively. No specimen tested positive for both HBeAg and anti-HBs, and 442 (88.4%) individuals were non-immune to HBV. Of the 250 ARV-naive individuals, 8 (3.2%), 33 (13.2%), 2 (0.8%), 10 (4%), 2 (0.8%) and 1 (0.4%) were positive for HBsAg, anti-HBs, HBeAg, anti-HCV, HBsAg and HBeAg, and HBsAg and anti-HBs markers, respectively. Of the 250 ARV-experienced individuals, 7 (2.8%), 25 (10%), 1 (0.4%), 8 (3.2%), 1 (0.4%), and 0 (0%) were positive for HBsAg, Anti-HBs, HBeAg, anti-HCV, HBsAg and HBeAg, and HBsAg and anti-HBs markers, respectively. In summary, seroprevalence of HIV/HBV and HIV/HCV co-infections was lower in Addis Ababa, Ethiopia, than in Sub-Saharan Africa and globally. HBV and HCV infections were not significantly different between HIV positive subjects who were or who were not on ARV. This suggests that the two groups have equal chance of being infected with these two viruses; despite this, disease progression could be different.

INTRODUCTION

Viral hepatitis is a global public health problem affecting millions of people every year, causing disability and death. About 80% of countries, including all countries in the African region, have recognized this infection to be an urgent public health issue (WHO, 2011). Globally, about 400 million people are infected with hepatitis B virus (HBV), and 180 million are infected with hepatitis C virus (HCV). These two infections account for 60% of cirrhosis and 80% of hepatocellular carcinoma and cause one million deaths worldwide each year, mostly in low- and middle-income countries (WHO, 2013). The prevalence of HBV is estimated at 5–7% in central, eastern and southern Africa. However, the prevalence of HCV is even higher in some areas, reaching levels of up to 10% (WHO, 2012). The seroprevalence of HBV and HCV infections in Ethiopia was reported to be 7% (Abebe et al., 2003) and 2% (Frommel et al., 1993), respectively.

Because of common routes of transmission, people are frequently co-infected with viral hepatitis and HIV: an estimated 5–25% of people living with HIV are also infected with either HBV (2–4 million) and/or HCV (4–5 million) (WHO, 2013). The mean HIV/HBV and HIV/ HCV co-infections in 20 Sub-Saharan African countries were reported as 15% and 7%, respectively (Barth et al., 2010). Past studies held in Ethiopia showed prevalence of
HIV/HBV and HIV/HCV co-infections to be 3.9% (Shimelis et al., 2008) and 4.5% (Frommel et al., 1993), respectively.

Although antiretrovirals (ARVs) have significantly improved the health and survival rates of people with HIV infection, co-infection with either HBV or HCV has become the leading cause of non-AIDS-related deaths among this population (Koziel & Peters, 2007). There is a significantly elevated relative incidence of severe liver disease, hepato cellular carcinoma, changes in cognitive and psychiatric function, decreased quality of life, and increased prevalence of diabetes mellitus in people with HIV/HCV co-infection (Koziel & Peters, 2007; Graham et al., 2001). CD4+ T-cell recovery was negatively affected by the presence of ongoing HCV replication in highly active antiretroviral therapy (HAART) HIV co-infected individuals and fast decline of CD4+ T-cells in pre-HAART patients (Taye & Lakew, 2013). Likewise, HBV infection in HIV co-infected patients increases the risk of cirrhosis, end-stage liver disease, and death from liver disease (Thio et al., 2002).

Most HIV positive persons have not been tested for viral hepatitis and therefore their treatments have not been optimized for possible co-infections. The HBV status of most HIV patients in Africa, including Ethiopia, is not well known, which means that many are unknowingly receiving mono-therapy for HBV infection in the context of their antiretroviral therapy (ART) for HIV. A substantial proportion of HIV positive patients with chronic HBV show detectable HBV viraemia despite being treated with anti-HBV active ARVs (Soriano et al., 2010). In patients with HIV/HBV co-infection, greater immunocompromise was associated with continued HBV viraemia while on lamivudine, an effective drug against both HBV (at 100 mg day⁻¹) and HIV (at 300 mg day⁻¹), as the sole anti-HBV agent in the first-line ART regimen (Saha et al., 2013). In addition to this, mono-therapy with lamivudine has been shown to induce HBV resistance in 24% of HBV mono-infected patients after 1 year, increasing to 71% after 5 years of treatment (Rusine et al., 2013). Besides, resistance to lamivudine confers partial or complete cross-resistance to the HBV inhibitors emtricitabine, telbivudine and entecavir, thus limiting future treatment options for HBV infection. Lamivudine is among the first-line HAART regimens in Ethiopia and it is considered the best tolerated ARV agent (FMOH, 2007).

Initial laboratory screening to diagnose HBV infection in an asymptomatic individual generally consists of testing for hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs) and hepatitis B core antibody (anti-HBc). The first sign of infection is the characteristic appearance of HBsAg in infected cells (Miller, 2005). HBsAg is the HBV structure that encloses the viral components and plays a major role in cell membrane attachment to initiate the infection process (Lee & Ahn, 2011). It is produced and secreted through a complex mechanism that is still not fully understood. In clinical fields, HBsAg has long served as a qualitative diagnostic marker for HBV infection (Lee & Ahn, 2011). On the other hand, during the course of chronic HBV infection, presence of HBcAg (extracellular form of HBsAg) is often associated with active and usually continuing liver disease (Hsu et al., 2002). HBcAg positive status is a major factor to be associated with higher HBV DNA levels (Saha et al., 2013). Biologically, virus clearance is classically characterized by the emergence of anti-HBs in the serological profile (Koziel & Peters, 2007).

The optimal approach to detect HCV infection is to screen for a history of risk of exposure to the virus and to test those who have an identifiable risk factor and those who are blood donor candidates for anti-HCV. Screening should not be deferred because of absence of symptoms, signs or elevated hepatic transaminase levels, as HCV is often clinically silent until late stages, and transaminase levels may not be sufficiently elevated to trigger screening (Ghany et al., 2009).

Owing to the seriousness of HIV/HBV or HIV/HCV co-infection, it is important to know the seroprevalence of the diseases in patients who are and who are not on ARV. The global public health response to viral hepatitis recognizes that surveillance and control are vital to ensure that testing, care and treatment are available to all who need these services in every country of the world (WHO, 2013). This study aimed at investigating the serological pattern of HIV/HBV and HIV/HCV co-infections among ARV-naïve and -experienced HIV co-infected adults in Addis Ababa, Ethiopia.

**METHODS**

**Study settings and context.** This cross-sectional study was conducted in Addis Ababa, Ethiopia, on frozen serum and plasma samples collected from HIV-infected adults seeking voluntary counselling and testing (VCT) and ART services in the private Bethzatha Health Service Hospital and in Bethel Teaching General Hospital. The two healthcare facilities were purposely selected as they have service centres in different corners of Addis Ababa, which makes the study sample the best possible representative of the target population. The study population consisted of all HIV positive ARV-naïve and -experienced clients.

**Specimen and data collection.** A total of 500 HIV positive serum and plasma samples collected from both ARV-naïve (n=250) and ARV-experienced (n=250) subjects were used to conduct this study. The samples were collected from VCT attendants at Bethethaza Hospital and its VCT centres, and from ART therapy attendants at Bethzatha Hospital and Bethel Teaching General Hospital. Demographic data, including age, sex and address, and sample code numbers were collected from registration logbooks. An assessment was made between code numbers labelled on test tubes and the registration books for confirming the data.

**Serological test.** Randomly selected samples were stored at 2°C in an icebox and transported quickly from their respective sources to the Microbiology and Immunology Laboratory of the Akilu Lemma Institute of Pathobiology, Addis Ababa University, for serological tests. The samples were tested for HBsAg, anti-HBs, HBcAg and HCV.
antibody (anti-HCV) using a rapid immunochromatographic assay designed for testing HBV markers (HBsAg, anti-HBs, HBeAg) (Wondof Rapid HBV Markers Test; Biotech) and for anti-HCV testing (dBest One Step Rapid Test; Ameritek). The principle of the techniques was based on qualitative two-site sandwich immunosays employing recombinant antigens representing targeted regions of the genome and result interpretation was based on colour formation. Sensitivity and specificity of the HBV test were 94.2 % and 99.5 % respectively, whereas for the anti-HCV test they were 93.3 % and 99.5 % respectively.

Samples testing positive for HBsAg and anti-HCV markers were confirmed by third generation enzyme linked immunosorbent assay (ELISA) (Hepanostika; Organon) in Bethzata Diagnostic Laboratory, and sera that tested similarly by ELISA techniques were used for analysis in this study. Sensitivity and specificity of the ELISA were 99.66 % and 99.30 % respectively. Given that HIV testing had been done for the study subjects within the two health institutions, re-examination was not compulsory. The general procedure of HIV testing in both institutions was similar and used the national serial testing algorithm for detection of antibodies to HIV-1 and HIV-2 (FMOH, 2007; Manyazewal et al., 2013).

**Measurement.** The dependent variables were detection of HBV antibodies and HBV markers. The independent variables were age, sex and ARV status. Critical quality control measures were employed on pre-analytical, analytical and post-analytical procedures. The quality of reagents and instruments was assessed by running quality control samples before starting the actual work and throughout the study. Test kits with high specificity and sensitivity were used to run the test, and known positive and negative controls were run in parallel with test samples. ELISA was employed to confirm HBsAg and anti-HCV markers screened by rapid test devices.

**Statistical analysis.** Retrospective quantitative data extracted from the registration book were checked for completeness and consistency by the principal investigator. Data entry and analysis were carried out using Statistical Package for the Social Sciences (SPSS) version 13 software for Windows. Descriptive statistical methods were used to explore the socio-demographic characteristics of the study participants and the outcome variable. Associations between the socio-demographic characteristics of the study participants and outcome variable were investigated using bivariate analysis. Chi-squared and Fisher’s exact test with 5 % level of significance were used to measure the association between the variables and infection rates.

**Ethical concern.** Ethical clearance was obtained from the Akilu Lemma Institute of Pathobiology Ethical Review Committee, Addis Ababa University. An official letter of co-operation was written to Bethzata Health Service and Bethel Teaching General Hospitals. In order to ensure confidentiality of the information, names and addresses of study participants were not included in the data sheet. Only demographic data of participants were used for client identification and data selection purposes. Leftover frozen samples were used throughout the study.

**RESULTS**

Of the total of 500 subjects, 51.2 % were female. Of 250 samples collected from ARV-naive subjects and another 250 samples collected from ARV-experienced subjects, females accounted for 54.4 % and 48 %, respectively. The majority of the study participants were in the age range 30–39 years, accounting for 36.8 % of the total subjects. Only one sample was collected from a subject aged less than 14. The baseline demographic characteristics of the study participants are summarized in Table 1.

Of the 500 subjects tested, 15 (3 %), 58 (11.6 %), 3 (0.6 %) and 18 (3.6 %) were positive for HBsAg, anti-HBs, HBeAg and anti-HCV markers, respectively. Three (0.6 %) patients tested positive for both HBsAg and HBeAg, whereas (0.2 %) tested positive for both HBsAg and anti-HBs. No patient tested positive for both HBeAg and anti-HBs, and 442 (88.4 %) patients were not immune to HBV. Of the 250 ARV-naive subjects tested, 8 (3.2 %), 33 (13.2 %), 2 (0.8 %), 10 (4 %), 2 (0.8 %), and 1 (0.4 %) were positive for HBsAg, anti-HBs, HBeAg, anti-HCV, HBsAg and anti-HBs, and HBsAg and anti-HBs, respectively. The cumulative prevalence of HBV and HCV markers in ARV-naive and -experienced study subjects is summarized in Table 2.

In Table 3, prevalence of HBsAg by gender indicated that HBV infection among males and females is not statistically significant [8 (53.3 %) versus 7 (46.7 %) respectively, \( P=0.721 \)]. Prevalence of HCV was higher for males than females but the difference was not statistically significant.

<table>
<thead>
<tr>
<th>Table 1. Demographic characteristics of ARV-naive and -experienced study subjects categorized on the basis of a conventional age-ranging scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td><strong>Female, n (%)</strong></td>
</tr>
<tr>
<td>&lt;19</td>
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<tr>
<td>20–29</td>
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<tr>
<td>30–39</td>
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<tr>
<td>40–49</td>
</tr>
<tr>
<td>&gt;50</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
Results indicated that HIV positive subjects in the age category 30–39 years were predominantly infected with HCV, with a prevalence of 5.7%. However, a relatively higher HBsAg prevalence of 5.2% was observed within the age group 40–49 years. Results observed in each age group category are described in detail in Table 4.

DISCUSSION

In this study, the overall seroprevalence of HBV among the 500 HIV positive adults was 3%, which is similar to the 3.9% reported from a study held on HIV positive patients who were attending VCT centre and ART clinic in Addis Ababa, Ethiopia (Shimelis et al., 2008). The slight disparity in the prevalence might be due to difference in sample size, study setting or study period. The prevalence, however, was lower than the Sub-Saharan Africa (15%) and global prevalences (5–25%), which might be due to the particularly lower prevalence of HBV infection in Ethiopia, although further studies are required to explain the reasons in detail. The existence of this antigen in 3% of the study subjects could shed light on the risk of liver damage to the patients.

The simultaneous presence of HBsAg and anti-HBs in this study was clinically insignificant. Though the mechanism underlying the simultaneous presence of both markers in a patient remains unknown, it suggests a selection of HBV immune escape mutants (Lada et al., 2006), which could lead to drug resistance. Likewise, that only 3 of 500 (0.6%) patients were positive for both HBsAg and HBeAg suggests that almost all patients have a better prospect of survival free of hepatic complications (Thio et al., 2002; Hsu et al., 2002). HBeAg is detected in a very few patients, and this potentially indicates that infected individuals have lower levels of HBV (Hsu et al., 2002). However, the absence of HBeAg alone does not guarantee lower viral replication as the patient could be infected with a variant form of HBV, the Mediterranean variant, which decreases or abolishes the production of HBeAg.

Likewise, the seroprevalence of anti-HCV observed in this study, 3.6%, was slightly lower than the 4.5% observed in

Table 3. HBV and HCV markers and their prevalence among males and females

<table>
<thead>
<tr>
<th>Sex</th>
<th>Female, n (%)</th>
<th>Male, n (%)</th>
<th>Total, n (%)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>294 (51)</td>
<td>236 (48.7)</td>
<td>485 (97)</td>
<td>0.72</td>
</tr>
<tr>
<td>Positive</td>
<td>7 (46.7)</td>
<td>8 (53.3)</td>
<td>15 (3)</td>
<td></td>
</tr>
<tr>
<td>Anti-HBs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>226 (51.1)</td>
<td>216 (48.9)</td>
<td>442 (88.4)</td>
<td>0.93</td>
</tr>
<tr>
<td>Positive</td>
<td>30 (51.7)</td>
<td>28 (48.3)</td>
<td>58 (11.6)</td>
<td></td>
</tr>
<tr>
<td>HBeAg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>254 (51.1)</td>
<td>242 (48.9)</td>
<td>497 (99.4)</td>
<td>0.59</td>
</tr>
<tr>
<td>Positive</td>
<td>2 (66.7)</td>
<td>1 (33.3)</td>
<td>3 (0.6)</td>
<td></td>
</tr>
<tr>
<td>Anti-HCV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>250 (51.9)</td>
<td>232 (48.1)</td>
<td>482 (96.4)</td>
<td>0.63</td>
</tr>
<tr>
<td>Positive</td>
<td>6 (33.3)</td>
<td>12 (66.7)</td>
<td>18 (3.6)</td>
<td></td>
</tr>
</tbody>
</table>
a similar study conducted on serum samples collected in the year 1994 as part of a representative household community survey in Addis Ababa, Ethiopia (Ayele et al., 2002). The observed differences may primarily be due to the significant difference in the study period.

As there is an effective vaccine for HBV, immunization has been a central strategy for most countries to reduce the burden of hepatitis B, especially in people who are infected with HIV (WHO, 2013). However, this study has witnessed that the majority of HIV positive people have not been immunized against HBV, indicating that they do not have protective levels of antibodies against HBV (Rao et al., 2008). Immediate screening of everyone with HIV and immunizing all who test negative for HBV markers, irrespective of their ARV status, is therefore the best approach to minimize HIV/HBV co-infection.

Similarly, lack of consensus about HBV and HCV screening in public health and treatment guidelines may contribute to suboptimal screening and late diagnosis in HIV-infected populations (Taylor et al., 2012). Thus, current HIV care and treatment algorithms ought to urge that everyone with HIV should be screened for HBsAg, anti-HBs and anti-HCV before starting on ART. To facilitate this, the current national ART supply chain package should incorporate distribution of HBV and HCV test kits to health facilities. Also, all patients that are confirmed positive for HIV/HBV or HIV/HCV co-infection should be counselled to use appropriate precautions to prevent transmission of the infections to close contacts.

The results of this study show that HBV and HCV infections were not significantly different between HIV positive subjects who were or who were not on ART. This suggests that the two groups have equal chance of being infected with these two viruses; despite this, disease progression could be different, and needs further study. Starting ART without knowing HBV or HCV status of a patient infected with HIV diminishes the effectiveness of the treatment, leading to a more complex morbidity which could lead to death. Screening all HIV positive subjects for HBV and HCV before they start taking ARVs is thus a significant step to better manage these co-infections and minimize the risk of drug resistance.

Though this study has generated very useful data on the prevalence of HBV and HCV infections in ARV-naive and -experienced adults, certain limitations deserve due consideration. Some important data were missing during data collection; the types of ARVs that patients were taking, duration of treatment, adherence, blood transfusion history, and other risk factors should have been collected. In addition, HBeAg and anti-HBc testing was not included in the study, which could have given further information on whether HBsAg- and anti-HBs-negative samples were in the ‘window period’ - the period between the onset of HBV infection and the appearance of detectable antibodies to the virus. Despite these limitations, the large sample size and the use of quality-assured laboratory techniques and procedures were strengths of the study.

In summary, seroprevalence of HIV/HBV and HIV/HCV co-infections was lower in Addis Ababa, Ethiopia, than in Sub-Saharan Africa and globally. HBV and HCV infections were not significantly different between HIV positive subjects who were or were not on ART. This suggests that the two groups have equal chance of being infected with these two viruses; despite this, disease progression could be different. Given that the majority of HIV positive subjects were not immune to HBV, hepatitis B vaccination is recommended for all HIV-infected adults who test negative for HBV markers, irrespective of their ARV status. All patients that are confirmed positive for HIV/HBV or HIV/HCV co-infection should also be counselled to use appropriate precautions to prevent transmission of the infections to close contacts.

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