Assessment of hepatitis B virus and hepatitis C virus infections and associated risk factors in HIV infected patients at Debretabor hospital, South Gondar, Northwest Ethiopia

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Objective: To assess hepatitis B and hepatitis C virus infections and associated risk factors among HIV infected patients at Debretabor hospital.

Methods: A cross-sectional study was conducted among HIV/AIDS patients attending Debretabor hospital from February to April, 2012. Venous blood samples were collected from study participants for HBsAg and anti HCV antibody tests. Bivariate and multivariate analyses were used to identify associated variables with HBsAg and anti HCV positivity. Variables having \( P<0.05 \) was taken as statistically significant association.

Results: From a total of 395 HIV infected patients included in this study, 234 (59.2%) were females and 161 (40.8%) males with mean (SD) age of 36.31 (±9.91) years. The prevalence of HBsAg and anti HCV antibody was 6.1% and 1.3%, respectively. In multivariate analysis, multiple sexual partner (AOR=8.1, 95% CI=1.8-33.97) and history of opportunistic infections (AOR=3.17, 95% CI=1.3-7.7) were statistically associated with HBsAg positivity. History of blood transfusion (AOR=5.61, 95% CI= 1.03-36.59) was associated with presence of anti-HCV antibody.

Conclusions: The prevalence of HBsAg and anti HCV antibodies in HIV coinfected patients was intermediate. However, it is relevant for HIV infected patients since viral hepatitis co-infections in HIV patients can cause multiple complications. Therefore, routine HIV and HCV screening with reliable diagnostic markers need to be carried out for close monitoring and better management in HIV patients.

1. Introduction

Chronic viral hepatitis is a major health problem worldwide. Five hundred million people estimated to be currently infected with hepatitis B virus (HBV) or hepatitis C virus (HCV) globally. These two viruses are the cause of one million deaths each year[1].

Hepatitis B virus is a DNA virus replicates in hepatocytes and damage the liver by immune response to the virus. The virus is transmitted vertically at birth, horizontally through unprotected sex, sharing of injecting equipment and close contact between infants and neonates. Transmission through unscreened blood products is another route of transmission since blood remains infectious for several
weeks even when dried[23]. Human immunodeficiency virus (HIV) infected patients are commonly co-infected with HBV due to similar modes of transmission. Worldwide, about two to four million of HIV patients are estimated to be coinfected with HBV[3,4]. Globally, a prevalence of 5%–15% has been reported for chronic HBV infection among HIV patients[5]. In Ethiopia, some hospital–based studies documented from 3.9% to 14% hepatitis B surface antigen (HBsAg) prevalence in HIV patients[6,7]. HIV–HBV co–infected patients are reported to be at higher risk for developing chronic hepatitis B[8], decreased hepatitis B e antigen (HBeAg) clearance rate, increased HBV replication and higher HBV DNA viral loads[9]. Recent studies have pointed out that individuals co–infected with HBV are at risk of faster progression of HIV infection and cirrhosis and more likely to lose previously developed protective anti–hepatitis B surface antibody (anti–HBsAb) [10,11]. Hepatic immune reconstitution inflammatory syndrome may occur following initiation of antiretroviral therapy (ART)[12], reactivation of HBV after discontinuation of ART regimen containing anti–HBV agents and increased prevalence of antiviral resistance[13,14].

Hepatitis C virus is an RNA virus which is is transmitted by blood–to–blood contact. Sharing injecting equipment and blood transfusion are the most frequently observed routes of transmission[15]. Approximately four to five million HIV patients are coinfected with HCV globally[3]. In these patients, HIV virus weakens the immune response to HCV resulting in a lower probability of spontaneous viral clearance of HCV infection, higher levels of HCV RNA in the blood, more rapid progression to HCV–related end stage liver disease and increased risk of antiretroviral associated liver toxicity[16–18]. The problems listed above highlight the importance of preventing further spread of HBV and HCV infections in HIV–infected patients as well as diagnosing existing infections. Quality of treatment and life in HIV infected patients coinfected with HBV and/or HCV can be improved by appropriate management and monitoring[14].

Hence, magnitude of HBV and HCV infections in patient populations in different areas as well as risk factors for the transmission in this areas should be investigated to take measures for reduction of such transmission with sound evidences in the populations.

However, little emphasis is given for viral hepatitis coinfections in HIV patients in Ethiopia and recent ART guidelines do not recommend routine screening tests in Ethiopia[19]. According to the guidelines, the only test to be performed for liver related complications is determination of alanine aminotransferase (ALT) levels. HIV co–infection results lower ALT levels in HIV infected individuals with positive HBeAg than in HIV–negative similar patients[20]. In addition, different studies also reported that serum ALT has limited predictive value to identify viral coinfection[21–24].

Prevalence of hepatitis B and hepatitis C virus infections has been studied in different parts of Ethiopia[6,7,25–31]. The distribution of the viruses and risk factors for their transmission have not been well addressed in varying community groups like HIV infected patients. Therefore, the aim of this study was to assess seroprevalence of HBV and HCV infections and associated risk factors in HIV infected patients at Debretabor hospital, South Gondar, Northwest Ethiopia.

2. Materials and methods

2.1. Study design and area

A cross sectional study was conducted at Debretabor hospital found in Debretabor town, South Gondar Zone of the Amhara regional state from 16 February to 10 April, 2012. The town has a latitude and longitude of 11°51’N and 38°17’E with an elevation of 2 706 m above sea level. It is 100 km far from Southeast of Gondar, 50 km east of Lake Tana and 666 km from the capital city of Ethiopia, Addis Ababa.

Debretabor hospital is the only hospital in the area and currently provides health services to more than 2.3 million people. It has 88 beds for inpatient services in four wards (medical, pediatric, surgical and gynecology/obstetrics). The hospital also provides outpatient health services including HIV related services.

2.2. Study subjects and sampling procedures

All HIV infected patients attending Debretabor hospital during the study period were eligible for the study. Newly HIV infected patients who received their HIV status after the data collection started and HIV patients less than 18 years old were excluded due to difficulty of obtaining consent.

Study participants were selected based on systematic random sampling technique. A total of 5 453 (1 952 on ART and 3 501 pre ART) HIV infected patients attended Debretabor hospital before the data collection period. Among them, 1 234 HIV patients were expected to attend the clinic in the study period based on their appointment dates. To obtain the pre–planned sample size of approx. 400 patients one–in–three patients was investigated. Of the first three subjects one individual was randomly selected by lottery method, then every 3rd individual was selected to participate in the study.

2.3. Data collection procedure

2.3.1 Socio–demographic and other risk factors

Structured questionnaire was designed and two interviewers (professional nurses) working in Debretabor hospital HIV clinic were selected to collect data from HIV patients.

2.3.2. Blood sample collection and processing

Three milliliters of venous blood was collected using red top vacutainer tube. Clotting, serum was separated by...
centrifugation at 3000 r/min for 10 min for HBsAg and anti HCV. The sera were tested for HBsAg using commercially available test kit according to the manufacturer’s instruction (One step HBsAg test, Ameritech–China, Ltd. Seattle, Washington, USA). Similarly, sera were screened for anti HCV antibody using rapid test kit which works based on principle of immunochromatographic assay (Wondfo one step hepatitis C virus test, Wondfo Biotech Co., Ltd., Guanzhou, PRC).

Three milliliters of venous blood were collected using K3EDTA test tube for CD4+$^+$ count. The whole blood was incubated in CD4$/CD8^+$ reagent vial and a fixative was added to measure the CD4+$^+$ count by using FACS count analyzer (BD FACS count, Sanjose, USA) according to manufacturer’s instruction.

### 2.4. Quality control, data processing and analysis

To assure the quality of the data, the questionnaire was pre–tested at Debretabor Health Center in 21 (5%) HIV infected patients. Possible restructuring and adjustment was made after pretest. Two–day training was given for data collectors. Every day, the collected data was reviewed and checked for completeness by the principal investigator. Standardized operating procedures and manufacturer’s instructions were strictly followed. Known positive and negative samples confirmed by ELISA technique were used in each test procedures for HBV and HCV testing kits and each test kit also had internal control system.

The collected data was analysed using SPSS v.16.0 statistical package software. Descriptive and summary statistics were calculated. Association between each exposure and seropositivity of HBV or HCV infection was determined by using univariate analysis. Those variables with overall $P$–values less than 0.2 in the univariate analysis were entered into a multivariate model. Odds ratio was used as a measure of strength of association. Variables having $P<0.05$ were taken as statistically significant association with HBsAg and anti HCV antibody serostatus.

### 2.5. Ethical consideration

Ethical clearance was obtained from the Ethical Committee of University of Gondar School of Biomedical and Laboratory Sciences, and official permission from Debretabor hospital.

The study participants were informed about the purpose of the study and written informed consent was obtained from each participant. Each participant was informed as they had the right to withdraw from the study at any point in time.

Sample taken from each patient was coded and results obtained were kept confidential. The results were notified to study participants. Individuals found to be positive for HBV and/or HCV were linked to physicians for monitoring and further management.

### 3. Results

#### 3.1. Socio–demographic of study participants

Of the total 422 selected patients, 395 HIV–infected patients participated with non–response rate of 6.3% ($n=27$) due to insufficient blood sample and refusal to consent. Out of 395 patients, 234 (59.2%) were females and 161 (40.8%) males with mean ($\pm$SD) age of 36.31 ($\pm$9.91) years. The largest group were illiterate ($n=168$, 42.5%) followed by those who completed elementary school ($n=105$, 26.6%), and high school ($n=65$, 16.5%). A total of 174 (44.1%) were married. With regard to religious beliefs, 384 (97.2%) were followers of Orthodox Christianity. Among the 395 patients, 296 (74.9%) were urban residents and 99 (25.1%) were from rural settings (Table 1).

#### 3.2. Clinical characteristics and Laboratory findings of study participants

According to the WHO AIDS clinical staging criteria, 38.5%, 23.8%, 29.1% and 8.6% of the patients were classified as stage I, stage II, stage III and stage IV, respectively. A total of 87 (22%) of the patients reported history of opportunistic infection. Eighty three (21%) of the participants had a body mass index of less than 18.5 kg/m$^2$. A total of 208 (52.7%) patients received ART. Altogether 136 (34.4%) and 118 (29.9%) had CD4$^+$ T cell count between 200–350 cell/mm$^3$ and <200 cells/mm$^3$, respectively (Table 1). The mean ($\pm$SD) CD4$^+$ count of the study participants was 317.02 ($\pm$180.80) cells/mm$^3$.

#### 3.3. Seroprevalence of HBV and HCV among the study participants

Of the total 395 HIV infected patients participated in the study, HBsAg and anti–HCV antibody was detected in 24 (6.1%) and 5 (1.3%), respectively. Serological signs of any viral hepatitis (HBsAg and/or anti–HCV) was found in 28 (7.1%) of HIV patients, one HIV infected patient (0.3%) was identified positive for both HBsAg and anti–HCV coinfections. The prevalence of HBV infection was 6.8% and 5.6% among male and female patients, respectively. The prevalence of HBsAg was 10% in patients with single marital status, 11.5% in patients with history of opportunistic infections and 20% in patients with multiple sexual partners.

#### 3.4. Risk factors associated with HBV and HCV infections among study participants

In bivariate analysis, none of the sociodemographic variables, CD4$^+$ count, experience of ART, hospital admission, surgical history, dental procedures and sharing of sharps materials were significantly associated with HBsAg or anti HCV positivity (Table 1). In a multivariate analysis, history of opportunistic infections and multiple sexual partners were independently and significantly associated with HBsAg
positive. The odds of study participants with history of opportunistic infections (AOR=3.17, 95% CI=1.3-7.7, P=0.010) and multiple sexual partners (AOR=8.1, 95% CI=1.8-33.97, P=0.027) were around three and eight times higher for acquiring HBV infection than those who were not exposed (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>Variables</th>
<th>HBsAg status</th>
<th>COR (95% CI)</th>
<th>AOR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
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<tr>
<td>Sex</td>
<td>Male</td>
<td>1.61 (1.18-2.16)</td>
<td>1.61 (1.18-2.16)</td>
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<td></td>
<td>Female</td>
<td>1.18 (1.10-1.26)</td>
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<td>Age (years)</td>
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<td>35-44</td>
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<td>≥54</td>
<td>1.36 (0.2-2.93)</td>
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<td>Marital status</td>
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<td>Widowed</td>
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<td></td>
<td>Divorced</td>
<td>4.4 (4.6)</td>
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<td>History of OIs</td>
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<td></td>
<td>Yes</td>
<td>10 (11.5)</td>
<td>2.73 (1.2-6.4)</td>
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<td>Blood transfusion</td>
<td>No</td>
<td>118 (8.8)</td>
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<td>Yes</td>
<td>118 (8.8)</td>
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<td>Dental procedure</td>
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<td>123 (5.1)</td>
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<td>0.266</td>
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<td>No. of sexual partners</td>
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</tr>
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<td>≥2</td>
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### Table 3

<table>
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<th>Variables</th>
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<th>AOR (95% CI)</th>
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<td></td>
<td>Yes</td>
<td>187 (7.0)</td>
<td>1.0 (0.0)</td>
<td>1.000</td>
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<td>Hospital admission</td>
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<td>Yes</td>
<td>272 (7.0)</td>
<td>1.0 (0.0)</td>
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</tr>
<tr>
<td>Blood transfusion</td>
<td>No</td>
<td>383 (6.1)</td>
<td>1.0 (0.0)</td>
<td>1.000</td>
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<tr>
<td></td>
<td>Yes</td>
<td>383 (6.1)</td>
<td>1.0 (0.0)</td>
<td>1.000</td>
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<tr>
<td>Dental procedure</td>
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<td>306 (6.9)</td>
<td>1.0 (0.0)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>306 (6.9)</td>
<td>1.0 (0.0)</td>
<td>1.000</td>
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<tr>
<td>No. of sexual partners</td>
<td>0</td>
<td>208 (6.9)</td>
<td>1.0 (0.0)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>208 (6.9)</td>
<td>1.0 (0.0)</td>
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<tr>
<td></td>
<td>≥2</td>
<td>3 (4.0)</td>
<td>1.0 (0.0)</td>
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</table>

### 4. Discussion

In 395 HIV infected patients in our area, the prevalence of HBsAg was 6.1%. This study is in agreement with previous studies conducted in Gondar among antenatal clinic attendees (7.3%/29), in Amhara and Tigray regions of Ethiopia.
among blood donors (6.2%)[27], in Kenya (6%)[32] and India (6.3%) among HIV infected patients[33].

However, the prevalence of HBsAg was found lower compared to the 10.9% in Gondar City among street dwellers[31]. Similarly, it is also lower than the 14.0% prevalence reported in Shashemene and 20% in immigrants from Ethiopia among HIV infected patients[7,25]. Moreover, this finding is relatively lower compared to some studies done in African countries like 11.5% in Nigeria and 12.2% in Gambia among HIV infected patients[21,34]. The discrepancy might be due to the differences in diagnostic methods followed in which in these studies ELISA technique was used whereas in this study serological test was applied. The lower rate of HBsAg prevalence in this study might also be partly due to the effect of some HIV drugs like lamivudine to eliminate HBsAg[35].

In contrast, HBsAg prevalence in this study is relatively higher than a previous study done in Uganda and Rwanda among HIV infected patients, where a prevalence of 2.4% and 4.1% was reported, respectively[36]. It is also higher than the 3.9% HBsAg prevalence reported among HIV infected patients in Addis Ababa, Ethiopia[6]. This difference also might be due to differences in accessibility of information about the transmission and prevention of HBV infection in that population whereas in this study population might lack information about transmission and prevention of the virus.

The prevalence of anti HCV in this study was 1.3%. This prevalence is similar with a study conducted in Kenya (1%), Gambia (0.6%) and Uganda (0.6%) among HIV infected patients[32,34,36]. Moreover, this finding is in agreement with the prevalence among blood donors in Amhara and Tigray regional states in which 1.7% was reported[28]. This finding is also in line with a study conducted among antenatal clinic attendees in Gondar Health Center, Northwest Ethiopia where the prevalence was reported to be 1.3%[29].

In contrast, the finding in this study is much lower compared to a study conducted in Iran in which prevalence of 77% was reported among HIV infected patients[37]. The finding in this study is also relatively lower than previous studies conducted in other African countries where anti HCV prevalence of 18.1% in Tanzania[22] and 3.6% in Ghana[38] were reported. The lower prevalence of anti HCV in this study might be due to the difference HCV infection in different geographical area and the lower proportion of high risk groups such as intravenous drug users as well as differences including the use of more reliable diagnostic methods to detect HCV infection in those countries.

The rate of anti-HCV positivity was also lower as compared to a study conducted in different parts of Ethiopia. For example, the 10.5% and 4.5% anti HCV prevalence reported in Hawassa city[27] and Addis Ababa[26] among HIV infected patients, respectively. This might be due to the difference in diagnostic methods in which these studies were carried out by ELISA method but in this study by serological antibody. A recent study showed that patients with severe immunosuppression may have a false negative serology due to impaired antibody formation[39]. On the other hand, the difference also might be a variation in geographical distribution of HCV in the country.

The prevalence of all three viruses (HIV–HBV–HCV) coinfections was 0.3% among HIV infected patients. This finding is in agreement with a study conducted in Kenya which has documented that 1 (0.3%) patient was infected with all three viruses among 378 consecutive HIV positive individuals[32]. However, the finding was much lower as compared to a study done in Iran which has reported a 9.2% prevalence of all three viruses[37]. The difference might be due to the presence of high risk groups such as intravenous drug users in that country, but in Ethiopia these exposures assumed to be rare.

In this study, 3.8% of the patients had history of multiple sexual partners after knowing their HIV serostatus. Among patients who had this exposure, the prevalence of HBsAg (20%) was significantly higher than that who had not such exposure (20% vs. 4.3%, AOR=8.1, 95% CI=1.8–35.97, \( P=0.024 \)). The finding is in agreement with similar findings done in Brazil and in Mexico city which had reported a strong statistically significant association between HBV infection and having multiple sexual partners previously[40,41]. This finding supports the fact that people who have multiple sexual partners will be prone to HBV infections as well as sexually transmitted disease[42].

The higher prevalence of HBsAg was observed in HIV patients with history of OIs (11.5%) than those patients without such infections (4.5%) and the difference was statistically significant (AOR=3.17, 95% CI=1.3–7.7, \( P=0.011 \)). Regarding this point, our study is in agreement with a recent data from Taiwan that showed coinfected HIV patients with HBV were more likely to develop new opportunistic illnesses[43]. This significant association might be due to the fact that opportunistic infections may cause wounds on the surface of the body which might increase the probability of HBV transmission and infect the patients. On the other hand, HIV infected patients might have low immunity when coinfected with HBV that could result the occurrence of opportunistic infections.

Of the risk factors reported by patients, blood transfusion was found to be a statistically significant risk factor for HCV infection in this study (AOR=5.61, 95% CI=1.03–36.59, \( P=0.047 \)). This result was in agreement with a study conducted in Iran among HIV patients reported a significant association between anti HCV positivity and history of blood transfusion[44].

On the other hand, previous studies conducted in Addis Ababa and Hawassa city had reported no significant association between HCV and blood transfusion history among HIV infected patients[26,27]. The difference might be due to patients infected with HIV have lower hemoglobin value probably requiring frequent blood transfusion without following good screening methods of blood donors such as ELISA technique and HCV RNA in the study area. This supports that although transfusion remains the mainstay of therapy for acute or severely symptomatic anemia of any cause, there are several inherent risks such as transfusion reactions and transmissible infection (e.g. viral hepatitis)[45]. This study is not without limitation because of the small number of anti HCV positivity among study participants with history of known risk factors, it was not possible to report the adjusted effects of these factors on the prevalence of anti HCV which is indicated by a wide confidence interval. Moreover, a commercially available rapid test kit was used to detect both HBsAg and anti HCV prevalence.

In conclusion, the prevalence of HBV and HCV infections was found to be intermediate in HIV infected patients in the study area. These indicate a high burden for HIV patients since complications related with these viruses and
HIV coinfections is high. Having opportunistic infections and multiple sexual partners were found statistically associated factors for HBV infection, and having history of blood transfusion was the only statistically associated factor for HCV infection. Following this observation, we recommend that the Federal Ministry of Health needs to incorporate routine HBV and HCV screening in the ART treatment guideline. Moreover, Health institutions giving HIV related services should provide appropriate massage for HIV patients on risk factors such as multiple sexual partners and having opportunistic infections for the transmission of HBV, and blood transfusion for HCV infection.

Conflict of interest statement
We declare that we have no conflict of interest.

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