Hepatitis C prevalence among HIV-infected patients in Guinea-Bissau: a descriptive cross-sectional study

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SUMMARY

Objectives: To estimate the prevalence and determine the clinical presentation of risk factors of hepatitis C virus (HCV) among HIV-infected patients in Bissau, Guinea-Bissau.

Methods: In this cross-sectional study, we included individuals who had a routine blood analysis performed during the period April 28 to September 30, 2011. Patient samples were tested for HCV antibodies (anti-HCV) with a chemiluminescence test (Architect, Abbott, USA) and INNO-LIA HCV Score (Innogenetics, Belgium). HCV viral load and genotype were analyzed using an in-house real-time PCR method.

Results: In total, 576 patients were included (417 HIV-1, 104 HIV-2, and 55 HIV-1/2). Ten (1.7%) patients were anti-HCV-positive and eight (1.4%) patients had detectable HCV RNA; all were genotype 2. In a multivariable logistic regression analysis, age >50 years was associated with anti-HCV reactivity (p < 0.01). No subjective symptoms or objective signs were more prevalent among patients with detectable HCV RNA compared to patients without detectable HCV RNA. Biochemically, detectable HCV RNA was associated with elevated amylase (83.3% vs. 38.6%, p = 0.03), but not with the liver enzymes alanine aminotransferase and aspartate aminotransferase.

Conclusions: The prevalence of anti-HCV was low and comparable to similar settings, and genotype analysis confirmed the presence of genotype 2 in West Africa.

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1. Introduction

Approximately 34 million people are infected with HIV worldwide, of whom one to two million have an HIV-2 infection, an epidemic mainly confined to West Africa. The Western African country Guinea-Bissau is currently experiencing a rise in HIV-1 prevalence and at the same time holds the world’s highest prevalence of HIV-2. An estimated 170 million people are chronically infected with hepatitis C virus (HCV) and more than three million are infected annually. Based on the RNA sequence homology, HCV has been classified into six major genotypes and several subtypes; genotype 2 has previously been found in Guinea-Bissau. HCV shares a route of transmission with HIV, and the greatest risk of transmission occurs with direct percutaneous exposure to infectious blood. HCV may also be transmitted sexually, but the risk is considered relatively low. Although HCV is prevalent in Sub-Saharan Africa, the predominant modes of
transmission are unclear. Active surveillance of HCV is rarely performed due to resource constraints and unreliable serological tests, the lack of molecular (PCR) based tests, and no available HCV-specific antiviral therapy. Since reliable HCV tests are not available for widespread screening in low-resource settings, physicians must often rely on clinical intuition. Unfortunately, data on symptoms and objective signs of chronic HCV infection in Sub-Saharan Africa are scarce.

Almost five million people are co-infected with both HIV and HCV, but the prevalence of HIV/HCV co-infection varies geographically; in Sub-Saharan Africa the prevalence of HCV among HIV-infected patients ranges between 0% and 22%. The rate of spontaneous HCV clearance is lower among HIV co-infected patients than among individuals without HIV infection. In regions with increasing availability of antiretroviral treatment (ART), life expectancy for HIV-infected patients is rising; thus, HCV-related morbidity may become more important. Certain- ly, HIV/HCV co-infection has been found to be associated with an increased incidence of end-stage liver disease and a poorer survival than for HCV infection alone. This is probably due to a more rapid liver disease progression and liver damage among severely immunosuppressed patients infected with HCV. Furthermore, HCV infection increases the frequency of hepatotoxicity of ART and may affect the physicians’ choice of ART regimen. The risk factors, demographic and clinical features of HIV and HCV co-infection in Sub-Saharan Africa are poorly studied and a better understanding is necessary to develop clinical strategies.

In this study we aimed to estimate the prevalence, risk factors, and clinical presentation of HCV among HIV-infected patients in Bissau, Guinea-Bissau.

2. Methods

2.1. Study design and sample collection

The study was conducted at the outpatient ART centre of the Hospital National Simão Mendes (HNSM) in Bissau, in collaboration with the Bandim Health Project and the National HIV Programme. The outpatient ART centre of HNSM is the largest ART centre in Guinea-Bissau, providing care for citizens of Bissau and operating as a reference centre for the other HIV clinics in the country. At the first visit to the clinic, HIV testing is performed and basic demographic information is collected. Blood sampling of patients is usually performed at the clinic the following day and subsequently whenever the physicians request analyses, according to national guidelines. Blood samples are sent to the National Public Health Laboratory for CD4 cell count analyses on a daily basis. Routines at the HIV clinic have been described previously.

All HIV-infected patients aged >15 years attending for routine blood analysis at the HIV clinic at HNSM and who provided enough blood to perform hepatitis serological analyses (>0.5 ml plasma) were consequently enrolled in the study from April 28 to September 30, 2011. No patients were excluded. Questionnaires were used to collect demographic information, symptoms, and potential risk behaviours and were completed on the day of blood sampling; physical examinations were carried out immediately after the interview. Patients were asked about symptoms of liver disease (nausea, vomiting, abdominal pain, skin itching, diminished libido, and diminished appetite) and risk factors (number of sexual partners, age at first marriage, ever bought/sold sexual services, use of condoms, circumcision, previous surgery, dentist treatment, previous blood transfusion, tattoo, traditional scarifications, ever been to prison/arrest, injection drug use, variola vaccination scar, and BCC vaccination scar). The physical examinations were performed with a focus on objective signs of liver disease (icterus, dilated abdominal veins, gynaecomastia, ascites, enlarged liver, tenderness beneath right curvature, axillary alopecia, and oedema).

2.2. HIV testing

Screening for HIV was done with a rapid test in the clinic (Determine HIV-1/2 Assay; Abbott Laboratories). HIV type discrimination was performed with Genie III HIV-1/HIV-2 (Bio-Rad, Steenovoorde, France).

2.3. HCV serology

Plasma samples were tested for hepatitis B surface antigen (HBsAg) and HCV antibodies (anti-HCV) at the Department of Clinical Immunology, Aarhus University Hospital, Denmark using commercially available chemiluminescence assays (anti-HCV and HBsAg, Architect; Abbott Laboratories, Abbott Park, IL, USA). All anti-HCV reactive samples were tested for HCV RNA. Samples testing anti-HCV reactive but HCV RNA-negative were subsequently confirmed with INNO-LIA HCV Score (Innogenetics, Belgium), which was considered conclusive in the case of mismatch with the chemiluminescence test.

2.4. HCV virology

HCV quantification was performed at the Department of Clinical Biochemistry, Section of Molecular Diagnostics, Aalborg University Hospital, Denmark. Samples were analyzed using inhouse real-time PCR methods. Primers from the 5’ non-coding untranslated region (UTR) of the virus were used.

The limit of quantification was 20 IU/mL HCV genotyping was carried out with genotype-specific primer pairs, and a common beacon probe from the 5’ non-coding UTR of the genome was used.

2.5. Statistical analysis

We analyzed the data using the Chi-square test for categorical variables. Continuous variables were compared using the two-sample t-test (normal distribution) or Wilcoxon rank sum test (non-normal distribution). Groups with a sample size of ≤5 were compared using Fisher’s exact test. Abnormal biochemical and haematological values were defined in accordance with reference levels used at HNSM. For the analysis of risk factors for anti-HCV, we used logistic regression and included variables associated with anti-HCV in the Chi-square test, t-test, and Wilcoxon rank sum test (p < 0.10). A p-value below 0.05 was considered significant. Data were analyzed using Stata IC 11.0 (StataCorp LC, College Station, TX, USA).

2.6. Ethics

All patients provided voluntary signed informed consent, or a fingerprint if illiterate, prior to inclusion. Patients consented to donate a blood sample and to the use of data provided for this study. The Danish ethics committee gave consultative approval (case number 1010050) and the study was finally approved by UCEPS, the National Ethics Committee of Guinea-Bissau (N. ref. 016/CNES/2011).

3. Results

3.1. Patient characteristics

During the study period, 930 patients had a blood sample taken for analyses and 576 patients provided sufficient blood to be included in the study. The HIV type discriminatory test revealed...
that 417 (72.4%) were HIV-1-infected, 104 (18.1%) were HIV-2-infected, and 55 (9.5%) were HIV-1/2 dually infected. Three hundred and ninety-four patients (68.4%) were female. The median age at the time of inclusion was 38 years (interquartile range [IQR] 31–47 years). Within 30 days of the interview, the CD4 cell count was available for 556 patients (96.5%); the median CD4 cell count was 286 cells/µl (IQR 164–434 cells/µl). Three hundred and two patients (52.4%) received ART. Overall 191 patients (33.2%) had been diagnosed with HIV within 30 days of inclusion into our study and only two of the newly diagnosed patients had initiated ART. The most frequently used ART combination at inclusion was zidovudine, lamivudine, and nevirapine (43.3%), followed by zidovudine, lamivudine, and efavirenz (15.1%) and zidovudine, lamivudine, and ritonavir-boosted indinavir (14.8%). The median duration of ART was 514 days (IQR 249–876 days).

3.2. HCV serology, viral load, and genotyping

Twenty-four patients (4.2%) were reactive for anti-HCV in the chemiluminescence test (Figure 1). Anti-HCV-positive and HCV RNA-negative samples had a confirmatory anti-HCV test performed (HCV INNO-LIA Score) in which 2/16 (12.5%) were reactive; the adjusted anti-HCV prevalence was 10/576 (1.7%) and the specificity of the chemiluminescence test was 97.5%. HCV RNA was detected in 8/24 samples (33.3%); HCV RNA prevalence was 1.4%. HCV RNA ranged between 65 × 10^5 and 7800 × 10^6 IU/ml and all were genotype 2. Only one patient was co-infected with hepatitis B virus (HBsAg-positive) and HCV. This patient was also dually infected with both HIV-1 and HIV-2.

3.3. Characteristics and risk factors of HCV infection

Patients with a past or current HCV infection were compared to uninfected patients (Table 1). The median age was significantly higher among anti-HCV-positive patients (52 years, IQR 43–54) compared to anti-HCV-negative patients (38 years, IQR 31–46; p < 0.01). The anti-HCV prevalence increased with age: 0/131 (0.0%) in patients aged <30 years, 4/319 (1.3%) in patients aged 30–49 years, and 6/99 (6.1%) in patients aged ≥50 years (p < 0.01).

HCV was not associated with anti-HCV status; anti-HCV prevalence among HIV-1-infected patients was 7/417 (1.7%), among HIV-2-infected patients was 1/55 (1.8%), and among HIV-1/2 dually infected patients was 2/104 (1.9%) (p = 1.00). In the patient group aged ≥50 years, the anti-HCV prevalence among HIV-1-infected patients was 4/46 (8.7%), among HIV-2-infected patients was 2/40 (5.0%), and among dually infected patients was 0/13 (0.0%) (p = 0.48). Furthermore, in this patient group the anti-HCV prevalence was 2/39 (5.1%) in men and 4/60 (6.7%) in women (p = 0.75). There was no significant difference in median CD4 cell count when comparing anti-HCV-positive and negative patients (269 vs. 287 cells/µl; p = 0.50).

Potential risk factors were investigated for association with anti-HCV positivity (Table 2). In the multivariable analysis, HCV remained significantly associated with age ≥50 years with an odds ratio of 7.7 (95% confidence interval 2.1–28.4). Only one patient reported previous intravenous drug use; this patient was anti-HCV-negative.

3.4. Clinical presentation of HCV infection

To evaluate the clinical presentation of HCV-infected patients, we compared patients with active HCV infection (HCV RNA-positive) and patients without active HCV infection (anti-HCV- and HCV RNA-negative). We did not include patients with hepatitis B virus infection (HBsAg) in the analysis. No subjective symptoms or objective signs were significantly more prevalent among patients with active HCV compared with patients without active HCV infection (data not shown). None of the study participants were icteric, had dilated abdominal veins, or gynaecomastia.

The only biochemical marker associated with chronic HCV infection was elevated serum amylase (83.3% vs. 38.6%; p = 0.03).

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Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Anti-HCV-negative n = 566 (98.3%)</th>
<th>Anti-HCV-positive n = 10 (1.7%)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (%)</td>
<td>Male 178/564 (31.6) 2/10 (20.0)</td>
<td>Female 386/564 (68.4) 8/10 (80.0)</td>
<td>0.44</td>
</tr>
<tr>
<td>Age stratification (%)</td>
<td>≤30 years 131/539 (24.3) 0/10 (0.0)</td>
<td>30–49 years 315/539 (58.4) 4/10 (40.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>≥50 years 93/539 (17.3) 6/10 (60.0)</td>
<td>0.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-type (%)</td>
<td>HIV-1 410/56 (72.4) 7/10 (70.0)</td>
<td>HIV-2 102/568 (18.0) 2/10 (20.0)</td>
<td></td>
</tr>
<tr>
<td>HIV-1/2 54/566 (9.5) 1/10 (10.0)</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 cell count (%)</td>
<td>≤200 cells/µl 181/548 (33.0) 3/9 (33.3)</td>
<td>301–350 cells/µl 152/548 (27.7) 5/9 (55.6)</td>
<td></td>
</tr>
<tr>
<td>&gt;350 cells/µl 215/548 (39.2) 1/9 (11.1)</td>
<td>0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutritional status (%)</td>
<td>BMI ≤18.5 kg/m² 168/556 (30.2) 2/10 (20.0)</td>
<td>BMI &gt;18.5 kg/m² 388/556 (68.0) 8/10 (80.0)</td>
<td></td>
</tr>
<tr>
<td>Treatment status (%)</td>
<td>Naïve 272/566 (48.1) 2/10 (20.0)</td>
<td>On treatment 294/566 (51.9) 8/10 (80.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Ethnic group (%)</td>
<td>Mendinka 59/565 (10.4) 0/0 (0.0)</td>
<td>Fula 105/565 (18.6) 0/0 (0.0)</td>
<td>0.29</td>
</tr>
<tr>
<td>Papel 68/565 (12.0) 2/10 (20.0)</td>
<td>Mancanha 55/565 (9.7) 1/10 (10.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Balanta 129/565 (22.8) 4/10 (40.0)</td>
<td>Mandjak 40/565 (7.1) 2/10 (20.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other 109/565 (19.3) 1/10 (10.0)</td>
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<td></td>
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</tr>
</tbody>
</table>

HCV: hepatitis C virus; BMI: body mass index.
Active HCV infection was not associated with anaemia, thrombocytopenia, low albumin concentration, elevated creatinine, bilirubin, alkaline phosphatase, or elevated liver enzymes (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)).

Among patients with active HCV infection, 6/8 (75.0%) were on ART compared to 296/568 (52.1%) (p = 0.20). One patient with detectable HCV RNA had elevated ALT (37 IU/l) and AST (124 IU/l); this patient had been treated with a combination of zidovudine, lamivudine, and ritonavir-boosted indinavir for 2 years at inclusion date. Another HIV-infected patient had ALT within the normal range and slightly elevated AST (57 IU/l), but this patient did not receive ART.

4. Discussion

We have confirmed a low prevalence of hepatitis C in this region among HIV-infected patients. HCV infection was clinically silent and all patients were more than 30 years old, with the majority aged over 50 years.

A number of patients in this study were initially tested anti-HCV-positive using an ELISA-based chemiluminescence test, but subsequent testing for HCV RNA and anti-HCV by INNO-LIA revealed some of the initial tests to have been false-positive. Thus, our study provides a clear status on the number of patients exposed and chronically infected with HCV. Previous studies have reported that ELISA-based tests may overestimate anti-HCV prevalence in resource-limited settings. The reason for this may be cross-reaction to antibodies against other pathogens such as malaria and schistosomiasis. The validity of the confirmatory testing constitutes a major strength in our study. In fact, only a few Sub-Saharan African studies have used molecular methods to determine the prevalence of HCV. The tests used may, however, not be widely available for routine HCV screening in settings such as Guinea-Bissau.

A major limitation of this study is the relatively small number of patients tested positive for anti-HCV and HCV RNA. This decreased the power to detect any characteristics associated with HCV infection and thus the ability to infer cause and effect. For risk factor analysis, the sample size should ideally have been much larger. Furthermore, as patients were included from an HIV clinic in the capital city Bissau, the prevalence of HCV in this study may not reflect the status of the general population in Guinea-Bissau. A population-based survey among individuals aged ≥50 years performed in Bissau, the investigators found a tendency to a lower anti-HCV prevalence among those with HIV-2 infection (2.5%) and HIV-1 infection (0.0%) compared with the HIV-1 infected patients (5.1%; p = 0.07). The anti-HCV prevalence was higher in our study, which consisted of relatively immunosuppressed HIV-infected patients and individuals within a wider age span. Nonetheless, we found no association between a low CD4 cell count and anti-HCV status. The anti-HCV prevalence among HIV-positive and negative individuals was similar in a study of pregnant women in Abidjan, Ivory Coast and in a study of hospitalized patients in Uganda.

Hepatitis C prevalence among HIV-infected patients has previously been reported from other West African settings by testing for anti-HCV: Ivory Coast (1.2%), Gambia (1.2%), Senegal (1.6%), Ghana (3.6%), and Nigeria (3.8%). Furthermore, the proportion with active versus previous HCV infection in our study was 8/10 (80%), similar to other studies, and we confirm the presence of HCV genotype 2 in West Africa in accordance with other studies. In this perspective, the prevalence of anti-HCV in our study was comparable to previous West African studies.

A number of studies have attempted to address risk factors for HCV infection in Sub-Saharan Africa, but still the transmission routes are not clear. The aforementioned population-based survey from Guinea-Bissau found HCV infection to be associated with a history of having bought or sold sexual services and increasing age. Thus, the authors suggested that HCV genotype 2 transmissions occur through sexual intercourse. As in the survey, we found higher levels of HCV prevalence among patients not circumcised and certain ethnic groups in the univariable analysis, but this association was not significant in the multivariable analysis. Stratification into ethnic groups was based on linguistic and cultural similarities. Buying sexual services was not a risk factor for HCV infection in our study. Sexual transmission of HCV is considered a rare event but may occur. Cumulative exposure with increasing age may explain why HCV infection was found to be associated with increasing age in our study. Another explanation for this association is a cohort effect of patients infected many years ago. Studies performed in other Sub-Saharan African countries have found evidence to support the transmission of HCV during hospital admission and through non-sterile needle injections of antiparasitic drugs during the colonial era.

The majority (80%) of anti-HCV-positive patients in this study were men. In the population-based survey from Bissau, gender was not associated with HCV infection. A previous study has linked HIV-2 infection to clitoridectomy, and similar routes could cause HCV transmission among women, however our study did not find a significant association between circumcision and HCV infection (Table 2).

We found no symptoms or objective signs to be associated with active HCV infection, which is similar to a study from Uganda. The only biochemical marker associated with HCV infection in our study was elevated amylase, which is not specific for liver disease. Overall, physical signs and liver transaminase are poor predictors of chronic liver disease due to HCV infection and are mainly seen in end-stage liver cirrhosis. From this, we may conclude that HCV infection among HIV-infected patients in Guinea-Bissau is a silent disease. To diagnose HCV infection, screening of all patients is

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**Table 2**

Univariable and multivariable analysis of hepatitis C risk factors

<table>
<thead>
<tr>
<th>Risk factors of anti-HCV</th>
<th>Logistic regression, anti-HCV odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariable analysis</td>
</tr>
<tr>
<td></td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Never used condoms</td>
<td>5.6 (0.7–44.2)</td>
</tr>
<tr>
<td>Previously used condoms</td>
<td>1.00</td>
</tr>
<tr>
<td>Not circumcised</td>
<td>9.0 (1.1–71.3)</td>
</tr>
<tr>
<td>Circumcised</td>
<td>1.00</td>
</tr>
<tr>
<td>Ethnic group Papel, Mancança, Balanta, or Mandjako</td>
<td>8.41 (1.06–66.9)</td>
</tr>
<tr>
<td>Other ethnic group</td>
<td>1.00</td>
</tr>
<tr>
<td>Age &gt;50 years</td>
<td>7.2 (2.0–26.0)</td>
</tr>
<tr>
<td>Age &lt;50 years</td>
<td>1.00</td>
</tr>
</tbody>
</table>

HCV, hepatitis C virus; OR, odds ratio; CI, confidence interval.

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needed. In Guinea-Bissau rapid tests detecting anti-HCV are used and no confirmatory HCV RNA test is available, making the implications of HCV diagnosis less clear. In low-resource settings, ART affecting the liver such as nevirapine is often used. Even though HCV treatment may not be available in many low-resource settings, knowledge of the patient’s HCV status may be used by the clinician to guide decisions on ART in the co-infected patient. Furthermore, when initiating ART, concomitant HCV infection may contribute to an immune reconstitution syndrome (IRS), which could be confused with drug toxicity. Clinicians may also encourage patients to limit the use of any other substances that could worsen liver disease including alcohol.

Before sending patient blood samples to the laboratory for CD4 cell count, the samples were labelled with the date and a unique identification number. As only the staff at the HIV clinic and affiliated researchers had access to the database, the samples were anonymized for laboratory staff and anyone else handling the samples. We did not actively search for patients to provide them with the results of the HCV tests. In the case where a participant might want the result of their HCV test, the results could be traced from a different database and communicated to the patient.

In conclusion, this study the prevalence of HCV infection was comparable to that in other West African countries, and as reported by several other studies, the prevalence was higher among individuals aged ≥50 years. The transmission routes of HCV in Guinea-Bissau remain unclear, and we recommend that future studies investigate whether HCV prevalence is declining. Among the HIV-infected patients in Guinea-Bissau, HCV infection is a silent disease making screening tests necessary to diagnose co-infection. Although HCV treatment is not available in many low-resource settings, screening is recommended to gain knowledge and guide clinicians in the choice of an appropriate ART regimen for HIV co-infected patients.

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