Sero-prevalence and Correlates of Hepatitis B and C Co-infection Among HIV-infected Individuals in Two Regional Hospitals in Cameroon

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Received: August 20, 2016
Revised: September 12, 2016
Accepted: September 13, 2016

Abstract:

Background:
Liver disease related to Hepatitis B (HBV) and C (HCV) infection has become a major cause of morbidity and mortality in HIV/AIDS patients. Data on the prevalence of HBV and HCV in Cameroon remains inconclusive.

Objective:
We aimed to determine the sero-prevalence and correlates of Hepatitis markers in HIV/AIDS patients in two Regional Hospitals.

Methods:
A cross-sectional study carried out from December 2014 to March 2015. HIV/AIDS patients aged 21 were included and above, receiving care at HIV treatment centres. Data was collected using a structured questionnaire. Blood samples were collected to screen for Hepatitis with HBsAg and anti HCV antibody rapid immunochromatographic test kits. Correlates of hepatitis were investigated by logistic regression. STATA was used for data analysis.

Results:
We included 833 HIV/AIDS patients, 78.8% (657) were female. Mean age was 44 (SD 11) years. Prevalence of Hepatitis in general (total of two viral markers tested) was 8.9% (74/833), with 6.1% for HBsAg and 2.8% for Anti-HCV antibodies. From multivariate analysis, the likelihood of having hepatitis was independently increased by a history of surgical interventions [OR: 1.82(1.06-3.14)], and of sexually transmitted infections [OR: 2.20(1.04-4.67)].

Conclusion:
Almost one in ten participants with HIV/AIDS attending the BRH and LRH tested positive for either HBsAg or anti HCV antibodies. Screening for HBV and HCV should therefore be integrated to the existing guidelines in Cameroon as it can influence management. More studies are needed to evaluate the extent of liver disease and magnitude of HIV suppression in hepatitis and HIV coinfection in this setting.

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**Keywords:** Cameroon, Correlates, HBV, HCV, HIV/AIDS, Prevalence, Screening.

**BACKGROUND**

Human immunodeficiency virus (HIV) infection remains a major cause of morbidity and mortality worldwide [1]. An estimated 35 million (33.2-37.2 million) people were infected with HIV at the end of 2013 [2], 71% of whom were in sub-Saharan Africa (SSA). In Cameroon, the prevalence of HIV in adults is 4.3% [1, 3] with 600,000 people living with HIV [4].

Liver diseases are a major cause of morbidity and mortality among HIV/AIDS patients and they account for 15-17% of HIV-associated mortality worldwide [5]. The global mortality from liver diseases is second only to AIDS-related mortality and most of these liver diseases are of viral aetiology. Amongst the viral aetiologies 66% were linked to Hepatitis C virus (HCV), and 17% to Hepatitis B virus (HBV) [6]. HBV, HCV and HIV can co-exist [7]. The co-existence is quite permissive due to their shared modes of transmission which are mostly through sexual contact with infected individuals, contact with infected blood and blood products and vertical transmission. The prevalence of HBV in HIV approaches 10% worldwide and the risk of transmission is greatly enhanced by injection drug use and unprotected sex [8]. The reported prevalence of HCV in HIV varies significantly depending on the geographic region and mode of transmission [9, 10] ranging from 7% by sexual transmission to 91% for injection drug use [9].

With increasing access to antiretroviral therapy (ART), the burden of viral hepatitis in resource limited settings is expected to increase as is now the case in Europe and North America [11, 12]. There is therefore the concern that if this is not addressed, hepatitis virus related disease may threaten the success of ART programs in developing countries [12]. Understanding the prevalence and disease characteristics of HBV and HCV coinfection with HIV is thus essential [13]. Guidelines for the clinical management of HIV patients recommends screening for viral hepatitis but unfortunately this is not standard practice in Cameroon, as it is not included in the package of baseline laboratory tests. The few previous studies in this setting have reported the prevalence of HCV in HIV to vary between 0.6 to 12.4% while HBV in HIV was from 6.9 to 12.4% [14, 15]. Inspite of these findings, we still need more data with bigger sample sizes, for more accurate estimates of the prevalence of these coinfections especially as they are known to vary in different population groups, different regions even in the same geographical zone, and risk factors for acquiring each infection. A true prevalence of these coinfections is essential for evidence based policy making and resource allocation and planning regarding cost effective interventions especially in a context of limited resources. This will positively impact on general prevention and treatment strategies as in HBV/HIV where ART agents also possess anti HBV activity [16] and the increasing availability of affordable generic Direct acting antivirals (DAA) for HCV treatment. The objectives of this study were to determine the sero-prevalence and correlates of Hepatitis B surface antigen (HBsAg) and anti HCV positivity in HIV/AIDS patients in a regional hospital setting in Cameroon.

**METHODS**

**Study Design and Setting**

This was a hospital-based cross-sectional study carried out from 1st December 2014 to 31st March 2015 at the HIV treatment centres of the Buea (BRH) and Limbe Regional Hospitals (LRH). The BRH and LRH are both second-level referral hospitals in the South West Region, one of ten regions, in Cameroon. In Cameroon, referral hospitals are classified as level 1 (District), level 2 (Regional), level 3 (Central) and level 4 (National reference). The treatment centres function according to national guidelines with standardized HIV management protocols. Their activities include counselling, screening, follow up, dispensation of ART and Cotrimoxazole prophylaxis when indicated. Following pretest counselling, HIV is diagnosed by detection of HIV antibodies using two rapid tests (a highly sensitive first test when positive is followed by a more specific second test). First line ART is based on standard protocols consisting of two Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and one Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI). The preferred treatment regimen consists of Tenofovir/Lamivudine/Efavirenz. Second line treatment is reserved for those who have treatment failure to first line as defined by clinical, immunological, or virological parameters. It consists of two NRTI and one boosted protease inhibitor (Lopinavir). CD4 count testing is recommended six monthly.

**Study Population and Procedures**

The study population included in this study were HIV/AIDS infected individuals aged 21 and above who were...
enrolled in the HIV/AIDS treatment centres of the BRH and LRH. Participants were recruited by consecutive sampling as they presented for their monthly follow-up sessions in the different treatment centres. Written informed consent was obtained from each individual who agreed to participate. A face to face interview by a trained investigator (final year medical student) was conducted using a structured, pretested questionnaire. Patient files were used to complete the required information. Data collected was demographic (Age, sex), level of education (no education, Primary, Secondary and Tertiary), comorbidities (hypertension, diabetes mellitus, stroke, malignancy, heart failure, alcohol consumption-yes or no- and smoking), potential risk factors for acquiring viral hepatitis (scarifications, piecing, tattoo, blood transfusion, surgery, induced abortions, sexual partners -one or if more than one termed multiple-, intravenous (IV) drug use, history of sexually transmitted infection (STI) and liver disease), HIV care characteristics (duration since HIV diagnosis, HIV World Health Organisation (WHO) clinical staging, history of opportunistic infections, most recent CD4 count, ART regimen and duration). Information on comorbidities and potential risk factors was through questions requiring Yes or No response.

Blood Sample Collection and Sample Testing

Participants were initially counselled on hepatitis viral infections. Then, venous blood (4 mL) was collected into coded dry tubes per participant using vacutainer needles. The blood containing tubes were taken to the respective hospital laboratory for separation and hepatitis B and C screening. Serum from the dry tubes was obtained after centrifugation at 3000 rpm for 5 minutes. Then using a micropipette, 2ml of each participant's serum was tested for HBsAg and anti HCV antibodies using INTEC Products (Fujian China (Mainland) rapid screening kits (sensitivity and specificity at greater than 99%). Manufacturer's instructions were strictly followed. Each test kit was a lateral flow qualitative immunochromatographic assay. A positive control sample for hepatitis B and C was used to control the validity for each packet of the reagent before use. The result of the test for presence of HBsAg and or anti-HCV was recorded in the corresponding section of the questionnaire. The patients were individually and privately informed of their results. HBV/HIV coinfection was defined by a positive HBsAg and HCV/HIV coinfection by a positive anti HCV antibody test. Participants who tested positive for HBsAg were treated with Tenofovir containing ARV’s and those positive for Anti-HCV were referred to the Internist or gastroenterologist for further management. All results were recorded in their individual medical files. At the end of each day, the filled questionnaires were cross-checked and validated by the principal investigator.

Statistical Analysis

We used STATA 12.0 for windows (STATA corp, college station, TX, USA) for data analysis. Participant’s characteristics were reported as count and percentages and mean and standard deviation, and compared them across major subgroups via Chi square tests and equivalents for qualitative variables, and Student’s t-test for continuous variables. For further analysis, we categorized some continuous variables without any clinical cut-off at their medians and described hepatitis as carrying any of the 2 markers (HBsAg and anti-HCV antibodies). Thus associated factors of hepatitis were analysed by univariable logistic regression reporting Odds ratios (OR) and their 95% confident intervals (CI). All significant variables were mutually introduced in a final multivariable logistic regression model. A p-value <0.05 was used to indicate statistically significant results.

Ethical Considerations

Ethical Clearance was obtained from the Institutional Review Board of the Faculty of Health sciences, University of Buea (Approval number 2014/258/UB/FHS/IRB). Administrative authorization was sought and obtained from the Regional Delegation of the Ministry of Public Health. Thereafter, administrative approval was sought and obtained from the directors of both hospitals. Confidentiality, anonymity and privacy of all participants were guaranteed at all levels of this study. Written consent was provided by each and every participant.

RESULTS

Baseline Characteristics

A total of 833 patients with HIV were recruited, 632 from LRH and 201 from BRH. Table 1 depicts the baseline characteristics of the study population. The Mean age was 44 (SD 11) years and 78.9% were female. The median duration of HIV infection from diagnosis was 5 [IQR 3-9] years, and almost all the patients were on ART with a median duration of treatment of 5 [IQR 2-8] years. More than half of them (53%) were classified WHO clinical stage 3 and
median CD4 cell count was 419 [IQR 250-593] cells/mm$^3$.

Table 1. Baseline characteristics of the study population.

| Variables                                           | Frequency | Percentage |
|-----------------------------------------------------|-----------|------------|
| Age (n=833)                                         |           |            |
| 21-39                                               | 325       | 39.0       |
| 40-59                                               | 420       | 50.4       |
| ≥60                                                 | 88        | 10.6       |
| Sex (n=833)                                         |           |            |
| Female                                              | 657       | 78.9       |
| Male                                                | 176       | 21.1       |
| Level of Education (n=829)                          |           |            |
| No education                                        | 37        | 4.5        |
| Primary                                             | 446       | 53.8       |
| Secondary                                           | 286       | 34.5       |
| Tertiary                                            | 60        | 7.2        |
| Duration of HIV Infection from diagnosis (n=829)     |           |            |
| ≤1 year                                             | 39        | 4.7        |
| 1-5 years                                           | 376       | 45.4       |
| >5 years                                            | 414       | 49.9       |
| ART (n=831)                                         |           |            |
| No                                                  | 5         | 0.6        |
| Yes                                                 | 826       | 99.4       |
| ART Regimen (n = 826)                               |           |            |
| 1$^\text{st}$ Line                                  | 784       | 94.9       |
| 2$^\text{nd}$ line                                  | 42        | 5.1        |
| Duration of HIV Treatment (n=826)                   |           |            |
| ≤1 year                                             | 38        | 4.6        |
| 1-5 years                                           | 424       | 51.3       |
| >5 years                                            | 364       | 44.1       |
| WHO clinical staging (n= 710)                       |           |            |
| Stage 1                                             | 85        | 11.9       |
| Stage 2                                             | 188       | 26.5       |
| Stage 3                                             | 377       | 53.1       |
| Stage 4                                             | 60        | 8.5        |
| CD4 category, cells/mm3 (n=650)                     |           |            |
| <50                                                 | 37        | 5.7        |
| 50-199                                              | 92        | 14.1       |
| 200-499                                             | 276       | 42.5       |
| ≥500                                                | 228       | 37.7       |
| Opportunistic Infection (818)                       |           |            |

ART: antiretroviral therapy; CD4: cluster of differentiation 4; WHO: World Health Organisation.

Comorbidities and Risk Behaviours

Known hypertensive and diabetic patients were almost 5% and 2% respectively. 27.7% of participants admitted to alcohol consumption. Amongst probable risk factors for acquiring viral hepatitis B and C, scarification was the most practiced (53%), followed by induced abortion in women (28%) and alcohol consumption (27%). Twenty one percent reported a history of blood transfusion (21%) and 9% reported a history of STI. Only 6 participants declared being IV drug users, none of whom were co-infected (Table 2).

Prevalence and Correlates of Hepatitis

No study participant admitted to previous screening for HBV and HCV. The overall sero-prevalence of both Hepatitis B and C was 8.9% (95%CI 7.04%-11.02%). Hepatitis B surface antigen (HBsAg) and anti-hepatitis C antibodies were found respectively in 6.1% (51) and 2.8% (23) of the study participants. 80.4% (41/51) of HBSag
positive patients were on Tenofovir containing regimens. No participant had both markers for HBV and HCV.

Table 2. Comorbidities and risk factors by type of hepatitis.

| Variables                  | Total (N=833) | Hepatitis B (N=51) n(%) | Hepatitis C (N=23)n(%) |
|---------------------------|--------------|-------------------------|------------------------|
| **Co-morbidities**        |              |                         |                        |
| Hypertension              | 39 (4.68)    | 1 (1.96)                | 1 (4.35)               |
| Diabetes Mellitus         | 14 (1.68)    | 1 (1.96)                | 0 (0.00)               |
| Stroke                    | 11 (1.32)    | 1 (1.96)                | 1 (4.35)               |
| Malignancy                | 8 (0.96)     | 0 (0.00)                | 0 (0.00)               |
| Heart Failure             | 2 (0.24)     | 0 (0.00)                | 0 (0.00)               |
| Alcohol consumption       | 231 (27.7)   | 18 (35.29)              | 5 (21.74)              |
| Cigarette smoking         | 21 (2.5)     | 2 (3.92)                | 1 (4.35)               |
| **Risk Factors and Behaviours** |            |                         |                        |
| Scarification             | 444 (53.3)   | 26 (50.98)              | 11 (47.83)             |
| History of Induced Abortion | 187 (28.5)   | 6 (11.76)               | 4 (17.39)              |
| Blood transfusion         | 182 (21.9)   | 9 (17.65)               | 6 (26.09)              |
| Surgical intervention     | 180 (21.6)   | 15 (29.41)              | 8 (34.78)              |
| Multiple sexual partners  | 160 (19.2)   | 18 (35.29)              | 3 (13.04)              |
| History of STIs           | 76 (9.13)    | 10 (19.61)              | 2 (8.70)               |
| Piercing                  | 66 (7.9)     | 1 (1.96)                | 3 (13.04)              |
| Tattoo                    | 45 (5.4)     | 3 (5.88)                | 1 (4.35)               |
| History of Liver Disease  | 24 (2.9)     | 0 (0.00)                | 1 (4.35)               |
| IV drug users             | 6 (0.7)      | 0 (0.00)                | 0 (0.00)               |

STIs: sexually transmitted infections.

From univariable analysis, participants with a history of a surgical intervention [OR 1.73 (95%CI 1.02-2.92)], having multiple sexual partners [OR 1.76 (95%CI 1.03-3.03)] and a history of STIs [OR 2.09 (95%CI 1.07-4.10)] had an increased likelihood of having either of the two markers for hepatitis B and C. On the other hand, patients classified as WHO clinical stage 3 and 4 had higher chances of having hepatitis than those classified as stage 1 and 2 [OR 1.63 (95%CI 0.95-2.89) P=0.07] as well as those with a history of opportunistic infections [OR 1.58 (95%CI 0.96-2.62) P=0.07] though not statistically significant. From multivariable logistic regression, surgical intervention [OR 1.82 (95%CI 1.06-3.14)] and history of STIs [OR 2.20 (95%CI 1.04-4.67)] were independent correlates of both hepatitis B and C when analysed together (Table 3). In Table 4, HBV/HIV and HCV/HIV co-infection were analysed separately. In the univariable analysis there was evidence of association between HBV/HIV coinfection with having multiple sexual partners and a history of STIs. However, this is not supported by the multivariable model. No such association was seen in HCV/HIV coinfection with all the parameters analysed.

Table 3. Correlates of the total of the two markers for hepatitis B and C co-infection in the study population.

| Factors                              | Univariate analysis | Multivariate analysis |
|--------------------------------------|---------------------|-----------------------|
|                                      | OR (95% CI) | P       | OR (95% CI) | P       |
| Age, ≥42 years                       | 1.17(0.73-1.89) | 0.52   |             |         |
| Sex: Male                            | 1.43(0.83-2.46) | 0.19   |             |         |
| HIV infection, ≥5 years              | 1.13(0.69-1.82) | 0.62   |             |         |
| ART duration, ≥5 years               | 1.12(0.68-1.80) | 0.65   |             |         |
| WHO, stage 3&4                        | 1.63(0.95-2.81) | 0.07   | 1.44(0.82-2.52) | 0.21   |
| CD4, ≥200cells/mm3                   | 0.74(0.39-1.40) | 0.36   |             |         |
| OIs, yes                             | 1.58(0.96-2.62) | 0.07   | 1.26(0.75-2.14) | 0.39   |
| Alcohol consumption, yes             | 1.19(0.71-2.00) | 0.50   |             |         |
| Smoking, yes                         | 1.73(0.49-6.05) | 0.38   |             |         |
| Scarification, yes                   | 0.86(0.53-1.39) | 0.55   |             |         |
| Tattoo, yes                          | 0.99(0.35-2.87) | 0.99   |             |         |
| Piercing, yes                        | 0.64(0.23-1.82) | 0.40   |             |         |
| Blood transfusion, yes               | 0.90(0.50-1.63) | 0.73   |             |         |
| Surgery, yes                         | 1.73(1.02-2.92) | 0.038  | 1.83(1.06-3.14) | 0.03   |
| Multiple sexual partners, yes        | 1.77(1.03-3.03) | 0.036  | 1.46(0.80-2.69) | 0.22   |
Factors Univariate analysis Multivariate analysis

| Factors                        | OR (95% CI) | P     | OR (95% CI) | P     |
|-------------------------------|-------------|-------|-------------|-------|
| History of STIs, yes          | 2.09(1.07-4.10) | 0.027 | 2.20(1.04-4.68) | 0.04  |
| History of liver disease, yes | 0.44(0.10-3.30) | 0.41  |             |       |

Odds ratio; CI: confidence interval.

Variables such as age, duration of HIV infection and treatment were categorized at their medians before being entered in univariable analysis and all variables that reached significance at the level of 10% in univariable analysis were entered by default in the final multivariable analysis.

# DISCUSSION

The overall prevalence of both HBsAg and anti HCV antibodies in this study was 8.9%. A history of previous surgery and sexually transmitted infections was shown to be independently associated with this overall prevalence. When considered separately, the sero-prevalence of HBsAg was 6.1%. There was some evidence that male gender, a history of opportunistic infections, multiple sexual partners and STIs were associated with HBV/HIV co-infection. Anti HCV antibody sero-prevalence was 2.8%. Neither socio-demographic, potential risk factors for acquiring hepatitis, nor HIV characteristics were shown to be associated with HCV/HIV co-infection.

We used rapid HBsAg and anti HCV antibody tests. They can be a powerful tool for screening at the point of care. They identify persons infected with these viruses so that preventive services, additional investigations, care and treatment can be offered immediately. Tested persons are thus: notified of their infection status, enabled to make informed decisions about medical care and options for treatment, able to be advised to take measures to limit hepatitis associated disease progression for example as regards alcohol intake, be vaccinated against Hepatitis B, and have a minimised risk of transmission to others [17]. The sensitivity of rapid testing has been questioned by some authors [18]. It remains unclear whether HIV serostatus affects test performance [18]. However another author [19] found HBsAg rapid diagnostic test as an accurate assay for screening for HBsAg in HIV infected patients in a SSA setting.

Liver disease remains an important modifier of health in persons with HIV [20]. The negative effects of HIV infection with progression of HBV and HCV infection is well established with high rates of viral persistence, higher hepatitis viral load and a more rapid progression to liver fibrosis and hepato-cellular carcinoma in co-infected patients [11]. It is therefore unfortunate that screening for HBV and HCV is not routine in Cameroon at the initial assessment of HIV positive patients. None of our study participants admitted to have been previously screened for HCV and HBV infection.
A sero-prevalence of HBsAg of 6.1% was similar to another study in the same region, using a similar methodology but with a smaller sample size [15]. Other studies conducted in Kenya and Ethiopia [21 - 23] found a similar prevalence as ours. One study in Cameroon with a smaller study population found a prevalence of 8.3%. The study setting, patient characteristics (Median CD4 count was 135) and methodology were different. Polymerase chain reaction (PCR) test was used to diagnose HBV and HCV infection [14]. A much higher prevalence of 11 to 20% was found in Nigeria [24, 25] Malawi [26] and Senegal [27]. In a systematic review and meta-analysis aimed at determining the prevalence of HBV and HCV in HIV infected individuals in SSA, the prevalence of HBsAg in HIV in Cameroon in the study used was 20% of 20 study participants [7, 28]. This is unlikely to reflect the burden of HBV in HIV. No recent studies have corroborated these findings.

Only 2.8% of our study participants were anti HCV antibody positive. This prevalence is much lower than the known prevalence of HCV in the general population in Cameroon which is around 13.8% [29]. However another author in Cameroon, using a similar methodology, also reported a prevalence below that of the general population [15]. A prevalence of HCV in HIV below 5% has been found in other African countries [23, 25, 30, 31]. Using data from studies carried out between 2002 and 2014, a systematic review aimed at determining the prevalence of HCV in HIV coinfection in SSA, found the overall pooled sero-prevalence of HCV coinfection in HIV infected individuals was 5.73% [32]. There were fewer studies included from our Central African region compared to West and South East Africa. Again, paradoxically, these prevalence for a high risk population, as HIV positive patients, were lower than that of the general population. However, general population prevalence for HCV may be reviewed in the future as large population surveys are now being done in these regions. For example two recent populations based studies in Cameroun found a prevalence of HCV of 2.55% and 1.1% from a study population of 23,990 [33] and 14,150 [34] respectively.

Prevalence rates of these HBV and HCV co-infections can vary according to the risk factors involved [35]. Such data is not always available in this setting. A history of previous surgery and STI was independently associated with the overall prevalence of both co-infections. Surgery in our setting may still have inherent risks related to lapses in asepsis and blood transfusions which both increase the risk of iatrogenic and nosocomial transmission of these infections [36]. A history of STI and to a lesser extent from this study, multiple sex partners were associated with both HBV and HCV coinfection. This was similar to those of another author [37]. Explanation for HBV than HCV coinfection is easier. Sexual transmission of HBV and HIV is more efficient than for HCV [38].

There were some limitations to this study. Diagnosis of HBV and HCV co-infections was based on the detection of HBsAg and anti HCV antibodies by use of rapid tests with no confirmation by ELISA. Neither was molecular testing done, whereas absence of HCV RNA has been described in 10 to 50% of anti HCV antibody positive patients in some studies [37]. In addition to finding out the sero-positivity of our study participants, additional data on the extent of liver disease, and the magnitude of HIV suppression by use of viral loads could have contributed more to an improved understanding of the impact of hepatitis and HIV coinfection. Also, information on some known risk factors for acquiring hepatitis, particularly sexual history and practices, was lacking. Lastly, our findings cannot be generalised as they reflect the sero-prevalence among individuals in only one region in Cameroon.

CONCLUSION

The prevalence of HBsAg and anti HCV antibodies was lower than expected for a high-risk group like this (patients living with HIV/AIDS) as well as that in published data for the general population in Cameroon. Nevertheless, as almost one in ten participants living with HIV/AIDS tested positive for one of these viral markers, screening for HBV and HCV should be integrated to the existing guidelines in Cameroon as it can influence management. A history of STI and undergone surgery was correlated with hepatitis B and C infection. More research is needed to confirm our data and to study the impact of these coinfections on the evolution of HIV/AIDS in this setting.

LIST OF ABBREVIATIONS

AIDS = Acquired immunodeficiency Virus
ART = Antiretroviral therapy
BRH = Buea Regional Hospital
CD4 = Cluster of differentiation 4
CI = Confidence interval
AUTHORS CONTRIBUTIONS

HNL, BHMN, YNM, DSME, and SAFBE conceived the study. DE collected the data while DSME, FKL entered the data. HNL, FKL, DSME and ODS analyzed the data and drafted the manuscript. HNL, SAFBE, BHMN, YNM, FKL and OSD proofread and corrected the manuscript. All authors agreed with the final manuscript to be submitted for publication.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The authors are thankful to the members of the HIV/AIDS treatment centres of the BRH and LRH and also the patients who accepted to participate in this study.

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