High Human Immunodeficiency Virus (HIV) Viral Load and Coinfection with Viral Hepatitis Are Associated with Liver Enzyme Abnormalities among HIV Seropositive Patients on Antiretroviral Therapy in the Lake Victoria Zone, Tanzania

Shabani Iddi,1 Caroline A. Minja,2 Vitus Silago,3 Asteria Benjamin,3 Jastine Mpesha,3 Shimba Henerico,4 Benson R. Kidinya,2 Stephen E. Mshana,3 and Mariam M. Mirambo3

1Department of Physiology, Weill Bugando School of Medicine, Catholic University of Health and Allied Sciences, P.O. Box 1464, Mwanza, Tanzania
2Department of Biochemistry and Molecular Biology, Weill Bugando School of Medicine, Catholic University of Health and Allied Sciences, P.O. Box 1464, Mwanza, Tanzania
3Department of Microbiology and Immunology, Weill Bugando School of Medicine, Catholic University of Health and Allied Sciences, P.O. Box 1464, Mwanza, Tanzania
4Bugando Medical Centre, P.O. Box 370, Mwanza, Tanzania

Correspondence should be addressed to Shabani Iddi; shabsizya2007@yahoo.co.uk

Received 17 December 2018; Revised 21 March 2019; Accepted 17 April 2019; Published 2 June 2019

Academic Editor: Glenda Gray

Copyright © 2019 Shabani Iddi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Liver enzymes abnormalities have been found to be common among patients on antiretroviral treatment (ART). Apart from the effects of ART on these changes, other factors that can potentially contribute to the abnormal levels of these enzymes have been found to vary in different geographical locations. This study investigated factors associated with liver enzymes abnormalities among human immunodeficiency virus (HIV) infected individuals on ART from the Lake Victoria zone, Tanzania. Methods. A cross-sectional study involving a total of 230 sera from HIV seropositive patients from different regions of the Lake Victoria zone was carried out in July 2017. All samples with required variables/parameters such as age, sex, ART regimen, and residence were serially included in the study. Hepatitis B virus (HBV) and Hepatitis C virus (HCV) detection and liver enzymes assays (alanine transaminase (ALAT) and aspartate transaminase (ASAT)) were assessed following the standard procedures. Data were analyzed by using STATA version 13. Results. The median age of the study participants was 38 (interquartile range [IQR]: 30–48) years. The overall prevalence of abnormal liver enzymes was 43.04% (99/230, 95% CI: 36.6–49.3). A total of 26.09% (60/230) had elevated ASAT while 23.9% (55/230) patients had elevated ALAT levels. ASAT levels were significantly high among patients with high HIV viral load (P=0.002) while ALAT levels were significantly high among those coinfected with hepatitis C virus (P=0.017) and hepatitis B virus (P<0.001). Conclusion. A significant proportion of HIV seropositive individuals on ART have abnormal levels of liver enzymes, which is significantly associated with high HIV viral load and viral hepatitis. This calls for the need to emphasize screening of viral hepatitis and provision of appropriate management among HIV seropositive individuals in this setting.

1. Background

In the era of highly active antiretroviral therapy (HAART), liver diseases have become one of the commonest nonacquired immune deficiency syndrome (AIDS) related causes of death. It accounts for 14–18% of mortalities among human immunodeficiency virus (HIV) infected patients [1, 2]. Among hospitalized HIV patients on HAART, almost half of deaths are attributed to liver diseases which range from asymptomatic or mild elevations of liver enzymes to cirrhosis, end stage liver disease, and other associated complications [3, 4]. Most of the studies have linked these
abnormalities to hepatotoxicity caused by antiretroviral drugs without considering other factors such as HIV viral load and viral hepatitis among many others that can potentially cause abnormalities of the liver enzymes [5–7]. Several factors may be involved in these patients making it difficult to clearly establish the etiology. Concomitant infections with viral hepatitis such as hepatitis B (HBV) and hepatitis C (HCV), other opportunistic infections, alcohol abuse, AIDS related malignancies, and many other entities might be associated with abnormality of liver enzymes [8–10]. However, epidemiology and distribution of these factors might differ from different geographical locations with limited reports from low- and middle-income countries. This study aimed at determining factors associated with liver enzyme abnormalities among HIV population attending different centers in the Lake Victoria zone, Tanzania. The findings from this study might help the health care providers to establish proper monitoring and management of HIV infected individuals by increasing awareness about other factors that can contribute to the abnormalities of liver enzymes in this population.

2. Methods

2.1. Study Design, Area, and Period. This was a cross-sectional hospital based study which was conducted in July 2017 at the Bugando Medical Centre (BMC). BMC is a consultant and teaching hospital with about 900 bed capacity located in the Northwestern of Tanzania serving the lake zone regions, namely, Mwanza, Mara, Kagera, Shinyanga, Simiyu, and Kigoma with estimated population size of thirteen million people. BMC process samples for viral load for all CTCs in the lake zone and in each day samples from all centers are received.

2.2. Sample Size Estimation, Study Population, Sampling, and Selection Criteria. Sample size was estimated by using Kish Leslie formula using the prevalence of 16.4% [11]; the minimum sample size was 211 sera. However, a total of 230 samples were collected. All blood samples collected from different care and treatment centers (CTCs) in different regions for viral load testing at BMC were eligible to be included in the study. Samples with required variables/parameters (age, sex, residence, ART regimen (first, second, or third line), reason for HIV viral load testing (first test, repeating test, or suspected treatment failure), and drug adherence) were serially included until the required sample size was reached.

2.3. Data Collection Procedure. Sociodemographic and other relevant information such as age, sex, residence, ART regimen (first, second, or third line), reason for HIV viral load testing (first test, repeating test, or suspected treatment failure), and drug adherence (good, fair, or poor) were extracted from laboratory request forms accompanying samples for viral load testing using a checklist. Blood specimens in vacutainer EDTA tubes (BD, Franklin Lakes, New Jersey, USA) collected from different CTCs in different regions for viral load testing at BMC were used in this study.

2.4. Laboratory Procedures. EDTA blood samples were centrifuged at 4000 revolutions per minute for 20 minutes to obtain plasma which was then used for viral load testing. Viral load testing was done using a COBAS TaqMan analyzer (Roche diagnostics, Germany) following the manufacturer’s instructions. Viral suppression was defined as viral load < 1000 copies/mL [12].

The liver enzymes (ASAT and ALAT) were analyzed using the CIBA CORNING 252 calorimeter (Ciba Corning Analytical Halstead, England). Liver enzymes were considered normal when values range from 2-40 IU/mL for ASAT and 2-41IU/mL for ALAT. In addition, all samples were analyzed for the presence of HBV antigens and HCV antibodies using immunochromatographic tests (Wondfo HBsAg and HCV antibodies, Guangzhou, China) following the manufacturer’s instructions.

2.5. Quality Control. The standard operating procedures were strictly followed for the quality assurance. Control materials with known ALAT and ASAT results were used to calibrate the calorimeter as per assay kit instructions before processing the samples for ASAT and ALAT. HBV and HCV kits were quality checked using known HBsAg and anti-HCV antibody positive and negative sera controls.

2.6. Data Management and Analysis. Every sample was given a unique identification number. All data were recorded in the log book, transferred to excel then transferred to STATA version 13 (San Antonio, Texas) for cleaning and analysis. Results were presented into proportions for categorical variables and median (IQR) for continuous variables. Chi square test was used to test the association between liver enzymes abnormalities and other factors such as viral hepatitis and HIV viral load followed by multivariate logistic regression analysis to establish independent predictors. Wilcoxon Rank Sum Mann–Whitney test was used to compare median for various groups. A P value of < 0.05 at 95% confidence interval was considered statistically significant.

2.7. Ethical Considerations. Ethical clearance for using patient’s samples was sought from the joint CUHAS/BMC research ethics and review committee with ethical clearance number CREC/381/2017. Permission to conduct the study was requested from hospital laboratory administration. All patient-related information was stored carefully and anonymously using codes.

3. Results

3.1. Sociodemographic and Clinical Data of the Participants. A total of 230 HIV seropositive individuals on ART with the median age of 33 (IQR: 30–48) years participated in this study. The slight majority of the participants 152 (66.1%) were females. A significant proportion of them were from Shinyanga (30.43%), Bunda (26.5%), and Butiama (21.3%). Other characteristics are shown in Table 1. The median age of participants with high ASAT levels was 24(IQR: 15–41) years while that of those with high ALAT levels was 33(IQR: 22–41) years. On Wilcoxon Rank Sum Mann–Whitney test, there was no significant difference on age among participants with high ASAT levels compared to their counterparts [38(IQR:
Table 1: Demographic and clinical characteristics of HIV positive individuals in the lake zone (N=230).

| Variables | Categories | Frequency (n) | Percent |
|-----------|------------|---------------|---------|
| Age*      |            | 37.35(1-75)   |         |
| Sex       | Female     | 152           | 66.09%  |
|           | Male       | 78            | 33.91%  |
| ART Regimen | 1g-A(TDF+3TC+EFV) | 111           | 48.26%  |
|           | 1b-A(AZT+3TC+NVP) | 40            | 17.39%  |
|           | 2H-A(TDF+FTC+ATV/R) | 5             | 2.17%   |
|           | 2s-A(AZT+3TC+ATV/R) | 1             | 0.43%   |
|           | Ic-P(AZT+3TC+EFV) | 4             | 1.74%   |
| ASAT      | Normal     | 170           | 73.91%  |
|           | High       | 60            | 26.09%  |
| ALAT      | Normal     | 175           | 76.09%  |
|           | High       | 55            | 23.91%  |
| Age*      | High ASAT  | 24(15-41)     |         |
|           | High ALAT  | 33(22-41)     |         |

*: age (in years) in median (IQR).

AZT = Zidovudine, EFV = Efavirenz, R = Ritonavir, NVP = Nevirapine, TDF = Tenofovir, 3TC = Lamivudine, ATV = Atazanavir, FTC = Emtricitabine.

29.5-47) vs. 38(IQR: 30-48) years, P=0.577 while the median age of participants with high ALAT levels was significantly low compared to their counterparts [33(IQR: 27-43) vs. 39(IQR: 30-49), P=0.036].

3.2. Prevalence of Liver Enzyme Abnormalities and Associated Factors among HIV Infected Patients. Overall, the prevalence of liver enzyme abnormalities was 43.04% (99/230, 95% CI: 36.6-49.3). A total of 60 patients (26.09%) had elevated ASAT level while 55 patients (23.9%) had elevated ALAT levels (Table 1). Regarding ASAT levels, on univariate analysis high HIV viral load (P=0.001), coinfection with HBV (P<0.001), and HCV (P=0.017) were significantly associated with elevated ASAT levels. Only high HIV viral load (OR: 2.86, 95% CI:1.45-5.65, P=0.002) was independently found to predict high levels of ASAT (Table 2). On the other side coinfection with HCV was significantly associated with elevated ALAT levels (P=0.017) on univariate analysis while none of the factors was found to predict ALAT levels on multivariate logistic regression analysis (Table 3).

4. Discussion

Liver enzyme abnormalities are common among human immunodeficiency virus (HIV) infected individuals and have been associated with multiple factors. However, the risk factors associated with these abnormalities tend to differ in different geographical locations. To the best of our knowledge, this is the first study to investigate factors associated with liver enzyme abnormalities in the Lake Victoria zone, Tanzania. The most salient finding in this study is high overall prevalence of liver enzyme abnormalities which was observed to be 43.09%. This was significantly high in comparison to previous studies in Cameroon and South Africa which observed the prevalence of (22%) and (23%), respectively [13, 14]. Furthermore, in comparison with a previous study in Rwanda [11] in East Africa, the prevalence reported in this study is indeed significantly high. These variations could be explained by genetic variations in metabolizing antiretroviral drugs which may affect the drug toxicity. In addition, elevated liver enzymes in HIV infected patients might be due to direct inflammation of hepatocytes by HIV virus through apoptosis, mitochondrial dysfunction, and permeability alteration in mitochondrial membrane that stimulates an inflammatory response [15]. This has been further confirmed in this study whereby liver enzyme abnormalities were significantly associated with high HIV viral load.

In this study, liver enzyme abnormalities were found to be significantly associated with viral hepatitis (HBV and HCV); this observation was similar to the previous studies [13, 16–19]. In addition, Cicconi et al. observed high levels of liver enzyme abnormalities among HIV-viral hepatitis coinfected patients than HIV monoinfected patients [20]. The possible explanation could be viral cytopathic effects [21]. Further studies to investigate the HBV and HCV viral load in relation to liver enzymes abnormalities in HIV infected patients are of paramount importance.

Another observation in this study was significant association between high ALAT levels with young age. This could be explained by the fact that young aged individuals can mount strong immunity than old aged individuals; therefore, the elevated ALAT could be associated with immunopathology following viral infections [22].

5. Limitation of the Study

One of the major limitations of this study could be the sensitivity of the assays which might affect the results. In addition, other factors that can cause liver damage such as alcohol abuse and opportunistic infections were not investigated.

6. Conclusion

This study observed high prevalence of liver enzyme abnormalities which was significantly associated with viral hepatitis
Table 2: Factors associated with high ASAT levels among HIV positive individual in the lake zone (N=230).

| Variable  | Categories | Normal ASAT (2-40 IU/L) | High ASAT (>40 IU/L) | P-value | OR [95%CI] | P-value |
|-----------|------------|--------------------------|----------------------|---------|------------|---------|
|           | n(%)       | n(%)                     |                      |         |            |         |
| Age*      |            |                          |                      |         |            |         |
| Sex       | Female     | 115 (75.66)              | 37 (24.34)           | 0.400   |            |         |
|           | Male       | 55 (70.51)               | 23 (29.49)           |         |            |         |
| IgA       | No         | 84 (70.59)               | 35 (29.41)           | 0.234   |            |         |
|           | Yes        | 86 (77.48)               | 25 (23.52)           |         |            |         |
| lbA       | No         | 145 (76.32)              | 45 (26.68)           | 0.071   | 1.4 (0.6-3.5) | 0.367   |
|           | Yes        | 25 (62.50)               | 15 (37.50)           |         |            |         |
| IcA       | No         | 138 (73.80)              | 49 (26.20)           | 0.933   |            |         |
|           | Yes        | 32 (74.42)               | 11 (25.58)           |         |            |         |
| IeA       | No         | 144 (73.10)              | 53 (23.90)           | 0.491   |            |         |
|           | Yes        | 26 (78.79)               | 7 (21.21)            |         |            |         |
| Adherence | Good       | 87 (79.09)               | 23 (20.01)           | 0.087   | 1.39 (0.68-2.80) | 0.357   |
|           | Poor       | 83 (69.87)               | 37 (30.83)           |         |            |         |
| Viral load| Suppression| 126 (80.25)              | 31 (19.75)           | 0.001** | 2.86 (1.45-5.65) | 0.002** |
|           | No suppression| 44 (60.27)         | 29 (39.73)           |         |            |         |
| HBV       | Negative   | 170 (77.98)              | 48 (22.02)           | <0.001* |            |         |
|           | Positive   | 0 (0.00)                 | 12 (100)             |         |            |         |
| HCV       | Negative   | 170 (74.56)              | 58 (25.44)           |         |            |         |
|           | Positive   | 0 (0.00)                 | 2 (100)              | 0.017** |            |         |

*: age (in years) in Median (IQR).
**: significant association.

Table 3: Factors associated with high ALAT levels among HIV positive individual in the lake zone (N=230).

| Variable  | Categories | Normal ALAT (2-41 IU/L) | High ALAT (>41 IU/L) | P-value | OR [95%CI] | P-value |
|-----------|------------|--------------------------|----------------------|---------|------------|---------|
|           | n(%)       | n(%)                     |                      |         |            |         |
| Age*      |            |                          |                      |         |            |         |
| Sex       | Female     | 116 (76.32)              | 36 (26.68)           | 0.910   |            |         |
|           | Male       | 59 (70.54)               | 19 (25.36)           |         |            |         |
| IgA       | No         | 91 (76.47)               | 28 (23.53)           | 0.888   |            |         |
|           | Yes        | 84 (75.68)               | 27 (25.32)           |         |            |         |
| lbA       | No         | 144 (75.79)              | 46 (24.21)           | 0.818   |            |         |
|           | Yes        | 31 (77.50)               | 9 (22.50)            |         |            |         |
| IcA       | No         | 142 (75.94)              | 45 (24.06)           | 0.911   |            |         |
|           | Yes        | 33 (76.74)               | 10 (23.26)           |         |            |         |
| IeA       | No         | 151 (76.65)              | 46 (23.35)           |         |            |         |
|           | Yes        | 24 (72.73)               | 9 (27.27)            | 0.625   |            |         |
| Adherence | Good       | 89 (80.91)               | 21 (19.09)           |         |            |         |
|           | Poor       | 86 (71.67)               | 34 (28.33)           | 0.101   | 1.61 (0.81-3.18) | 0.170   |
| Viral load| Suppression (<1,000 copies/mL) | 125 (79.62) | 32 (20.68) | 0.066 | 1.75 (0.88-3.49) | 0.107 |
|           | No suppression (≥1,000 copies/mL) | 50 (68.49) | 23 (31.51) |         |            |         |
| HBV       | Negative   | 171 (78.44)              | 47 (21.56)           |         |            |         |
|           | Positive   | 4 (33.33)                | 8 (66.67)            |         |            |         |
| HCV       | Negative   | 170 (74.56)              | 58 (25.44)           |         |            |         |
|           | Positive   | 0 (0.00)                 | 2 (100)              | 0.017** |            |         |

*: age (in years) in Median (IQR).
**: significant association.
patients are essential in this setting. Management of liver enzyme abnormalities in HIV-infected patients in the Lake Victoria zone, Tanzania. Therefore, monitoring and high HIV viral load among HIV infected patients in the Lake Victoria zone, Tanzania. Thereafter, monitoring and management of liver enzymes abnormalities in HIV infected patients are essential in this setting.

Data Availability
The data used to support the findings of this study are included in the article.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Authors’ Contributions
Shabani Iddi, Mariam M. Mirambo, and Stephen E. Mshana participated in the design of the study. Mariam M. Mirambo, Asteria Benjamin, and Jastine Mpesha participated in the data and sample collection. Asteria Benjamin, Jastine Mpesha, Caroline A. Minja, Vitus Silago, Shabani Iddi, and Shimba Henerico performed serological tests and viral load testing. Stephen E. Mshana and Benson R. Kidenya analyzed and interpreted the data. Mariam M. Mirambo and Shabani Iddi wrote the first draft of the manuscript. Stephen E. Mshana did a critical review of the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgments
The authors would like to acknowledge the technical support provided by the staff of BMC Laboratory, Care and Treatment Centers in the Lake Victoria zone, Tanzania, and the Department of Microbiology and Immunology, CUHAS-Bugando.

References
[1] F. J. Palella Jr., R. K. Baker, A. C. Moorman et al., “Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study,” Journal of Acquired Immune Deficiency Syndromes, vol. 43, no. 1, pp. 27–34, 2006.
[2] Group, DCoAEo-HdS, “Factors associated with specific causes of death amongst HIV-positive individuals in the D: A: D Study,” Aids, vol. 10, pp. 1537–1548, 2010.
[3] I. Bica, B. McGovern, R. Dhar et al., “Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection,” Clinical Infectious Diseases, vol. 32, no. 3, pp. 492–497, 2001.
[4] L. Martin-Carbonero, V. Soriano, E. Valencia, J. García-Samaniego, M. López, and J. González-Lahoz, “Increasing impact of chronic viral hepatitis on hospital admissions and mortality among HIV-infected patients,” AIDS Research and Human Retroviruses, vol. 17, no. 16, pp. 1467–1471, 2001.
[5] A. C. Collier, R. W. Coombs, D. A. Schoenfeld et al., “Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine,” The New England Journal of Medicine, vol. 334, no. 16, pp. 1011–1017, 1996.
[6] A. Kayode, O. Kayode, O. Aroyeun, and M. Stephen, “Hematological and hepatic enzyme alterations associated with acute administration of antiretroviral drugs,” Journal of Pharmacology and Toxicology, vol. 6, no. 3, pp. 293–302, 2011.
[7] M. S. Sulikowski, “Drug-induced liver injury associated with antiretroviral therapy that includes HIV-1 protease inhibitors,” Clinical Infectious Diseases, vol. 38, no. 2, pp. S90–S97, 2004.
[8] N. Crum-Cianflone, G. Collins, S. Medina et al., “Prevalence and factors associated with liver test abnormalities among human immunodeficiency virus–infected persons,” Clinical Gastroenterology and Hepatology, vol. 8, no. 2, pp. 183–191, 2010.
[9] M. S. Sulikowski, “Management of hepatic complications in HIV-infected persons,” The Journal of Infectious Diseases, vol. 197, no. 3, pp. S279–S293, 2008.
[10] M. S. Cappell, “Hepatobiliary manifestations of the acquired immune deficiency syndrome,” American Journal of Gastroenterology, vol. 86, no. 1, pp. 1–15, 1991.
[11] J. C. Dusingize, D. R. Hoover, Q. Shi et al., “Association of abnormal liver function parameters with HIV serostatus and CD4 count in antiretroviral-naive rwandan women,” AIDS Research and Human Retroviruses, vol. 31, no. 7, pp. 723–730, 2015.
[12] National AIDS Control Programme, National Guidelines for the Management of HIV and AIDS, Ministry of Health, Community development, Gender, Elderly and Children, Dare es Salaam, Tanzania, 6th edition, 2017.
[13] C. J. Hoffmann, S. Charalambous, C. L. Thio et al., “Hepatotoxicity in an African antiretroviral therapy cohort: the effect of tuberculosis and hepatitis B,” AIDS, vol. 21, no. 10, pp. 1301–1308, 2007.
[14] K. Lucent, A. Clement, N. Fon, P. Weldeji, and C. Ndikvu, “The effects of antiretroviral treatment on liver function enzymes among HIV-infected out patients attending the central hospital of Yaounde, Cameroon,” African Journal of Clinical and Experimental Microbiology, vol. 11, no. 3, pp. 174–178, 2010.
[15] M. B. Shiferaw, K. T. Tulu, A. M. Zegeye, and A. A. Wubante, “Liver enzymes abnormalities among highly active antiretroviral therapy experienced and HAART naive HIV-1 infected patients at Debre Tabor Hospital, North West Ethiopia: a comparative cross-sectional study,” AIDS Research and Treatment, vol. 2016, Article ID 1985452, 7 pages, 2016.
[16] M. J. Alter, “Epidemiology of viral hepatitis and HIV co-infection,” Journal of Hepatology, vol. 44, pp. S6–S9, 2006.
[17] L. Cooley and J. Sasadeusz, “Clinical and virological aspects of hepatitis B co-infection in individuals infected with human immunodeficiency virus type-1,” Journal of Clinical Virology, vol. 26, no. 2, pp. 185–193, 2003.
[18] D. Lincoln, K. Petoumenos, G. J. Dore et al., “HIV/HBV and HIV/HCV coinfection, and outcomes following highly active antiretroviral therapy,” HIV Medicine, vol. 4, no. 3, pp. 241–249, 2003.
[19] L. Wu, C. Jin, S. Bai et al., “The effect of highly active antiretroviral therapy on liver function in human immunodeficiency virus-infected pediatric patients with or without hepatitis virus co-infection,” Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences, vol. 20, no. 2, p. 127, 2015.
[20] P. Cicconi, A. Cozzi-Lepri, A. Phillips et al., “Is the increased risk of liver enzyme elevation in patients co-infected with HIV and hepatitis virus greater in those taking antiretroviral therapy?” AIDS, vol. 21, no. 5, pp. 599–606, 2007.
[21] P. A. Revill, M. Littlejohn, A. Ayres et al., "Identification of a novel hepatitis B virus precore/core deletion mutant in HIV/hepatitis B virus co-infected individuals," *AIDS*, vol. 21, no. 13, pp. 1701–1710, 2007.

[22] B. T. Rouse and S. Sehrawat, “Immunity and immunopathology to viruses: What decides the outcome?” *Nature Reviews Immunology*, vol. 10, no. 7, pp. 514–526, 2010.