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Short Communication

Absence of hepatitis delta infection in a large rural HIV cohort in Tanzania

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SUMMARY

Objectives: The epidemiological and clinical determinants of hepatitis delta virus (HDV) infection in Sub-Saharan Africa are ill-defined. The prevalence of HDV infection was determined in HIV/hepatitis B virus (HBV) co-infected individuals in rural Tanzania.

Methods: All HBV-infected adults under active follow-up in the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) were screened for anti-HDV antibodies. For positive samples, a second serological test and nucleic acid amplification were performed. Demographic and clinical characteristics at initiation of antiretroviral therapy (ART) were compared between anti-HDV-negative and -positive patients.

Results: Among 222 HIV/HBV co-infected patients on ART, 219 (98.6%) had a stored serum sample available and were included in the study. Median age was 37 years, 55% were female, 46% had World Health Organization stage III/IV HIV disease, and the median CD4 count was 179 cells/μl. The prevalence of anti-HDV positivity was 5.0% (95% confidence interval 2.8–8.9%). There was no significant predictor of anti-HDV positivity. HDV could not be amplified in any of the anti-HDV-positive patients and the second serological test was negative in all of them.

Conclusions: No confirmed case of HDV infection was found among over 200 HIV/HBV co-infected patients in Tanzania. As false-positive serology results are common, screening results should be confirmed with a second test.

1. Introduction

Worldwide, 5% of individuals with chronic hepatitis B virus (HBV) infection have serological evidence of exposure to hepatitis delta virus (HDV).[1] HBV/HDV co-infected individuals generally have more severe liver disease than HDV-uninfected individuals. Only limited data on HDV infection are available from Sub-Saharan Africa (SSA); prevalence estimates range from zero in hepatitis B surface antigen (HBsAg)-positive individuals in Mozambique to 53% in the Central African Republic.[2,3] The aim of this study was to determine the prevalence, as well as the main epidemiological and clinical determinants, of HDV infection in HIV/HBV co-infected individuals in rural Tanzania. To estimate the proportion of false-positive HDV screening tests, all positive samples were retested with a second serological assay and nucleic acid amplification.

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Table 1
Serology results for 11 patients with a positive anti-HDV screening test[a]

Patient	DiaSorin OD	DiaPro ratio
1	0.87	0.49
2	0.62	0.18
3	0.65	0.19
4	0.81	0.21
5	0.87	0.18
6	0.71	0.20
7	0.60	0.19
8	0.83	0.21
9	0.89	0.18
10	0.91	0.27
11	0.73	0.19

HDV, hepatitis delta virus; OD, optical density.
[a] Positive result: DiaSorin OD <0.9, DiaPro ratio (threshold/OD) >0.9.

2. Methods

The study was conducted within the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) in Ifakara, Tanzania.[4] All HIV/HBV-co-infected adults (≥15 years of age) with at least a baseline and one follow-up visit between January 2013 and December 2014, and who started antiretroviral therapy (ART) before December 2014, were included. All participants gave written informed consent. Ethical approval was obtained from the Ifakara Health Institute institutional review board and from the National Institute for Medical Research of Tanzania.

Serum HBsAg was tested using a rapid test (Abon Biopharm, Hangzou, China). The ETI-AB-DELTA-2 test (DiaSorin, Brussels, Belgium) was used to screen for anti-HDV antibodies; results were considered positive when the optical density (OD) was <0.9. A second serological test was used as confirmation (HDV-Ab kit; DiaPro, Milan, Italy, used on an ETI-Max (DiaSorin) platform). The result was considered negative when the ratio (threshold/OD) was <0.9. For the measurement of HDV viral load, total nucleic acids were purified from plasma (Qiagen EZ1 DSP kit), and cDNA (High Capacity cDNA Reverse Transcription Kit; Applied Biosystems) was subjected to real-time PCR according to Ferns et al.,[5] with minor modifications. The detection limit was 1000 genome equivalents per milliliter of plasma.

Anti-HDV prevalence was described with a 95% confidence interval (CI). Fisher's exact test and the Wilcoxon rank sum test were used to compare individual characteristics at ART start between anti-HDV-positive and negative individuals. Analyses were done using SAS version 9.3 software (SAS Institute, Cary, NC, USA).

3. Results

Of 222 HBsAg-positive patients on ART, 219 (98.6%) had a stored sample available for HDV serology. The median age of the patients was 37 years, 55% were female, 46% had World Health Organization stage III/IV HIV disease, and the median baseline CD4 count was 179 cells/μl.

The prevalence of anti-HDV antibody-positivity was 5.0% (11/219, 95% CI 2.8–8.9%) with the screening test. In all positive samples, the OD ranged between 0.60 and 0.91 (median 0.81, interquartile range (IQR) 0.65–0.87), while the positive controls had OD values between 0.05 and 0.09. Among 11 anti-HDV-positive samples, none was positive with the second anti-HDV test (negative controls all had a DiaPro ratio (threshold/OD) <0.9; Table 1). HDV RNA could not be amplified in any of the patients with a positive screening test. Positive real-time PCR results of spiked internal RNA controls confirmed that HDV RNA-negative results were true negatives (data not shown).

The age and sex distribution, as well as median body mass index, CD4 cell count, and alanine aminotransferase levels were similar between anti-HDV-positive and -negative individuals (Table 2). The estimated glomerular filtration rate was slightly lower in HDV-positive individuals compared to HDV-negative individuals ($p = 0.05$).

4. Discussion

In this cohort of over 200 HIV/HBV co-infected individuals in rural Tanzania, no confirmed case of active HDV infection was found. Five percent of the patients had a positive anti-HDV antibody screening test, but the second serology and nucleic acid amplification were negative in all of them. As no demographic or clinical predictor of anti-HDV false-positivity was identified, these results suggest that weakly positive (OD values 0.1–0.9) anti-HDV screening results should be confirmed with an additional test.

There appear to be no published data on HDV prevalence in Tanzania at the current time. In line with the results of this study, reports from neighboring countries Mozambique and Burundi have shown the absence of HDV infection, whereas an anti-HDV antibody prevalence of 53% has been described in the Central African Republic.[2,3,6] As there seem to be large differences in HDV prevalence across and even within countries, further research is needed to improve our knowledge on HDV infection in SSA.

Table 2
Baseline characteristics by anti-HDV screening result

Baseline characteristics	HDV screening test result		*p*-Value
	Anti-HDV non-reactive ($n = 208$)	Anti-HDV reactive ($n = 11$)[a]	
Sex, *n* (%)			
Female	114 (54.8)	5 (45.5)	0.55
Male	94 (45.2)	6 (54.6)	
Age, years, median (IQR)	37 (31–44)	37 (30–44)	0.77
BMI, kg/m^2, median (IQR)	20.3 (18–23)	20.8 (17–23.5)	0.98
CD4 cell count, cells/μl, median (IQR)	174 (66–275)	214 (84–337.5)	0.69
ALT, IU/ml, median (IQR)	23.0 (11.0–39.6)	26.6 (13.7–54.0)	0.36
eGFR, ml/min, median (IQR)	132.3 (110.6–146.1)	115.4 (105.1–124.4)	0.05
Initial ART regimen (%)			0.18
3TC/D4T/NVP	39 (18.8)	0	
3TC/AZT/EFV	63 (30.3)	5 (45.4)	
XTC/TDF/EFV	77 (37.0)	6 (54.6)	
Other	29 (13.9)	0	

HDV, hepatitis delta virus; IQR, interquartile range; BMI, body mass index; ALT, alanine aminotransferase; eGFR, estimated glomerular filtration rate based on the CKD-EPI formula; ART, antiretroviral therapy; 3TC, lamivudine; D4T, stavudine; NVP, nevirapine; AZT, zidovudine; EFV, efavirenz; XTC, lamivudine or emtricitabine; TDF, tenofovir.
[a] Using ETI-AB-DELTA-2 (DiaSorin).

All patients with a false-positive screening test had a weakly positive result. Thus, the cut-off recommended by the manufacturer appears inappropriate in the present study setting. Unfortunately it was not possible to calculate the specificity of the HDV antibody screening test as patients with a negative result were not tested with the second serology. However, if the patients with a negative screening test were all truly HDV-uninfected, the specificity would be 95% (95% CI 91–97%). Although no predictor of anti-HDV false-positivity was identified in this cohort, the power to detect such a difference was very limited. Govindarajan et al. suggested that lipemic serum or the presence of rheumatoid factor were associated with false-positive anti-HDV immunoassay results.[7] Unfortunately it was not possible to test these hypotheses as data on lipids were unavailable.

In summary, HDV infection was found to be rare in HIV/HBV co-infected individuals in rural Tanzania. To avoid the overestimation of HDV prevalence, future studies should confirm serological results with a second test, especially in patients with a weakly positive ETI-AB-DELTA-2 test result.

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Conflict of interest: The authors declare no conflicts of interest.

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