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BRIEF REPORT

Hepatitis E Virus Seroprevalence and Correlates of Anti-HEV IgG Antibodies in the Rakai District, Uganda

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A cross-sectional study was conducted of 500 human immunodeficiency virus (HIV)-infected adults frequency matched on age, sex, and community to 500 HIV-uninfected individuals in the Rakai District, Uganda to evaluate seroprevalence of anti-hepatitis E virus (HEV) IgG antibodies. HEV seroprevalence was 47%, and 1 HIV-infected individual was actively infected with a genotype 3 virus. Using modified Poisson regression, male sex (prevalence ratios [PR] = 1.247; 95% confidence interval [CI], 1.071–1.450) and chronic hepatitis B virus infection (PR = 1.377; 95% CI, 1.090–1.738) were associated with HEV seroprevalence. HIV infection status (PR = 0.973; 95% CI, 0.852–1.111) was not associated with HEV seroprevalence. These data suggest there is a large burden of prior exposure to HEV in rural Uganda.

Keywords. hepatitis E virus; viral hepatitis; HEV seroprevalence; Uganda; Rakai.

Hepatitis E virus (HEV) is an enterically transmitted virus that primarily causes mild or asymptomatic self-limiting disease. However, it can cause severe disease in pregnant women, with mortality rates up to 20% [1]. HEV is responsible for large outbreaks, often associated with flooding and poor sanitation in developing countries, but sporadic cases have also been reported. Genotype 1 and 2 HEV infections are often associated with large outbreaks and more severe disease in developing countries as compared to genotype 3 infections, which are more often associated with sporadic and more mild infections, often in developed countries [2]. There have been a limited number of studies on the seroprevalence and genotype distributions of HEV in sub-Saharan Africa, particularly outside of outbreak settings [3, 4]. Reports of HEV in Uganda have been limited to those of outbreaks of genotype 1 infections, and genotype distribution and seroprevalence of HEV outside of outbreaks has gone unreported. Additionally, there have been conflicting reports about the association of HEV and human immunodeficiency virus (HIV) or acquired immune deficiency syndrome (AIDS) in the literature [3]. Here, we sought to investigate the correlates of prior exposure to and current infection with HEV in the rural Rakai District of Uganda including any potential relationship with HIV status.

METHODS

Study Design and Participants

The design of this study has been previously described in detail [5]. This cross-sectional study was performed in Rakai District, Uganda from 2008 to 2009. Five-hundred HIV-infected patients were recruited from 5 HIV program clinics. Patients were frequency matched on age, sex, and community to 500 HIV-uninfected participants identified from the Rakai Community Cohort Study (RCCS), an ongoing prospective population-based cohort of more than 10 000 adults [6]. Written informed consent was obtained from all participants. This study was approved by Institutional Review Boards (IRBs) of the National Institute of Allergy and Infectious Diseases, Johns Hopkins Medical Institutions, Western IRB (Olympia, WA), the Scientific and Ethics Committee of the Uganda Virus Research Institute, and the Uganda National Council for Science and Technology. This study is registered on clinicaltrials.gov (#NCT00782158).

Laboratory Assays

HEV immunoglobulin G (IgG) serology was conducted using an in-house sandwich method enzyme immunoassay (EIA) designed to detect anti-HEV IgG in serum or plasma, as described previously [2]. Positive anti-HEV IgG status was defined by an optical density (OD) at, or above, the cut-off value plus 10% for that plate using the World Health Organization anti-HEV reference reagent (95/584).

All anti-HEV IgG-positive samples were subsequently tested for anti-HEV immunoglobulin M (IgM). Additionally, all anti-HEV IgG-negative samples collected from individuals infected with HIV and a CD4 cell count ≤200 cells/μL were tested for anti-HEV IgM to ensure immune status did not affect serology results [7]. HEV IgM serology was conducted using an in-house

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class-capture EIA designed to detect anti-HEV IgM specifically, in serum or plasma [8]. Positive anti-HEV IgM status was defined by an OD at, or above, the cut-off value, as described previously [8]. All HEV serology tests were run in duplicate on the same assay plate and discordant results were rerun for confirmation.

All samples positive for IgM anti-HEV, along with a random subset of samples with a high IgG anti-HEV titer, which may be indicative of more recent infection, or from HIV infected individuals with a CD4 count of less than 200 cells/μL, were tested for HEV RNA using a broadly reactive Taqman assay [9, 10]. Samples testing positive by Taqman assay were confirmed by nested polymerase chain reaction (PCR) [11, 12]. Positive nested PCR products were sequenced, and a BLAST search of GenBank nucleotide sequences was performed to determine the viral genotype.

Biomarkers analyzed in the current study included: alanine aminotransferase (ALT), aspartate transferase (AST), hepatitis B surface antigen (HBsAg), anti-schistosomiasis IgG, and HIV-1. Data on occupational status and alcohol use was obtained via interview [5].

Statistical Analyses

To compare results with previous publications in this same cohort, we estimated prevalence ratios (PR) with robust variance using modified Poisson regression [13]. Age in years, AST (U/L), and ALT (U/L) were all analyzed as continuous variables in the models. Multivariable analyses included all variables with P value ≤ .1 in univariate analyses and age, which was determined a priori to have biological plausibility. Stata version 13.1 (StataCorp, College Station, TX) was used for all statistical analyses.

RESULTS

Population Characteristics

Population characteristics stratified by HIV status are shown in Table 1. Briefly, approximately two-thirds of the study population were female and this did not differ significantly by HIV status. Median age was 37 among HIV-uninfected and 38 among HIV-infected participants, and this varied slightly by HIV status due to frequency matching. HIV-infected participants were more likely to have higher liver enzyme levels and lower eosinophil counts than HIV-uninfected participants. HIV-uninfected participants were less likely to have been previously infected with *Schistosoma* parasites compared to HIV-infected participants, but likelihood of chronic hepatitis B virus (HBV) infection did not differ by HIV status. Participants who were HIV-uninfected were more likely to use herbal medicines and less likely to be current alcohol users as compared to participants who were HIV-infected. Heavy alcohol use did not differ by HIV status. Reported occupation in fishing or agriculture did not differ by HIV status. Among participants infected with HIV, the median CD4 count was 450 cells/μL and 60% were receiving antiretroviral therapy (ART).

Table 1. Population Characteristics by HIV Status

Characteristic[a]	HIV positive (n = 500) n (% or IQR)	HIV negative (n = 500) n (% or IQR)	*P* value
Sex			.893
Male	166 (33)	164 (33)	
Female	334 (67)	336 (67)	
Age (median years)	38 (32–45)	37 (32–45)	.025
AST (median U/L)	27 (22–35)	23 (20–27)	<.001
ALT (median U/L)	22 (16–31)	19 (15–25)	<.001
Eosinophil count (median)	5.6 (2.7–12.7)	3.3 (1.8–6.6)	<.001
cHBV (HBsAg positive)			.133
Yes	23 (5)	14 (3)	
No	477 (95)	485 (97)	
Schistosomiasis IgG			.003
Yes	77 (15)	46 (9)	
No	423 (85)	453 (91)	
Herbal medicine use			<.001
Yes	10 (2)	464 (93)	
No	490 (98)	36 (7)	
Current alcohol use			.008
Yes	112 (22)	79 (16)	
No	388 (78)	421 (84)	
Heavy alcohol use			.204
Yes	11 (2)	9 (2)	
No	489 (98)	491 (98)	
Occupational fisherman			.101
Yes	5 (1)	1 (0)	
No	495 (99)	499 (100)	
Agricultural occupation			.200
Yes	399 (80)	380 (77)	
No	100 (20)	116 (23)	
ART			
Yes	302 (60)		
No	198 (40)		
CD4 (median cells/μL)	450 (319–643)		

Abbreviations: AST, aspartate transaminase; ALT, alanine transaminase; cHBV, chronic hepatitis B virus infections; HBsAg, hepatitis B surface antigen; ART, antiretroviral therapy; IQR, interquartile range.

[a]Data are n (%) or median (IQR).

HEV Serology and Nucleic Acid Test

HEV serology results were obtained for 985 participants due to 15 unavailable samples. Anti-HEV IgG serostatus did not significantly differ by HIV status. Specifically, among those not infected with HIV, 47.7% (234/491) were anti-HEV IgG positive and 46.4% (229/494) of persons living with HIV were anti-HEV IgG positive. Of the 985 samples tested for anti-HEV IgG, 463 samples (47%) tested positive for anti-HEV IgG (Table 2). Of 480 samples tested for anti-HEV IgM, 1 sample, which was positive for anti-HEV IgG, was positive for IgM. Of 41 samples that were tested for HEV RNA only the sample that was anti-HEV IgG and anti-HEV IgM positive was found to have detectable levels

Table 2. Correlates of Hepatitis E Virus IgG Seropositive Status

Characteristic	Univariate PR (95% CI)	*P* value	Multivariable adjPR (95% CI)	*P* value
Male sex	**1.297 (1.138, 1.479)**	**<.001**	**1.247 (1.071, 1.450)**	**.004**
Age (years)	1.002 (0.995, 1.010)	.538	1.002 (0.994, 1.009)	.647
Agricultural occupation	0.995 (0.846, 1.170)	.948		
ALT (U/L)	1.002 (0.999, 1.001)	.094		
AST (U/L)	**1.002 (1.000, 1.001)**	**.034**	1.001 (1.000, 1.003)	.128
Occupational fisherman	0.708 (0.228, 2.200)	.550		
Schistosomiasis (IgG)	1.095 (0.907, 1.323)	.345		
Current alcohol use	**1.220 (1.050, 1.418)**	**.009**	1.033 (0.867, 1.231)	.717
HBsAg	**1.443 (1.134, 1.836)**	**.003**	**1.377 (1.090, 1.738)**	**.007**
HIV positive	0.973 (0.852, 1.111)	.683		

PR estimates, 95% CIs, and *P* values determined using Poisson regression with robust variance.

Abbreviations: adjPR, adjusted prevalence ratio; ALT, alanine aminotransferase; AST, aspartate transferase; HBsAg, hepatitis B surface antigen; HIV, human immunodeficiency virus; PR, prevalence ratio.

of HEV RNA by Taqman assay. The 280-base pair nucleotide sequence isolated from the RNA positive sample was compared with other HEV isolates in the NCBI database by BLAST analysis and was determined to be a genotype-3 virus. The partial open reading frame 1 (ORF1) sequence from this participant was submitted to the GenBank database under the accession number MF092844. The individual with detectable HEV RNA was a 45-year-old HIV-positive male receiving ART with a CD4 cell count of 154 cells/μL, elevated ALT (106.2 U/L) and AST (298.5 U/L) enzyme levels, without chronic HBV or schistosomiasis, and had no self-reported history of heavy drinking.

Correlates of Anti-HEV IgG Seropositivity

In univariate analyses, male sex (PR = 1.297, $P < .001$), AST (PR = 1.002, $P = .034$), current alcohol use (PR = 1.220, $P = .009$), and chronic HBV infection (PR = 1.443, $P = .003$) were all associated with being anti-HEV IgG seropositive (Table 2). Age, agricultural occupation, occupational fishing, ALT, schistosomiasis, and HIV were not associated with antibodies to HEV. After adjustment for variables deemed important a priori (age) and in the univariate analyses, male sex (adjusted prevalence ratios [adjPR] = 1.247, $P = .004$), and chronic HBV infection (adjPR = 1.377, $P = .007$) remained significantly associated with prior exposure to HEV.

Discussion

There are limited data available on the prevalence of HEV-antibodies in sub-Saharan Africa outside of large outbreaks where there is a large coburden of HIV infection and liver disease [14]. However, HEV is known to cause waterborne outbreaks during floods in Asia, and has been reported in outbreaks among refugee populations without access to clean water in Africa [14]. Nearly half (47%) of this rural East African population was seropositive for anti-HEV IgG antibodies. Correlates of anti-HEV IgG antibodies included both male sex and chronic HBV infection. Male sex may be associated with specific occupational or environmental exposures that we were unable to capture in our study. The association with chronic HBV has been reported previously [3], and possibly reflects increased childhood risk because the majority of individuals in this population likely were infected with HBV at birth. Importantly, HIV was not associated with prior exposure to HEV in this population.

A large outbreak of HEV caused by a genotype 1 virus occurred in a population of refugees in Northern Uganda from October 2007 through August of 2009, resulting in more than 10 000 cases and 160 deaths [3]. In this study anti-HEV IgM and HEV RNA could only be detected in 1 individual infected with a genotype 3 virus. While genotypes 1 and 2 are more often associated with outbreaks in developing countries, genotype 3 HEV has a global distribution with natural reservoirs in humans and animals such as swine, and is generally responsible for a milder clinical disease [1]. This genotype has not been previously reported in Uganda, and has been reported less frequently in Africa than genotype 1 [3, 4]. Data identifying occupational exposure to animals might help explain some of the prevalence of HEV in this community. Unfortunately, data in this study were limited to self-reported agricultural work without additional details of type of agricultural work or contact with animals. Despite the high seroprevalence of anti-HEV IgG, it is clear that neither active nor chronic HEV infections were common in this population at the time of sample collection.

This analysis has a number of limitations. This was a cross-sectional study design and the analysis was based on data collected previously to examine the relationship of HIV-status and liver stiffness, not the correlates of prior exposure to HEV. Therefore, temporal associations cannot be established, and data on critical variables important to the epidemiology of HEV are lacking, such as occupational exposure to animals or primary water source, which may be relevant to the mode of HEV transmission. Previous studies have reported mixed findings on the association of HEV and HIV or AIDS [3]. The average age of HEV infection in developing countries is late childhood or early adulthood [1]. In our study, HEV infection and clearance may have occurred prior to HIV infection in many of the HEV seroprevalent persons where the average age was approximately 38 years, therefore limiting our ability to examine the relationship between HEV infection among persons infected with HIV. Finally, the samples in this study were collected in 2008, and therefore do not necessarily reflect current HEV seropositivity

in the Rakai District. More recent serosurveys among a broader age range would be helpful in defining the present burden of current and prior HEV infection in this population.

In this study we found a high prevalence of anti-HEV-IgG among rural Ugandans. There was little evidence of active or chronic HEV infection, despite a large outbreak of HEV occurring elsewhere in this country during the same time period. Male sex and chronic HBV infection were significantly associated with prior HEV exposure. These data suggest a large burden of exposure to HEV among HIV-infected and HIV-uninfected individuals in rural Eastern Africa, and that HIV infection was not associated with prior exposure to HEV.

Notes

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