Published in final edited form as:
J Acquir Immune Defic Syndr. 2015 February 1; 68(2): 123–127. doi:10.1097/QAI.0000000000000415.

HIV Through the Looking Glass: Insights Derived from Hepatitis B

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Abstract

Background—While higher levels of hepatitis B virus (HBV) replication in HIV-HBV co-infection may relate to liver disease progression, this has not been completely elucidated. We utilized expression of hepatitis B core antigen (HBcAg) in liver biopsies from HIV-HBV co-infected and HBV mono-infected patients as a marker for HBV replication, and related these findings to clinical and histological parameters.

Methods—Data from 244 HBV patients were compared to 34 HIV-HBV patients. Liver biopsies were scored for inflammation, fibrosis, HBcAg, and hepatitis B surface antigen (HBsAg). Univariate and multivariate analyses were performed.

Results—HBcAg, but not HBsAg, staining was stronger in HIV co-infected than in HBV mono-infected. Co-infected and HBV mono-infected had similar ALT, inflammatory and fibrosis scores, and hepatitis B e Antigen (HBeAg) status. HBcAg staining correlated with HIV after correcting for HBV DNA and HBeAg. CD4 counts and HIV RNA level did not correlate with intensity of HBcAg staining. HBV DNA levels were higher in HIV co-infected and correlated with HBcAg staining.

Conclusions—By looking at HBcAg as a reflection of HBV replication in HIV-HBV co-infected with controlled HIV, our findings suggest that these patients may have subtle immune function defects, which could lead to adverse liver disease outcomes.

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The data in the current manuscript was published in abstract form when it was presented as a poster at the annual meeting of the American Association for the Study of Liver Diseases, Boston, MA 25-28 October 2003

Keywords

co-infection; HBV; HIV; immune

Introduction

Persons with human immunodeficiency virus (HIV) infection are not infrequently co-infected with the hepatitis B virus (HBV), largely because these two viruses share similar modes of transmission. The frequency of HBV co-infection in HIV infected persons in the United States ranges from 5 to 10% but is higher in areas of the world where the background rates of HBV infection are higher and ranges from 20 to 30% in Asia and sub-Saharan Africa [1].

Co-infection with HBV is important in the natural history and outcome of chronic HIV infection. In recent years, liver disease has become a primary cause of disease progression, morbidity, and mortality in persons with the acquired immune deficiency syndrome (AIDS). Thus, chronic HIV infection appears to worsen the natural history and outcome of hepatitis B; and in return, HBV exacerbates the course of chronic HIV infection. In addition, the mortality rate of chronic HIV infection is increased in those with HBV co-infection [2-4].

The interactions between HBV and HIV infection that lead to worse clinical outcomes of both viruses are not completely understood. A major element appears to be the suppression of the immune response due to HIV infection causing an increase in HBV replication, which in turn causes worsening of the accompanying liver disease. In view of this, one might expect that improvement in therapies of HIV infection would improve the outcome of hepatitis B in co-infected individuals. However, with introduction of HAART therapy, the rates of death due to liver disease in HIV infected cohorts appeared to increase and it was only with satisfactory therapies of the concomitant hepatitis B using agents that were active against both HIV and HBV that improved morbidity and mortality could be demonstrated [5-7].

HIV-HBV co-infected patients with controlled HIV disease (relatively low viral load, and relatively high CD4 count) were observed to have liver biopsies with more widespread staining for HBcAg staining, a marker of active HBV replication, compared to those of HBV mono-infected patients. Patients with well-controlled HIV would be expected to have HBV levels within the normal range. However, this higher level of HBcAg staining suggested that HBV disease was not controlled. We hypothesized that increased staining seen in HIV-HBV co-infection was related to facilitated HBV replication in patients with a compromised immune system due to HIV. The aim was to identify factors that correlated with HBcAg and hepatitis B surface antigen (HBsAg) staining in hepatocytes of patients with HIV-HBV co-infection, focusing upon serum and tissue markers of HBV and HIV infection.

Methods

Patients

Liver biopsies performed between 1980 and 2002 on adult patients with chronic hepatitis B by members of the Liver Diseases Branch of the Clinical Center of the NIH were selected for review. Patients with concomitant liver diseases were excluded as were those who were receiving HBV antiviral therapy at the time of liver biopsy. Clinical and laboratory data were extracted from patient charts. In patients without available results, stored serum samples taken at or around the time of liver biopsy (within 2 months) were retrieved and tested for HBV DNA. Patients with insufficient clinical data were excluded from analysis.

HBV DNA levels were measured in stored serum samples by quantitative PCR (Roche COBAS TaqMan HBV Analyte Specific Reagent). HIV RNA levels were measured by Roche COBAS Amplicor HIV-1 Monitor Test. HIV antibodies were measured by Ortho VITROS Anti-HIV 1+2 assay. Only the first liver biopsy was included in patients with more than one assessment.

All patients studied were participants in clinical research studies being conducted at the National Institute of Allergy and Infectious Diseases and National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health and gave written informed consent for studies of the HIV and/or HBV infection.

Liver histology

Liver histology was evaluated by a hepatic pathologist without knowledge of the clinical features of the patients. Inflammatory activity was graded using a modification of the histology activity index (HAI) and fibrosis was staged using the Ishak fibrosis score. All liver biopsies were also read for the presence of HBcAg and HBsAg by immunoperoxidase staining and scored without knowledge of HIV status. The degree of HBV antigen staining was scored on a scale of 0 to 4+ based upon the proportion of hepatocytes with positive staining in which 0 = none, 1+ = <10%, 2+ = 10-50%, 3+ = 50-90% and 4+ = > 90% stained. The pattern of distribution of HBcAg was also recorded as either nuclear only, nuclear predominant, mixed nuclear and cytoplasmic and cytoplasmic only.

Statistical analysis

Univariate analyses were performed with t tests, Mann-Whitney or Chi-square tests, as appropriate, using a p value < 0.05 as significant. Multivariate analysis included variables with a p value lower than 0.10 in univariate analysis. Multicollinearity among variables included in the model was defined as an $R^2 \geq 0.75$ between any 2 variables.

Results

Between 1980 and 2002, 823 liver biopsies were performed at the National Institutes of Health in patients with chronic hepatitis B. Only 369 biopsies, however, were the initial biopsy and were from adult patients who were not already on HBV antiviral therapy. After initial analysis of these subjects, 83 were excluded because of insufficient clinical information and 8 because of a concurrent liver disease including hepatitis C (n=6), chronic

alcoholism (n=1) and primary sclerosing cholangitis (n=1). Of the remaining 278 patients, 34 (12%) were co-infected with HIV. A comparison of the demographic, clinical, laboratory and histological characteristics of patients with HBV mono-infection versus HIV-HBV co-infection are shown in Table 1. Patients with HIV-HBV co-infection were statistically significantly more likely to be male (100% vs 80%) and white (95% vs 64%) than those with HBV mono-infection. The HIV infected cohort was also slightly younger (39 vs 42 years) although the known duration of HBV infection was longer (9 vs 2.5 years) than those with HBV alone. Nevertheless, serum ALT levels, platelet counts and liver histology scores were similar between the two groups ($p > 0.05$ with all three parameters).

In contrast to the clinical and biochemical features, the viral factors were different between the two groups. HBV DNA levels were higher in co-infected patients (median = 4.2 billion vs 526 million copies/mL) and they were more likely to have HBeAg in serum (96% vs 80%). Additionally, staining patterns differed between the two groups (Figure S1). HBcAg staining was more frequent (22.7 vs 5.6%) and more intense (mean staining scores = 1.7 vs 2.5) in the HIV-HBV co-infected than the HBV mono-infected group (Figure S2A). Even with adjustments for gender, age, race, total inflammatory score and Ishak fibrosis score, the co-infected group had a higher odds ratio for both HBc staining (OR = 3.71, $p = 0.01$) and HBV DNA (OR = 1.75, $p < 0.01$) (Table 2). The pattern of staining was most often “Mixed nuclear and cytoplasmic” in the HIV-HIV co-infected and more frequently “nuclear only” in the HBV mono-infected subjects. In contrast, HBsAg staining was similar in the two groups, with mean staining score 1.1 vs 1.4 (Figure S2B).

Associations were sought between HIV status and clinical features as well as biochemical and HBV virological factors. The majority of HIV infected subjects had reasonable immunologic function. CD4 counts were > 200 cells/mm3 in 81% and HIV viral loads were < 10,000 copies/mL in 84% of patients. Nonetheless, these HIV-positive patients had higher levels of HBV DNA and more intense HBcAg in liver tissue than the mono-infected patients despite similar degrees of disease activity as shown by serum ALT levels and HAI scores as well as similar degrees of disease stage as shown by platelet counts and fibrosis scores (Figure S3A) Indeed, peripheral blood CD4 counts and HIV RNA levels did not correlate with HBV DNA levels nor with the intensity of HBcAg staining in the 18 patients with available data (all $p>0.20$). (Figure S3B, S3C)

HBV DNA levels correlated strongly with degree of HBcAg staining and were higher in those with HBeAg in serum. However, even after controlling for HBV DNA levels and HBeAg status, HBcAg staining intensity was still greater in HIV co-infected patients than in HBV mono-infected patients ($R^2=0.36$; $p<0.0001$). Examples of typical HBcAg staining pattern in an HBV mono-infected patient compared to the typical such staining in an HIV-HBV co-infected patient are shown in Figure S3.

Discussion

High serum HBV DNA levels, positive HBeAg, positive HBcAg, and increased serum ALT levels are signs of active HBV replication. In HBV mono-infection, active HBV replication is linked with increased severity of liver disease and liver-related mortality [8-11].

In our study cohort, HBV-HIV co-infected patients had CD4 counts mostly above 200, low HIV RNA levels, and similar ALT, platelet counts, inflammation and fibrosis scores when compared to HBV mono-infection. Co-infected patients had higher serum HBV DNA and increased HBcAg hepatocyte staining despite low HIV RNA levels and independent of CD4 counts, serum HBeAg status, ALT level, or hepatic inflammation. HBcAg localization has been previously linked to more severe disease [12-14]. Showing increased HBcAg supports the notion that the hepatitis B virus was actively replicating at a higher rate in HIV-HBV co-infected than mono-infected despite no indications of immunosuppression or advanced disease. This implies that subtle defects by the host immune system in even well controlled HIV lead to enhanced HBV replication.

Our work adds to other previously described work that suggested functional lymphocyte abnormalities and lower T4/T8 ratios in patients with HIV infection compared to those without may help explain the subtle deficits [15,16]. A recent large retrospective study from Taiwan looked at the correlation between HIV positive patients and hepatitis B and hepatitis C serum markers [17]. Our work expands their chart review with liver biopsy observations. While we did not find an association between CD4 counts and anti-HBcAg staining, our findings, in particular that HBcAg staining is correlated with HIV co-infection, are consistent with their conclusion that compromised immunity affects HBV markers.

Although increased HBV DNA and HBcAg expression does not directly measure disease severity, it does offer insights into how the disease state in HIV-HBV co-infection is altered compared to HBV alone. Despite what appears to be controlled HIV disease, HBV liver disease remains uncontrolled. While studies have reported that liver disease progression is accelerated in HIV-HBV co-infection, the reason for this is still not well understood. Studies have shown that HIV infected individuals possess features characteristic of an aging immune system or ‘immune senescence’ that is driven by chronic immune activation [18]. In another study looking at CD4 T-cell decline in pediatric HIV-1 infection, microbial translocation was associated with persistent monocyte/macrophage activation unrelated to viral replication or T-cell activation [19]. Similarly, microbial translocation has been linked to hepatitis C virus liver disease progression in patients co-infected with HIV [20]. It is important to explore these and other possible mechanisms as it affects clinical outcomes.

We acknowledge several limitations to our retrospective study. After exclusion criterion, the sample size of co-infected patients was relatively small. PCR detection of viral nucleic acid levels at a single time-point may encompass transient variations. However, we do not believe these variables significantly bias our results.

In summary, using liver biopsies from HBV or HIV-HBV patients, we find significantly increased HBcAg but not HBsAg staining in co-infected patients, despite similar fibrosis levels. We also find that HBV DNA levels were higher in co-infected patients, although CD4 counts were independent of HBV DNA levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Silke Williams for the collection of liver biopsy samples and Dr. Mark Raffeld and his team for staining the liver specimens. We are grateful to the physicians in the National Institute of Allergy and Infectious Diseases for the use of their patient data.

Conflicts of Interest and Source of Funding: This research was supported by the intramural research program of the National Institute of Diabetes and Digestive and Kidney Diseases (Z01 DK054514-02 LDB), National Cancer Institute, and the Clinical Center, National Institutes of Health, Bethesda, MD.

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Abbreviations

HIV	human immunodeficiency virus
HBV	hepatitis B virus
CD4	cluster of differentiation 4
HAART	highly active antiretroviral therapy
HBcAg	hepatitis B core antigen
HBsAg	hepatitis B surface antigen
HAI	histological activity index
HbeAg	hepatitis B e antigen
ALT	alanine aminotransferase

J Acquir Immune Defic Syndr. Author manuscript; available in PMC 2016 February 01.

Table 1

The demographic, clinical, laboratory and histological characteristics of HBV mono- and HIV-HBV co-infected patients.

Variable	HBV		HIV-HBV		P*
	n		n		
Total	195		22		
Sex					
male	155	79.5%	22	100.0%	0.017
female	40	20.5%	0	0.0%	
Race					
White	125	64.1%	21	95.5%	0.015
Black	17	8.7%	1	0.5%	
Asian	43	22.1%	0		
Other / Not available	10	5.1%	0		
Age (mean ± SD, years)	187	41.6 ± 12.9	22	38.8 ± 5.8	0.077
BMI (mean ± SD, Kg/m^2)	144	25.5 ± 4.2	16	25.2 ± 3.3	0.820
Time from HBV diagnosis (median, years)	164	2.5	22	9.3	<0.001
ALT (median, IU/dL)	128	97.5	21	143	0.226
Platelet count (median, platelets/mm^3)	115	183	22	159	0.179
HBV DNA (median copies/mL)	526,333,000		4,155,000,000		0.019
HBeAg (% positive)	80		96		0.051
HIV viral load (%)					
<50 copies/mL	-		63		-
50-10,000 copies/mL	-		25		-
>10,000 copies/mL	-		12		-
CD4 count (mean ± SD, cells/mm^3)	-		468 ± 270		-
CD4 count (% > 200 cells/mm^3)	-		81.3		-
Periportal (mean ± SD, scale 0-10)	195	3.8 ± 2.1	22	3.7 ± 1.9	0.860
Total inflammatory (mean ± SD, scale 0-18)	195	9.0 ±3.2	22	8.7 ± 3.7	0.631
Ishak fibrosis score (mean ± SD, scale 0-6)	192	2.9 ± 1.8	22	3.1 ± 1.4	0.643
HBsAg (mean ± SD, scale 0-4)	1.1 ± 0.8		1.4 ± 1.1		0.22
HBcAg (mean ± SD, scale 0-4)	1.65 ± 1.1		2.5 ± 1.2		0.0044

Variable	HBV	HIV-HBV	P*
	n	n	
HBcAg pattern			
1 = Nuclear only	36%	11%	0.0231
2 = Nuclear predominant	28%	26%	
3 = Mixed, nuclear and cytoplasmic	26%	58%	
4 = Cytoplasmic only	10%	5%	

The means were compared with t test, the medians were compared with Wilcoxon Two-Sample Test, and the proportions were compared with Fisher's exact test

Table 2

Odds Ratios of HBcAg in Relationship to HIV status and HBV DNA

Outcome: HBcAg (ordered value, according to the levels of staining)
Number of subjects included in analysis: 134
Adjusted for gender, age, race, total inflammatory score and Ishak fibrosis score.

Variable		Odds ratio	95% CI		p
Group	HBV	Ref.			
	HIV-HBV	3.71	1.34	10.27	0.012
DNA	Log 10	1.75	1.44	2.12	<0.001