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Hyperbilirubinaemia in HIV–HCV co-infected patients on antiretroviral therapy: drug effect or liver disease severity?

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ABSTRACT

Objective: Hyperbilirubinaemia (HB) is common in HIV and hepatitis C virus (HIV–HCV) co-infected patients and poses a unique challenge in management as it may be due to medications such as the protease inhibitors (PIs) or to hepatic dysfunction. There are no data on the relationship of HB to liver histology and PI use in this population. Clinicians caring for these patients are faced with the difficult task of determining whether increasing serum bilirubin is due to drug effects or progression of liver disease.

Methods: To address this gap in knowledge, we performed a retrospective analysis of 344 consecutive HIV–HCV co-infected patients undergoing liver biopsy to identify factors associated with HB. Demographic, clinical, laboratory data were collected. Advanced fibrosis was defined as bridging fibrosis or cirrhosis. Those with hepatitis B virus, hepatic decompensation or hepatocellular carcinoma were excluded.

Results: The prevalence of HB (range 1.3–9.4) was 33% and more common in those on a PI (46%) than those who were not (10%; $p \leq 0.001$) and mostly in those on indinavir (40%) or atazanavir (46%). Of the patients on these PIs, HB was not associated with fibrosis grade, demographics, or other clinical variables. Conversely, in those not on a PI, HB was associated with fibrosis grade ($p \leq 0.0001$) after adjusting for other clinical and demographic variables.

Conclusions: In the setting of indinavir or atazanavir use, HB is common and unrelated to underlying disease severity and the medications can be continued safely. Conversely, HB in HIV–HCV co-infected patients not on a PI is due to their underlying liver disease and suggests these patients require closer monitoring.

Summary box

What is already known about this subject?
Hyperbilirubinemia is a common side effect of protease inhibitors.

What are the new findings?
Uses biopsy data to confirm that development of unconjugated hyperbilirubinaemia in HIV-HCV co-infected patients being treated with protease inhibitors is likely due to benign drug effect rather than progression of liver disease.

How might it impact on clinical practice in the foreseeable future?
Reinforce clinician resolve in conservative management of unconjugated hyperbilirubinaemia in HIV-HCV co-infected patients.

INTRODUCTION

The HIV has become a manageable (though incurable) entity since the introduction of highly active antiretroviral therapy (HAART) in the mid-1990s.[1] As HIV-infected patients live longer, non-AIDS illnesses which were of secondary concern during the early days of the HIV/AIDS epidemic are becoming increasingly important sources of morbidity and mortality in the HIV-infected population.[1] In particular, liver disease accounts for an increasing proportion of non-AIDS deaths.[2–5] While multiple mechanisms of liver disease exist in this population, viral hepatitis, particularly co-infection with hepatitis C virus (HCV), is common in patients with HIV infection.[6] This HIV–HCV co-infected population represents a unique challenge to clinicians who must manage their patients' HIV while remaining vigilant for progression of their coexistent liver disease.

Management of the co-infected patient is complicated by the fact that many of the medications used in HAART are known to confer a significant risk of hepatic side effects including elevated transaminases and hyperbilirubinaemia (HB).[7 8] As a class, the protease inhibitors (PIs), and more specifically the drugs atazanavir (ATZ) and indinavir (IND) are implicated in causing HB.[7–10]

While the HB associated with these drugs is typically benign, the sudden development of any derangement in liver function tests in

a patient known to have chronic viral hepatitis poses a unique challenge to those caring for HIV–HCV co-infected patients. These clinicians, faced with laboratory values suggestive of worsening liver inflammation and injury, are tasked with determining whether or not these new aberrancies require further workup or alteration of an otherwise stable and efficacious treatment regimen. Although the frequency of increased bilirubin in those on ATZ or IND is known,[7 9 10] its relationship to underlying liver disease by histology is unknown. To address this gap in knowledge, the aim of this study was to determine if HB in an HIV–HCV co-infected patient being treated with a PI represents a benign drug effect, progression of liver disease, or a combination of the two.

PATIENTS AND METHODS

Patients

This retrospective analysis was performed on a prospective database of 344 consecutive adult (age 18 and over) patients co-infected with HIV and HCV who had undergone liver biopsy at our institution between 1996 and 2013. HIV and HCV infection were defined as the presence of anti-HIV and HCV RNA, respectively. Patients were excluded if they were co-infected with hepatitis B (defined by hepatitis B surface antigen positivity), showed signs or symptoms of hepatic decompensation, or had been diagnosed with hepatocellular carcinoma. All patients biopsied had been on a stable HAART regimen prior to biopsy.

Methods

Cross-sectional data collected prospectively at time of liver biopsy included demographic, laboratory, histological, and HAART treatment regimen data. Data collected included age, gender, race, CD4 count, HCV genotype, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, bilirubin, albumin, haemoglobin, platelets, AST to Platelet Ratio Index (APRI) score,[11] Fibrosis-4 (FIB-4) score,[12] inflammation/fibrosis score (Ishak),[13] the presence of advanced fibrosis (defined as Ishak 4–6), the presence of steatosis >5%, steatohepatitis, non-alcoholic steatohepatitis,[14] and which (if any) PI the patient was currently taking. Biopsies were interpreted by hospital pathologists with experience in liver pathology as routine pathology (ie, with full access to the patient chart and without blinding). Length of biopsy core was used as a surrogate measure of biopsy quality.

Statistical analysis

All data were assessed for normality and are expressed as mean±SD unless otherwise noted. Statistical analysis was performed using JMP (V.11, SAS 2014). The primary end point for all analyses was the presence of HB (>1.2 mg/dL, the upper limit of normal in our laboratory) at time of biopsy. A secondary end point of HB of 1.8 g/dL (1.5 times the upper limit of normal) was chosen with the intent of illustrating similar results with what many practicing clinicians might consider a more significant hyperbilirubinaemia. Univariate analysis was performed to determine factors associated with HB, after which multiple logistic regression was used to determine factors independently associated with HB. Differences were assessed by Student t test, χ^2, or Fisher's exact test as appropriate. Pearson's correlation was used to assess relationships of continuous variables, and multiple linear regression was used to assess for independent predictors of HB.

RESULTS

Patient characteristics

Patient characteristics at time of biopsy and the number of patients on various PIs are shown in table 1. Mean age was 46 (±8.7) years, 75% of patients were male, and 84% were black. Of the cohort, 155 (45%) were being treated with a PI. Those on a PI were less likely to have HIV RNA>400 copies/mL (27% vs 42%; p=0.02), had lower CD4 counts (452 vs 551 cells/mm^3), and were more likely to be on an nucleoside reverse transcriptase inhibitor (NRTI) (96% vs 71%; p<0.001) compared with those who were not. There were no differences in liver enzymes or fibrosis in PI-treated versus non-PI-treated patients. Among those on a PI, 26% (40) were being

Table 1 Demographic, clinical laboratory, and histopathological data

Demographics	
Total patients	344
Age (years)*	46±8.7
Male	75%
Race (African American)	84%
Laboratory	
CD4 (cells/mm^3)	505±320
Genotype 1	91%
AST (U/L)†	61 (range 43–99)
ALT (U/L)†	64 (range 43–101)
Alkaline phosphatase (U/L)†	108 (range 82–139)
Bilirubin (mg/dL)†	0.6 (IQR 0.4–1)
Hyperbilirubinaemia (>1.2 mg/dL)	15.4%
Albumin (g/dL)*	3.9±0.6
Haemoglobin (g/dL)*	13.9±2.4
Platelets (10^9/L)*	210±78
APRI†	0.6 (range 0.1–10)
FIB-4†	1.81 (range 0.33–42)
Biopsy data	
Inflammation score*	6.44±2.8
Fibrosis score*	1.48±1.34
Steatosis >5%	19%
Steatohepatitis	9%
NASH	4%
Advanced fibrosis (Knodell score 3–4)	31%

*Mean±SD.
†Median (range).
ALT, alanine transaminase; AST, aspartate transaminase; NASH, non-alcoholic steatohepatitis.

treated with ATZ, 10% (15) with IND, 26% (40) with lopinavir/ritonavir, 22% (34) with nelfinavir, 4% (6) with darunavir, and 12% (20) with other miscellaneous PIs. The mean biopsy length was 20 mm and 94% were >10 mm. The mean histologic activity index was 7.88 (±3.6) with mean inflammatory score of 6.44 (±2.8) and fibrosis score 1.46 (±1.3) with 30% having advanced fibrosis. When those with biopsy length <10 mm were excluded, the results did not change.

Factors associated with HB

Of the total cohort of 344 patients, 53 (15.4%) exhibited some degree of HB, with values ranging from 1.3 to 9.4 mg/dL with 8% having a level 1.5 times upper limit of normal. Total bilirubin correlated with unconjugated bilirubin (r=0.44, p=0.005). In those with an increased total bilirubin, the unconjugated fraction was 1.08 (±0.58) with a range of 0.1–2.6. Univariate analysis (table 2) identified the following factors to be significantly different in patients with and without HB; CD4 count (p=0.03), AST (p<0.0001), ALT (p=0.005), alkaline phosphatase (p<0.0001), platelet count (p=0.0037), APRI score (p=0.0012), FIB-4 score (p<0.0001), Ishak score (p<0.0001), total inflammation (p=0.0007), fibrosis score (p<0.0001), the presence of advanced fibrosis (p=0.0001), and PI use (p<0.0001). Patients treated with PIs were significantly more likely to have increased bilirubin (24% vs 7.9%; p<0.001). In particular, the use of ATZ or IND was more strongly associated with HB than any of the other PIs. Of PI-treated patients with HB, 48% were using ATZ or IND, versus only 10% in the non-HB group (p<0.0001). There was no significant difference in the presence of HB due to age, sex, gender, albumin, haemoglobin, or presence of steatosis >5%. Subsequent multiple logistic regression identified only CD4 count (p=0.04), alkaline phosphatase (p=0.005), advanced fibrosis (p=0.005), and the use of IND or ATZ (p<0.001) as significant independent predictors of HB. As liver biopsy has been largely replaced by non-invasive assessments for advanced fibrosis such as FIB-4, we repeated our analysis utilising FIB-4 as a continuous variable and a high FIB-4 (>3.25) as categorical variable as a surrogate for advanced fibrosis. This repeat analysis again identified CD4 (p=0.07), alkaline phosphatase (p=0.002), FIB-4 (p=0.001) and use of IND or ATZ (p<0.0001) as independent predictors of increased bilirubin. When we repeated our analysis with a higher cut-off of total bilirubin (1.8 mg/dL) to minimise laboratory variation, alkaline phosphatase (p<0.0001), FIB-4 (p=0.04), and use of IND or ATZ (p<0.0001) remained predictors of increased bilirubin. In the subset of patients treated with a PI, HB was not associated with any other clinical variables. However, in patients not on a PI, HB was strongly associated with advanced fibrosis (9% vs 29%, p<0.0001) after adjusting for significant clinical and demographic variables as detailed above.

Table 2 Results of univariate analysis, primary end point bilirubin >1.2 mg/dL

Factor	Total bilirubin ≤1.2	Total bilirubin >1.2	p Value
N	291	53	
Age	46±8.7	46±9.2	NS
Gender (% male)	74	81	NS
HIV category (% CD4>400 cells/mm^3)	37	23	NS
CD4 count (cells/mm^3)	521±332	417±226	0.03
AST (U/L)	75±54	132±110	<0.0001
ALT (U/L)	77±61	103±8.5	0.005
Alkaline phosphatase (U/L)	115±60	172±110	<0.0001
Albumin (g/dL)	3.9±0.4	3.9±1.1	NS
Haemoglobin (g/dL)	13.9±1.5	13.4±1.9	NS
Platelets (10^9/L)	215±77	181±80	0.0037
APRI	1.11±1.5	2.37±1.7	0.0012
FIB-4	2.23±1.9	4.72±6.4	<0.0001
Knodell score	7.49±3.5	10.01±3.17	<0.0001
Total inflammation	6.21±2.9	7.68±2.4	0.0007
Fibrosis score	1.32±1.25	2.36±1.49	<0.0001
Advanced fibrosis (%)	25	59	0.0001
Steatosis (>5%)	14	22	NS
PI use (% on atazanavir or indinavir)	10	48	<0.0001

ALT, alanine transaminase; AST, aspartate transaminase; NS, not significant (p>0.05); PI, protease inhibitor.

DISCUSSION

As long-term prognosis of HIV continues to improve, clinicians are tasked with identifying and treating non-HIV comorbidities. Of these various comorbidities, liver disease has become an increasingly frequent non-HIV cause of death, particularly in patients co-infected with HIV and HCV. Co-infected patients represent a significant portion of the HIV-infected population, with the rate of HIV–HCV co-infection in the USA and Europe estimated to be approximately 33%, varying widely depending on the presumed mode of HIV transmission.[15 16]

As such, prompt recognition, evaluation, and treatment of liver disease in this patient population is of great importance. While HIV–HCV co-infected patients are at risk for developing primary hepatic dysfunction, a number of HAART medications are commonly implicated in the development of abnormal liver function tests. The severity of these adverse effects is broad, with presentations ranging from benign, self-limited HB to severe drug-induced hepatotoxicity. The majority of hepatotoxicity was observed with first-generation PIs introduced in the mid-1990s, most significantly high-dose ritonavir (incidence of severe hepatotoxicity 5.3–9.5%), with recent iterations of PIs such as ATZ, IND, nelfinavir and ritonavir-boosted PIs being much less likely to cause significant elevation in liver enzymes.[7] Instead, these drugs are commonly implicated in the development of

HB via drug-induced inhibition of the bilirubin conjugation enzyme bilirubin UDP-glucuronosyltransferase. This results in impaired conjugation and excretion of bilirubin, creating a pattern of unconjugated HB similar to that found in Gilbert's syndrome.[17 18]

Despite the availability of a number of validated noninvasive indices such as FIB-4 and APRI to aid in the evaluation for liver inflammation and fibrosis, liver biopsy remains the gold standard for diagnosis.[19] Although relatively safe, the procedure is costly and not without risk. The ability to confidently separate benign drug effect from progression of liver disease would allow clinicians managing co-infected patients the freedom to observe these patients rather than progress through an expensive and potentially invasive workup. Additionally, as liver biopsy is supplanted by less-invasive methods of assessment of liver disease such as ultrasound elastography,[20–22] it becomes only more important that the question of whether PI-associated HB is a truly benign process be answered while histological data remains readily available.

In designing this study, we hypothesised that PI-associated HB in the HIV–HCV co-infected patient followed the same pattern of that in the HIV monoinfected patient, that is, that the development of HB represented a benign process unrelated to progression of liver inflammation or fibrosis. Although a number of small studies examining this exist,[23 24] no previous publications include liver histology data in their determination of the presence or absence of liver inflammation or fibrosis making our study unique. In addition, as liver biopsies are now infrequently performed in staging HCV, our data confirming the use of FIB-4 as a surrogate of fibrosis provides broader applicability of our results.

While our study provides a novel example of biopsy-proven pathology (or lack thereof), it is not without shortcomings. As this was a cross-sectional analysis, we were unable to obtain information or observe trends in bilirubin levels before/after the initiation of PI therapy. In addition, we were unable to obtain fractionated bilirubin for a number of our patients. Furthermore, while we choose the upper limit in our laboratory for total bilirubin, our findings using a higher cut-off found use of IND or ATZ associated with increased bilirubin independent of fibrosis. While it would have been ideal to demonstrate the typical, primarily unconjugated HB seen with PI use, it is important to note that our data reflect the laboratory information which is likely to be readily available to clinicians in a 'real' clinical setting as most providers tend to order comprehensive metabolic panels which typically do not fractionate bilirubin.

In conclusion, our data suggest that, in co-infected patients being treated with a PI typically associated with HB such as ATZ or IND, a strategy of observation as would be used in a HIV monoinfected patient is safe and effective. However, in patients who are not being treated with a PI or in those with other evidence of hepatic dysfunction (increased International Normalised Ratio, ascites, etc), a rise in serum bilirubin likely represents progression of liver disease and should prompt referral to a hepatologist for further evaluation.

Competing interests None declared.

Ethics approval VCU IRB.

Provenance and peer review Commissioned; externally peer reviewed.

Data sharing statement Raw data used in this study are available on request to the corresponding author via email.

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