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The insulin-like growth factor axis and risk of liver disease in hepatitis C virus/HIV-co-infected women

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Abstract

Objective—Insulin-like growth factor (IGF) I stimulates the proliferation of hepatic stellate cells (HSC), the primary source of extracellular matrix accumulation in liver fibrosis. In contrast, insulin-like growth factor binding protein (IGFBP) 3, the most abundant IGFBP in circulation, negatively modulates HSC mitogenesis. To investigate the role of the IGF axis in hepatitis C virus (HCV)-related liver disease among high-risk patients, we prospectively evaluated HCV-viremic/HIV-positive women.

Design—A cohort investigation.

Methods—Total IGF-I and IGFBP-3 were measured in baseline serum specimens obtained from 472 HCV-viremic/HIV-positive subjects enrolled in the Women's Inter-agency HIV Study, a large multi-institutional cohort. The aspartate aminotransferase to platelet ratio index (APRI), a marker of liver fibrosis, was assessed annually.

Results—Normal APRI levels (< 1.0) at baseline were detected in 374 of the 472 HCV-viremic/HIV-positive subjects tested, of whom 302 had complete liver function test data and were studied. IGF-I was positively associated [adjusted odds ratio comparing the highest and lowest quartiles ($AOR_{q4–q1}$), 5.83; 95% confidence interval (CI) 1.17–29.1; P_{trend} = 0.03], and IGFBP-3 was inversely associated ($AOR_{q4–q1}$, 0.13; 95% CI 0.02–0.76; P_{trend} = 0.04), with subsequent (incident) detection of an elevated APRI level(> 1.5), after adjustment for the CD4 T-cell count, alcohol consumption, and other risk factors.

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Conflicts of interest: None.

Conclusion—High IGF-I may be associated with increased risk and high IGFBP-3 with reduced risk of liver disease among HCV-viremic/HIV-positive women.

Keywords

aspartate aminotransferase to platelet ratio index; APRI; hepatitis C virus (HCV); HIV; IGFBP-3; IGF; liver disease

Introduction

Recent laboratory data suggest that the insulin-like growth factor (IGF) axis could affect the risk of liver fibrosis in hepatitis C virus (HCV)-viremic patients. IGF-I, a peptide hormone with mitogenic and antiapoptotic activity, stimulates the proliferation as well as collagen gene expression and accumulation of hepatic stellate cells (HSC) [1,2], the primary source of excess extracellular matrix accumulation in liver fibrosis. IGF-I may also promote the chemotaxis of HSC to areas of liver injury [3]. In contrast, insulin-like growth factor binding protein (IGFBP) 3, the most abundant IGFBP in circulation, negatively modulates HSC mitogenesis [4], in keeping with the sequestration by IGFBP-3 of IGF-I, and its direct (IGF-I-independent) antimitotic activity.

The liver is the primary source of IGF-I and IGFBP-3 in circulation, and in patients with chronic HCV infection there is upregulation of IGF-I receptor expression in HSC as well as hepatocytes [1,5]. A sustained virological response after HCV immunotherapy, in contrast, is associated with a decrease in IGF-I receptor messenger RNA levels [5]. It has also been observed that HCV-positive patients with hepatocellular carcinoma have higher IGF-I and lower IGFBP-3 levels in circulation than other HCV-positive patients [6]. Compared with uninfected patients, however, HCV-viremic patients have, on average, low IGF-I and also low IGFBP-3. Indeed, in patients with advanced liver disease, IGF-I and IGFBP-3 levels are considered a measure of residual liver capacity, and low values for both proteins are associated with mortality in individuals with cirrhosis [7].

These earlier data leave unanswered whether in individuals with early liver disease, those with high residual liver capacity, are circulating IGF-I and IGFBP-3 levels predictive of subsequent fibrosis? Therefore, we conducted a pilot investigation of enrollment serum levels of IGF-I and IGFBP-3 and their associations with the incident detection of an elevated aspartate aminotransferase (AST) to platelet ratio index (APRI; a marker of liver fibrosis), and persistent elevation of alanine aminotransferase (ALT; a marker of liver injury).

Subjects and methods

Study population and data collection

Women in this pilot investigation were subjects in an IGF substudy based in the Women's Interagency HIV Study (WIHS), a large prospective cohort of HIV-positive (N = 2058) and HIV-negative (N = 568) women. The WIHS methods have been reported in detail elsewhere [8]. Briefly, between October 1994 and November 1995 HIV-positive and negative women 13 years of age and older were enrolled in the WIHS from similar clinical and outreach sources in six US cities. On an ongoing semiannual basis, WIHS participants undergo an interview as well as a physical examination, during which a blood sample is collected. The WIHS protocol was approved by each local institutional review board, and all participants signed informed consent. As part of the substudy, we selected a random sample of 1450 HIV-positive women. Of those selected, 1422 had enrollment serum available. These women included 472 who were HCV viremic, of whom 374 (79%) had normal baseline APRI (< 1.0), and 302 had complete liver function test data for inclusion in our analysis of

APRI elevation. Similarly, 315 (67%) of the 472 HCV-viremic/HIV-positive women had normal enrollment ALT, of whom 256 had complete data for inclusion in our analysis of ALT elevation.

Laboratory testing

HCV serostatus was determined at baseline in all WIHS subjects using a commercial second or third-generation enzyme immunoassay. HCV viremia was determined for HCV-seropositive women using either the COBAS Amplicor Monitor 2.0, which has a linear range of 600–5×10^5 IU/ml, as previously described [9], or the COBAS Taqman assay, which has a linear range of 10–2.0×10^8 IU/ml (both from Roche Diagnostics, Branchburg, New Jersey, USA). Previous testing demonstrated a high correlation of HCV-RNA levels in the two assays, and a 10% resample of patients with undetectable HCV RNA in the Amplicor assay were also undetectable in the TaqMan assay (data not shown). Platelet count, serum levels of AST and ALT were determined annually (every other visit) in fresh specimens in Clinical Laboratory Improvement Amendment-certified laboratories. The upper limit of normal for AST and ALT were 47 and 53 IU/ml, respectively.

All serum specimens used to measure IGF-I and IGFBP-3 levels in this study were stored at –70°C until tested. Total IGF-I and IGFBP-3 levels were measured using commercially available enzyme linked immunosorbent assays from Diagnostic Systems Laboratories, Inc. (Webster, Texas, USA; DSL kit #10-2800 for total IGF-I and DSL kit #10-6600 for IGFBP-3), in accordance with the manufacturer's recommendations.

Outcome measures

The primary endpoint was the incident detection of APRI elevation. APRI is calculated as APRI = ([AST/upper limit of normal for AST]/platelet count [$\times 10^9$]) × 100. Although liver biopsy results were unavailable for most HCV-viremic women in the WIHS (a limitation common to large prospective HIV-positive cohorts), a growing body of published literature has confirmed that APRI elevation accurately distinguishes patients with and without significant liver fibrosis [10,11]; i.e. area under the curve of the receiver operator characteristic values typically above 80% [10,11], including in HIV-positive patients [11,12]. Consistent with these earlier data, an APRI value of less than 1.0 was defined as normal, and cases were women who had a normal APRI at baseline but subsequently developed an APRI greater than 1.5. The incident detection of persistent ALT elevation (> 1 year) was our second endpoint. ALT is a marker of acute and chronic hepatic injury. Although a single ALT measurement is not a sensitive or specific indicator of the presence of liver disease, HCV-viremic patients with persistently normal ALT have low rates of advanced fibrosis [13]. Our specific operational case definitions are reported in the Statistical methods section.

Statistical methods

Follow-up data were truncated at 2 years, because IGF-I and IGFBP-3 levels were measured only at baseline. That is, unlike the fairly stable levels observed in healthy adults, levels of IGF-I and IGFBP-3 in patients with liver disease may vary meaningfully over time given the role of the liver as the primary source of IGF-I and IGFBP-3 in circulation. Only women with complete data during the 2-year observation period were included so that all subjects had a similar opportunity to experience outcomes, and to minimize concerns regarding competing risks (i.e. death or loss to follow-up unrelated to liver disease). As above, our major endpoint, the incident detection of elevated APRI, was defined as APRI elevation at one year (visit 3) or 2 years (visit 5) of follow-up in a woman with normal APRI levels at baseline (visit 1). The incident detection of persistent ALT elevation was defined as ALT elevation that began at visit 3 or at visit 5 and persisted at least one year (visit 7 data were

used only to categorize ALT elevation at visit 5 as persistent/non-persistent). The comparison group was women with consistently normal ALT. Multivariate logistic regression was then used to estimate the associations of IGF-I and IGFBP-3 with the incident detection of APRI elevation or persistent ALT elevation. In these models, the levels of IGF-I and IGFBP-3 were expressed as quartiles, in keeping with what has commonly been done in epidemiological studies of IGF and cancer [14]; the quartile serocutoffs were defined in 150 random HIV-uninfected WIHS participants (see footnote to Table 1) [15]. All models included baseline IGF-I and IGFBP-3 levels, as well as baseline values for age, alcohol consumption, and the CD4 T-cell count (because of their perceived biological significance). Careful control for the CD4 T-cell count was considered particularly important, because of its associations with both IGF-I and HCV disease progression. Therefore, we conditioned on CD4 T-cell stratum (i.e. CD4 T cells < 200, 200–500, > 500 per ml) by stratification within each model, and then also included the absolute value of the CD4 T-cell count as a variable (to control for the exact CD4 T-cell level within each stratum); a weighted average was used to determine the effect estimate for each parameter. Additional covariates are shown in Table 1. Any factors that were statistically significant when added to the model or that meaningfully altered the odds ratios (OR) for IGF-I and IGFBP-3 were included in the final multivariate model.

Results

Table 1 summarizes the characteristics of the 302 HCV-viremic/HIV-positive women with normal APRI at baseline. A total of 29 of these women, or approximately 10%, had incident elevation of APRI. In univariate models, only heavy alcohol use [OR 3.55; 95% confidence interval (CI) 1.52–8.30] had a statistically significant association with APRI elevation (Table 2). In multivariable models that controlled for the CD4 T-cell count, age and alcohol consumption, the risk of incident detection of APRI elevation was positively associated with IGF-I [adjusted odds ratio comparing the highest and lowest quartiles ($AOR_{q4–q1}$) 5.83; 95% CI 1.17–29.1; $P_{trend} = 0.03$) and inversely associated with IGFBP-3 ($AOR_{q4–q1}$, 0.13; 95% CI 0.02–0.76; $P_{trend} = 0.04$; Table 2). These associations and their statistical significance were unaltered by the addition of other factors in our multivariable models, including the HIV-RNA level, clinical AIDS at baseline, body mass index, smoking, injection drug use, and the use of hepatotoxic medications.

Similar strong effects were observed for the associations of IGF-I and IGFBP-3 with incident detection of persistent ALT elevation. A total of 27 of the 256 women (11%) with normal ALT levels at baseline had incident development of persistent ALT elevation, and were compared with 192 women (75%) who had consistently normal ALT. Women with transient ALT elevation ($N = 37$) were not assessed. In multivariate logistic regression analysis that controlled (as above) for age, CD4 T-cell count, and alcohol consumption, the incident detection of persistent ALT elevation was significantly associated with high IGF-I ($AOR_{q4–q1}$ 8.29; 95% CI 1.50–45.9; $P_{trend} = 0.02$) and inversely, albeit non-significantly, associated with high IGFBP-3 ($AOR_{q4–q1}$ 0.47; 95% CI 0.09–1.79; $P_{trend} = 0.31$). The addition of other factors to this model did not alter the IGF-I and IGFBP-3 results (data not shown).

Discussion

In a pilot study of HCV-viremic/HIV-positive women, we observed a significant positive association between circulating levels of IGF-I and the incident detection of APRI elevation, a marker of liver fibrosis. Serum IGFBP-3, in contrast, was inversely associated with the risk of APRI elevation. These results were obtained in statistical models that controlled for several factors associated with liver disease in HCV-viremic/HIV-positive women,

including age, race, CD4 T-cell count, injection drug use, and alcohol consumption, as well as other risk factors. Similar results were also obtained using the incident detection of persistent ALT elevation as our endpoint. Collectively, these data suggest that IGF-I and IGFBP-3 are each independently associated with progressive liver disease in HCV-viremic/HIV-positive women.

To our knowledge, this pilot investigation is the first prospective study of the IGF axis and its associations with HCV-related liver disease in patients who did not already have evidence of advanced fibrosis at enrollment. The observed relationships are consistent with our a priori hypotheses, and their biological plausibility has been partly established by previous laboratory studies (as discussed in the Introduction). Most importantly, the findings, if correct, suggest that the IGF axis could be a target for the development of interventions to reduce the risk of liver disease in HCV-viremic/HIV-positive women, or could be useful as biomarkers of high risk for liver disease. It will be important, therefore, for future studies to obtain a more comprehensive understanding of the relationship between the IGF axis and HCV-related liver disease by examining additional components of the IGF axis, the use of repeated measures over time, studying both women and men, as well as studying HIV-negative in addition to HIV-positive populations.

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Table 1

Selected characteristics of hepatitis C virus-viremic/HIV-positive women with normal aspartate aminotransferase to platelet ratio index at baseline ($N = 302$).

Variable	N (%)[a]
Race/ethnicity	
African-American	184 (61)
Hispanic	63 (21)
Other	4 (1)
Caucasian	51 (17)
Age (years)	
< 30	17 (6)
30–35	74 (25)
36–45	175 (58)
> 45	36 (12)
Body mass index (kg/m^2)	
Underweight (< 18.5)	8 (3)
Normal (18.5–25.0)	130 (45)
Overweight (> 25.0–30.0)	85 (30)
Obese (> 30.0)	63 (22)
Injection drug use	
Never	42 (14)
Former	188 (62)
Current	72 (24)
Alcohol consumption	
Abstains	131 (45)
Light (<3 drinks/week)	63 (22)
Moderate (3–13 drinks/week)	54 (18)
Heavy (> 13 drinks/week)	45 (15)
Hepatotoxic medications[b]	
No	166 (55)
Yes	136 (45)
CD4 T-cell count (per ml)	
< 200	62 (21)
200–500	139 (47)
> 500	97 (33)
HIV-RNA level (copies/ml)	
< 4000	99 (33)
4001–20 000	59 (20)
20 001–100 000	67 (22)
> 100 000	77 (25)
Clinical AIDS at baseline	
No AIDS	186 (62)

Variable	*N* (%)[a]
AIDS	114 (38)
IGFBP-3 (‘quartile’)[c]	
Q1	103 (34)
Q2	84 (28)
Q3	66 (22)
Q3	49 (16)
IGF-I (‘quartile’)[c]	
Q1	111 (37)
Q2	80 (26)
Q3	71 (24)
Q4	40 (13)

[a]Percentages (%) do not always add up to 100% as a result of rounding. The total number of subjects does not always equal 302 because of the unavailability of the indicated data.

[b]Hepatotoxic drugs (per US treatment guidelines) [15] included: azithromycin, clarithromycin, delavirdine, efavirenz, fluconazole, isoniazid, itraconazole, ketoconazole, nevirapine, protease inhibitors, rifabutin, rifampin, voriconazole.

[c]Serocutoffs for insulin-like growth factor (IGF) I and insulin-like growth factor binding protein (IGFBP) 3 were defined using the quartile values obtained in a random subsample of HIV-uninfected women enrolled in the WIHS. For IGF-I these serocutoffs were: 173, 219, and 275 ng/ml. For IGFBP-3 these serocutoffs were: 2497, 3094, and 3660 ng/ml.

Table 2

Associations with incident detection of aspartate aminotransferase to platelet ratio elevation.

Variable	Univariate OR (95% CI)	Multivariate OR[a] (95% CI)
Race/ethnicity		
Caucasian	Reference	–
African-American	1.95 (0.56–6.85)	
Hispanic	1.38 (0.31–6.07)	
Other	NA	
Age (years)	1.01 (0.95–1.07)	1.02 (0.95–1.09)
Body mass index (kg/m^2)		
Underweight (< 18.5)	–	–
Normal (18.5–25.0)	Reference	
Overweight (> 25.0–30.0)	0.45 (0.16–1.27)	
Obese (> 30.0)	1.04 (0.42–2.57)	
Injection drug use		
Current	1.49 (0.28–8.06)	–
Former	2.77 (0.63–12.24)	
Never	Reference	
Alcohol consumption		
Light or abstains	Reference	Reference
Moderate or heavy	3.55 (1.52–8.30) **	3.38 (1.36–8.44) **
Liver toxic medications[b]		
No	0.74 (0.34–1.60)	–
Yes	Reference	
CD4 T-cell count (per 100 cells/ml)	1.11 (0.99–1.22)	0.82 (0.61–1.11)
HIV-RNA level (copies/ml)		
< 4000	Reference	-
4001–20 000	2.07 (0.80–5.34)	
20 001–100 000	0.92 (0.29–2.94)	
> 100 000	1.05 (0.33–3.38)	
Clinical AIDS at baseline		
No AIDS	Reference	
AIDS	0.94 (0.43–2.06)	–
IGFBP-3 (‘quartile’)[b]		P_{trend} = 0.04
Q1	Reference	Reference
Q2	0.34 (0.12–0.98) *	0.23 (0.07–0.80) *
Q3	0.44 (0.15–1.26)	0.26 (0.07–1.04)
Q4	0.48 (0.15–1.53)	0.13 (0.02–0.76) *
IGF-I (‘quartile’)[b]		P_{trend} = 0.03
Q1	Reference	Reference

Variable	Univariate OR (95% CI)	Multivariate OR[a] (95% CI)
Q2	0.73 (0.26–2.06)	0.97 (0.31–2.99)
Q3	0.99 (0.37–2.70)	1.32 (0.35–4.92)
Q4	1.64 (0.55–4.67)	5.83 (1.17–29.1)*

CI, Confidence interval; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; OR, odds ratio.

***$P < 0.001$

NA, not analysed.

*$P < 0.05$

**$P < 0.01$

[a]Multivariate analyses conditioned on CD4 T-cell stratum (i.e. CD4 T cells < 200, 200–500, > 500 per ml) by stratification within each model, and also included the absolute value of CD4 T-cell count as a variable (to control for the exact CD4 T-cell level within each stratum); a weighted average was used to determine the effect estimate for each parameter.

[b]See details in Table 1.