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Hepatic Steatosis and Histologic Findings in HIV/HCV Co-infected Patients

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ABSTRACT

Background: Hepatic steatosis (fatty liver) is associated with liver fibrosis progression in HIV-uninfected patients (pts) with chronic HCV infection. The prevalence, severity and effects of steatosis are poorly understood in HIV/HCV co-infected pts. We hypothesized that steatosis would be associated with more extensive liver fibrosis, host factors such as diabetes and obesity, and antiretroviral therapy (ART).

Methods: Hepatic sections were assessed by a single pathologist for steatosis/steatohepatitis and hepatitis grade (0-4) and stage (0-4) in 48 HIV/HCV co-infected, HBV sAg negative pts. We retrospectively reviewed pt records to elucidate risk factors for steatosis.

Results: Of 48 pts analyzed, 38% were female, 44% Black, 29% Hispanic and 27% White. Median age was 43 yrs, median duration since HIV diagnosis was 9 yrs, and median CD4 was 418 cells/ul at time of biopsy. 46% had an AIDS diagnosis and 85% had history of ART. 65% were on ART at biopsy, including 35% on protease inhibitors (PIs) and 29% on d4T. Of pts with available HCV genotypes, 41 were 1a or b, 2 were 2a, and 1 was 3a (the type associated with steatosis in HIV-negative pts). Of 45 pts with available HCV viral loads, 23 were >1,000,000 copies/ml. Median BMI was 25.9 and similar in men and women; 17% of pts were diabetic. Steatosis was present on 56% of biopsies, with grade 2-3 (of 3) in only 4%. Steatosis was associated with degree of perisinusoidal fibrosis (p=.029) and bridging fibrosis (p=.029). Overall fibrosis stage was generally more advanced in the steatosis group with a median stage of 2 compared to 1 in the group without steatosis (p=.063). Inflammation was not associated with steatosis, though there was a higher median grade in pts with steatosis (2 vs.1-2, p=.087). Steatosis was not associated with diabetes (odds ratio [OR] 1.36, 95% confidence interval [CI] 0.28-6.49, p=0.70), alcohol use (OR 2.0, CI 0.53-7.60 p=0.31), BMI>25 (OR 2.05, CI 0.62-6.75, p=0.24), antiretroviral use (OR=0.18 CI 0.02-1.59, p=0.12), duration of exposure to d4T (OR 1.01 per month, CI 95% 0.97-1.04, p=0.75) or PIs (OR 1.00, CI 95% 0.98-1.02, p=0.99).

Conclusions: In HIV/HCV co-infected pts, hepatic steatosis is common and is associated with more advanced histological findings, including perisinusoidal and bridging fibrosis. Duration of particular antiretroviral therapies was not associated with steatosis in this small study.

BACKGROUND

- Studies have shown that patients with HIV and HCV co-infection have an accelerated course of progression to cirrhosis and end-stage liver disease.
- The presence of high-grade steatosis on liver biopsy specimens is associated with liver fibrosis progression in HIV-negative patients with HCV infection. This correlation is independent of other causes of fatty liver such as alcohol or diabetes, although alcohol and steatosis appear to act together to increase fibrosis.
- Risk factors elucidated for steatosis in HIV-negative patients include an elevated body mass index, visceral obesity, HCV genotype and possibly lipid abnormalities.
- It is not known whether particular antiretroviral agents are associated with the development of steatosis in patients with HIV/HCV co-infection.
- The associations between several metabolic complications and use of specific classes of antiretrovirals in HIV-infected patients provide biologic plausibility to support the hypothesis that use of antiretrovirals may be associated with steatosis in co-infected patients.
  - Certain HIV protease inhibitors (PIs) appear to cause insulin resistance and hyperlipidemia.
  - Lactic acidosis with hepatic steatosis is a rare complication of therapy with nucleoside reverse transcriptase inhibitors likely as a result of mitochondrial toxicity
  - Visceral obesity, which has been associated epidemiologically with use of protease inhibitors, may be a component of the lipodystrophy syndrome seen in patients with HIV infection.

Objectives:
- To determine if the presence of hepatic steatosis is associated with more advanced histologic findings on liver biopsies from patients with HIV/HCV co-infection.
- To identify potential risk factors for steatosis in patients with HIV/HCV co-infection.

METHODS

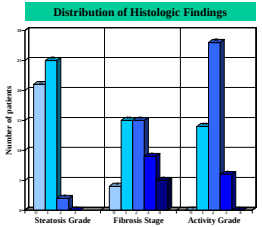
- This study included HBV sAg-negative, HIV/HCV co-infected patients who underwent liver biopsies during a four year period (1999-2002) with available medical records and liver pathology specimens. A pathologist blinded to clinical characteristics assessed the hepatic sections for the macrosteatosis grade, the hepatitis inflammatory grade and fibrosis stage, and the steatohepatitis necroinflammatory grade and fibrosis stage. A retrospective chart review was performed to elucidate potential risk factors for steatosis.
- Histologic Scoring: The steatosis and steatohepatitis scoring system described by Brunt *et al* (A J Gastroenterology 1994) and the hepatitis scoring system described by Scheuer (Hepatology 1991) was used.
  - Steatosis was graded based on the percent of hepatocytes with macrovesicular steatosis: Grade 0, no steatosis; Grade 1, 1-33%; Grade 2, 34-66%; Grade 3, >66%
  - Other histologic features related to steatohepatitis such as hepatocellular ballooning degeneration, intraacinar (lobular) inflammation, portal tract inflammation, perisinusoidal fibrosis, portal fibrosis, and bridging fibrosis were also scored 0-3.
  - Hepatitis scoring included: inflammatory grade: 0-4 and fibrosis stage: 0-4.
- Of the 88 patients identified, forty patients were excluded for the following reasons: fine needle aspirate only (3), positive HBV surface antigen (2), negative HCV viral RNA (3), interferon therapy predating biopsy (2), incomplete medical record or unavailable liver biopsy (30).
- Statistical Analysis: The hepatitis inflammatory grade and fibrosis stage as well as other histologic findings were compared in patients with and without steatosis using the Mann-Whitney test. An exploratory analysis was performed to identify clinical characteristics associated with steatosis using univariate and multivariate logistic regression. Odds ratios with 95% confidence intervals were calculated.
- Sample size: Based on a sample size of 100, a steatosis prevalence of 40%, and an alpha level of 0.05, we estimated having 80% power to detect an odds ratio of 3.3 for the association between a covariate and the presence of steatosis if the prevalence of the covariate was 25% in control patients without steatosis.

RESULTS

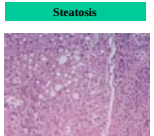
Patient Characteristics

n=48	
Male sex	30 (63%)
Race/Ethnicity	
Black	21 (44%)
Hispanic	14 (29%)
White	13 (27%)
Median age, yrs (range)	43 (32-56)
Normal ALT	13 (27%)
HCV RNA, copies/ml	
≤1,000,000	13 (27%)
>1,000,000	32 (67%)
Unavailable	3 (6%)
HCV Genotype	
1A	35 (73%)
1B	5 (11%)
Mixed 1A & 1B	1 (2%)
2a	2 (4%)
3a	1 (2%)
Unavailable	4 (8%)
HIV RNA, copies/ml	
≤ 400	28 (58%)
>400	20 (42%)
CD4 cell count, cells/mm^3	
Median (range)	418 (5-1847)
<200	6 (13%)
Diabetes	8 (17%)
Alcoholism	8 (17%)
Median BMI, kg/m^2 (range)	25.9 (19.9-36.0)
Antiretroviral therapy history	
History of any	41 (85%)
History of PI	36 (75%)
History of d4T	29 (61%)
ART at time of biopsy	
Any	31 (65%)
PI	17 (35%)
d4T	14 (29%)

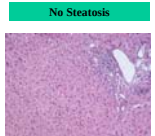
Distribution of Histologic Findings



Steatosis

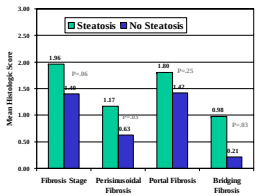


No Steatosis

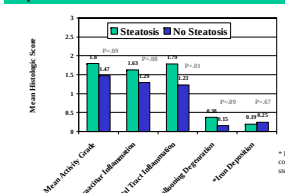


Hematoxylin and eosin-stained liver biopsy specimens from two patients included in this study. Photograph on left demonstrates grade 2 macrosteatosis and stage 4 fibrosis. Photograph on right demonstrates grade 0 macrosteatosis and grade 0 fibrosis. (Original pictures 20x (left), 10x (right))

Hepatic Fibrosis and the Presence/Absence of Steatosis



Hepatic Inflammation and the Presence/Absence of Steatosis



* Iron deposition was used as a control. No association with steatosis was expected.

Antiretroviral Therapy (ART) &the Presence/Absence of Steatosis

	Steatosis (pts, n=27)	No Steatosis (pts, n=21)	Odds Ratio	95% CI	P value
ART ever	21 (78%)	20 (95%)	0.18	0.02-1.59	0.12
ART at biopsy	16 (59%)	15 (71%)	0.58	0.17-1.97	0.38
d4T use ever	16 (59%)	13 (62%)	0.90	0.28-2.88	0.85
PI use ever	20 (74%)	16 (80%)	0.89	0.24-3.35	0.87
d4T at biopsy	8 (30%)	6 (30%)	1.05	0.30-3.70	0.94
PI at biopsy	9 (33%)	8 (40%)	0.81	0.25-2.67	0.73

Duration of Antiretroviral Therapy & the Presence/Absence of Steatosis

	Steatosis Mean (+/- SE)	No Steatosis Mean (+/- SE)	Odds Ratio (per 6 mos)	95% CI	P value
d4T, mos	14.5 (+/- 3.6)	12.9 (+/- 3.7)	1.03	0.84-1.27	0.75
PI, mos	26.4 (+/- 4.7)	26.3 (+/- 6.1)	1.00	0.87-1.15	0.99
NNRTI, mos	5.5 (+/- 2.0)	10.4 (+/- 2.3)	0.75	0.53-1.07	0.11
NRTI, mos	58.3 (+/- 9.2)	63.3 (+/- 8.7)	0.98	0.90-1.06	0.69

Clinical Characteristics & the Presence/Absence of Steatosis

	Steatosis (n=27)*	No Steatosis (n=21)*	Odds Ratio	95% CI	P value
Age, yr (Mean +/- SE)	45 (+/- 1)	43 (+/- 1)	1.42 (per 5 yr)	0.85-2.35	0.18
Sex (Male)	19/27 (70%)	11/21 (52%)	2.16	0.66-7.10	0.21
BMI, kg/m^2 (Mean +/- SE)	26.8 +/- 0.7	25.2 +/- 0.7	1.15 (per kg/m^2)	0.96-1.38	0.12
Alcoholism	5/25 (20%)	3/20 (15%)	1.42	0.29-6.81	0.67
Diabetes	5/27 (19%)	3/21 (14%)	1.36	0.29-6.50	0.70
Glucose, mg/dL (Mean +/- SE)	122 +/- 13	199 +/- 9	1.28 (per 30 mg/dL)	0.88-1.86	0.21
Total Cholesterol, mg/dL (Mean +/-SE) LDL cholesterol, mg/dL (Mean +/-SE) Triglycerides, mg/dL (Mean +/-SE)	160 +/- 7 83 +/- 6 215 +/- 24	173 +/- 9 95 +/- 6 199 +/- 23	0.75 (per 30 mg/dL) 0.63 (per 30 mg/dL) 1.17 (per 100 mg/dL)	0.46-1.24 0.30-1.30 0.64-2.14	0.27 0.21 0.61
Lipodystrophy	7/27 (26%)	3/21 (14%)	2.10	0.47-9.36	0.33
Years since HIV Diagnosis (Mean +/- SE)	9.9 +/- 0.9	9.2 +/- 0.8	1.22 (per 5 years)	0.60-2.5	0.59
History of AIDS diagnosis	9/26 (35%)	13/21 (62%)	0.31	0.09-1.01	0.052
Nadir CD4 count, cells/mm^3 (Mean +/- SE)	313 +/- 40	218 +/- 40	1.30 (per 100 cells)	0.94-1.81	0.11
CD4 count, cells/mm^3 (Mean +/- SE)	532 +/- 53	409 +/- 55	1.21 (per 100 cells)	0.95-1.54	0.12
Log_{10} HIV RNA at biopsy, copies/mL (Mean +/- SE)	3.02 +/-0.21	2.99 +/- 0.14	1.04	0.55-1.96	0.91
HCV RNA >1,000,000 copies/mL	19/26 (73%)	13/19 (68%)	1.25	0.34-4.59	0.73

* Some denominators are less than 27 for 'steatosis' and 21 for 'no steatosis' because of missing information..

Multivariate Analysis of Factors Associated with Steatosis*

	Odds Ratio	95% CI	P value
Age (per year)	**1.29**	**1.03-1.63**	**0.03**
BMI (per kg/m^2)	**1.54**	**1.10-2.16**	**0.01**
Glucose (per 30 mg/dL)	1.82	0.94-3.55	0.08
Total Cholesterol (per 30 mg/dL)	0.64	0.28-1.45	0.28
History of AIDS Diagnosis	**0.08**	**0.007-0.86**	**0.04**
CD4 Count (per 100 cells/mm^3)	1.15	0.85-1.55	0.38
Duration of PI Use (per 6 mos)	1.15	.93-1.42	0.21
Duration of NNRTI use (per 6 mos)	0.68	0.40-1.16	0.16

* Backward-selected logistic regression was performed. The following covariates with P<0.40 were removed from the model: sex (P=0.66), lipodystrophy (P=0.91), history of antiretroviral therapy (P=0.79), and duration of stavudine use (P=0.47)

LIMITATIONS

- Retrospective design:
  - Limited documentation of quantity and duration of alcohol use.
  - Limited documentation of antiretroviral history for patients with treatment prior to initiating care at our HIV clinic.
- Sample size: This is a preliminary report of an ongoing study. Statistical power was limited by the sample size.
- Disease severity: Steatosis grades in this study were mild (only 2/48 patients with grade>1) compared to most studies of steatosis in HCV mono-infected patients. This may have limited our ability to ascertain risk factors for steatosis.
- Population: This study sample reflects a healthier subset of the general population of HIV/HCV co-infected patients since the major indication for liver biopsy was evaluation for possible HCV therapy.

CONCLUSIONS

- Steatosis, or fatty liver, was common in patients with HIV/HCV co-infection in this study, although it was generally mild (Grade 1 of 3).
- The presence of steatosis on liver biopsy was associated with more advanced stages of perisinusoidal fibrosis and bridging fibrosis. The overall fibrosis stage was more advanced when steatosis was present, although this difference was not statistically significant (P=0.06).
- The presence of steatosis on liver biopsy was associated with more advanced grades of portal inflammation. The overall activity grade was more advanced when steatosis was present, although this difference was not statistically significant (P=0.09).
- Increasing age, increasing BMI, and the absence of a history of AIDS diagnosis were associated with steatosis on multivariate analysis.
- History of antiretroviral therapy and duration of particular antiretroviral therapies were not associated with steatosis in this small study.

ACKNOWLEDGEMENTS

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