

Hepatic Steatosis is Associated with Dideoxynucleoside Analogues and HCV Genotype 3 in HIV/HCV Co-Infected Patients



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Background:

In patients with HCV alone, the presence of hepatic steatosis is associated with increased fibrosis progression rates. Host factors, such as increased body mass index (BMI), hypertriglyceridemia, and insulin resistance, and viral factors such as HCV genotype 3 infection, are associated with an increased risk of steatosis [1-4].

There is great rationale to also study the role of steatosis in HIV/HCV co-infected patients since fibrosis progression rates are faster in this patient population compared to patients with HCV alone [5]. Patients who develop lipodystrophy have the same host risk factors that may predispose them to developing hepatic steatosis - namely increased BMI, hypertriglyceridemia, and insulin resistance.

Mitochondria play a major role in fat oxidation and energy production but also leak reactive oxygen species (ROS). The basal formation of ROS leads to oxidation of hepatic fat deposits. This contributes to lipid peroxidation, which alters mitochondrial (mt)DNA and impairs the transfer of electrons along the respiratory chain, further increasing the formation of ROS. Steatosis is the substrate for this vicious cycle and the presence of steatosis itself has been referred to as "the first hit" in the development of steatohepatitis. Secondary steatosis can be caused by alcohol or specific medications [6].

Little is known about the prevalence of steatosis in HIV/HCV co-infected patients or the additional factors that may play a role in the development of fatty liver, such as antiretroviral medications [7-9]. Certain nucleoside analogues (NA), such as didanosine (ddI) and stavudine (d4T) are associated with mitochondrial toxicity, lactic acidosis, and hepatic steatosis. Whether chronic administration of ddI or d4T is associated with an increased risk of hepatic steatosis and fibrosis progression in HIV/HCV co-infected patients is unknown.

In one small study of 106 HIV/HCV co-infected patients, an association between steatosis and fibrosis was found; in another study of 113 subjects a low prevalence of steatosis was demonstrated in a predominantly African-American cohort [7,8].

Objectives:

- To evaluate the role of hepatic steatosis in fibrosis progression in a longitudinal cohort of HIV/HCV co-infected patients attending four clinics in New England with high rates of injection drug users (IDU).

Specific Aims:

- To determine if the severity of hepatic steatosis is associated with higher BMI, hypertriglyceridemia, hypercholesterolemia or genotype 3 infection
- To determine if antiretroviral medications are associated with higher rates of steatosis and necro-inflammatory changes
- To determine if hepatic steatosis is associated with higher rates of fibrosis progression in HIV-infected patients with HCV

Retrospective chart reviews of 179 subjects with liver biopsies were conducted in four teaching hospitals in New England serving community and incarcerated patients. Data on demographics, medications, and laboratories was abstracted from medical records; all biopsies were read by one pathologist who was blinded to the clinical information. Steatosis was graded as absent, minimal, mild, moderate or severe. Data were analyzed using SPSS version 11.5.

Results:

179 patients with HIV/HCV co-infection who had undergone liver biopsy for evaluation of chronic hepatitis C from 1998-2000 were analyzed. Fifty-eight percent were taking HAART; 69% were taking nucleoside analogues (NA). The distribution of HCV genotypes were 1 (68%); 2(9%); 3(19%); 4 (5%).

The demographic data, as shown below, are stratified by presence or absence of steatosis:

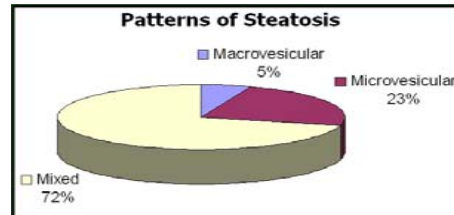
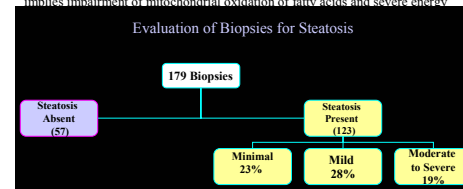
Feature	Steatosis Present	Steatosis Absent	P value
Male	104(83%)	41 (72%)	0.10
Female	22 (17%)	16 (28%)	
Age	43(39-47)	43(37-47)	0.56
Race:			0.61
-Caucasian	60 (48%)	31 (54%)	
-Hispanic	34 (27%)	15 (26%)	
-A.A.	32 (25%)	11 (20%)	
BMI (kg/m ²)	26.3	25.2	0.067
Heavy ETOH	80 (64%)	33 (58%)	0.47
Diabetes	17 (14%)	4 (7%)	0.22
HCV RNA	850, 000	874, 000	0.45
Genotype 3	24 (22%)	5 (10%)	0.083
Genotypes 1,2,4	87 (78%)	45 (90%)	

Evaluation of biopsies for steatosis

The patterns of steatosis were analyzed, since different types of steatosis have different implications for severity of disease.

- In macrovesicular steatosis, hepatocytes contain a single large vacuole of fat (triglyceride) which fills up the cell and displaces the nucleus to the periphery.

- In microvesicular steatosis, hepatocytes are filled up with numerous small lipid vesicles which leave the nucleus in the center of the cell. Its presence implies impairment of mitochondrial oxidation of fatty acids and severe energy



Factors Not Associated with Steatosis

- Age
- Race
- Ethanol use
- Duration of HIV
- CD4 cell count
- HIV RNA
- HCV RNA
- Protease inhibitors
- Cholesterol
- ALT
- AST
- INR
- Alkaline phosphatase
- Total bilirubin.

Analysis of Factors Associated with Steatosis:

In a univariate analysis, we found borderline significance for the association between nucleoside analogue use and steatosis.

Multivariate analysis indicated borderline association with HCV genotype 3 and non-D nucleoside analogues, and significant association with D-nucleoside analogues.

Univariate Analysis: Nucleoside Analogue Use and Steatosis

Types of NAs	Steatosis Present	Steatosis Absent	p value
No NA	32 (25%)	24 (42%)	0.054
Non D	45 (36%)	19 (33)	
D-nuc	49 (39%)	14 (25%)	

Multivariate Analysis of Factors Associated with Steatosis

Characteristic	Multivariate OR (95% CI)	Multivariate p value
HCV Genotype 3	3.38 (0.86-13.2)	0.08
1,2 and 4	1.00	
Types of NAs		0.02
None	1.00	
Non D	2.65 (0.95-7.41)	(0.062)
D-nucleoside	4.63 (1.55-13.8)	(0.006)

Histology score and steatosis

Steatosis correlated with higher fibrosis scores as interpreted by a pathologist who was blinded to the medication history of the patient

Histology	Steatosis Present	Steatosis Absent	P Value
Knodell:			
-Total HAI	6 (4-9)	6 (3-8)	0.077
-Grade	5 (3-7)	4 (2-6)	0.23
-Stage	3 (1-3)	1 (1-3)	0.029

Conclusions:

- Steatosis was associated with HCV genotype 3 infection and higher fibrosis scores.
- A trend towards significance was seen for hypertriglyceridemia and hyperglycemia and the presence of steatosis
- Hepatic steatosis was prevalent in this racially diverse HIV/HCV co-infected population.

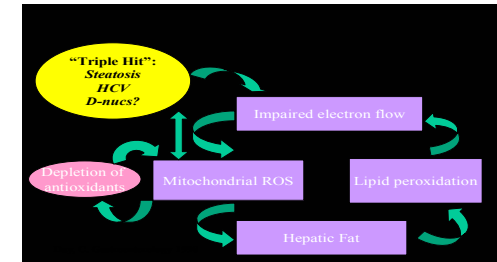
Discussion:

The mere presence of oxidizable fat within the liver is enough to trigger lipid peroxidation. However, many patients with steatosis never develop hepatic necroinflammation or fibrosis. This suggests that in addition to steatosis ("the first hit"), progression to steatohepatitis requires the presence of some other factor ("a second hit"). That second hit can be alcohol, drugs, or anything which will trigger an increase of ROS.

Over the past two years it is also becoming clear that you can acquire hepatic steatosis through two pathways. Host-related factors, including visceral adiposity, hyperlipidemia and insulin resistance, have been found to correlate with increased steatosis in patients with HCV alone. Viral-related factors include HCV genotype 3 infection. Most importantly, steatosis is associated with higher rates of fibrosis progression in patients with chronic hepatitis C.

In our study, steatosis was associated with the use of dideoxynucleoside analogues, particularly ddI and d4T. Through their deleterious effects on mitochondria and oxidative phosphorylation, the use of NAs may increase hepatic steatosis. Of great concern, the majority of our patients had evidence of microvesicular steatosis, which implies significant mitochondrial energy crisis.

In summary, steatosis has been shown to correlate with higher fibrosis progression rates in patients with HCV alone. Steatosis may be a contributing factor in fibrosis progression in HIV/HCV coinfected patients, as well. These patients may be at increased risk for steatosis due to host factors (visceral adiposity, hypertriglyceridemia, hypercholesterolemia, and insulin resistance secondary to the lipodystrophy syndrome) and due to specific antiretroviral medications (dideoxynucleoside analogues). Hepatic steatosis may be even more significant in those patients who are infected with HCV genotype 3.



Limitations:

- Estimates of fibrosis progression rates were limited to Lemuel Shattuck Hospital where data on age of onset of IDU were obtained for all 75 subjects
- Since this was a retrospective study, data on use of antiretroviral medications is limited to the time of the liver biopsy.
- BMI data were only available on 82 patients

References

- [1] Hourigan, L. et al, *Hepatology* 1999;29:1215-1219
- [2] Adinolfi, L. et al, *Hepatology* 2001;33:1358-1364
- [3] Castera, L. et al, *Gut* 2003;52:288-292
- [4] Kumar, D. et al, *Hepatology* 2002;36:1266-1272
- [5] Benhamou Y. et al, *Hepatology*: 1999;30:1054.
- [6] Day, C. *Gastroenterology* 1998;114:842-5
- [7] Marks, K. et al, AASLD 2004, Abstract #675
- [8] Sulkowski, M. et al, CROI 2004, Abstract #72
- [9] Agarwal, K. et al, AASLD 2004, Abstract #978