

Liver histology and hepatic mitochondrial function in HIV-1-infected patients on antiretroviral therapy with chronic transaminase elevation

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ABSTRACT

Background: Few data exist on liver histology and mitochondrial function in HIV-infected patients with chronic ALT/AST elevation of unknown origin.

Methods: We investigated prospectively mitochondrial function and mitochondrial DNA (mtDNA) content in liver biopsies from HIV-infected pts with ALT elevation>ULN for more than 6 months and treated with cART. Pts with chronic alcohol abuse, HCV or HBV-co-infection, autoimmune or genetic liver diseases were excluded. In a subgroup of sixteen pts with available liver biopsies, mitochondrial respiratory chain (complexes I, II, and IV) and citrate synthase activities were evaluated in liver homogenates using spectrophotometric assays while the mtDNA was quantified by real-time PCR.

Results: Thirty pts were included with a median age of 46 yrs [31-67], a median BMI of 23 kg/m² [22-24], a viral load<200 copies/mL [202-94500] in 19 (63%) pts and a median [IQR] of 565 [421-611] copies/mL of HIV RNA at the time of liver biopsy. The median duration of HIV infection was 13 yrs [8-15]. The median exposure time to cART was: 118 months [83-133] for NRTIs, 41 months [22-46] for NNRTIs and 53 months [36-90] for PIIs. The median time to cART was: 80 UI [66-135]. Histologic abnormalities were found in 20/30 (67%) pts. Steatosis was present in 10/30(33%) pts (mild [0%-30%] in 9 pts and severe >30% in 1 patient) and was associated with concomitant lobular inflammation and/or hepatocellular ballooning in 16/18 pts consistent with the diagnosis of NASH according to the NAS-score. Liver fibrosis was found in 16/30 pts: 13 pts as F1 and 5 pts as F2, including 3 pts with cirrhosis (Metavir-score). Compared to pts without NASH, pts with NASH had significantly higher fasting glucose levels (median 5.2 [5.0-5.5]mmol/L NASH vs 4.9 [4.4-5.3]mmol/L, p<0.01) and fasting insulin levels (median 19.4 [14.7-32.8]µU/L vs 10.9 [8.7-14.4]µU/L, p<0.007). In the subgroup of sixteen patients, 9 were classified as NASH. Between patients with and without NASH respectively, no significant difference in mitochondrial function (complex IV activity: 94/43[156] vs 76/26-52[9]nanomoles/min/mg of protein) and liver mtDNA amount (median 6920/3684-19893 vs 5584/4113-2742/copies/cells) was observed.

Conclusions: HIV infected pts on cART with chronic ALT elevation have a high rate of liver lesions with predominant histologic patterns of NASH related to insulin resistance. Despite long exposure to cART, in our population, the liver abnormalities may not account to mitochondrial dysfunction.

OBJECTIVE

The objectives of this study were to describe liver lesions of HIV-infected patients with unexplained chronic ALT/AST elevation and to identify factors potentially linked to these abnormalities. In addition, the role of a possible mitochondrial dysfunction was evaluated.

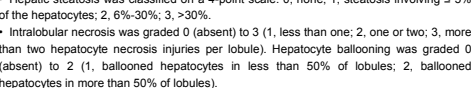
PATIENTS AND METHODS

Patients with ALT/AST elevation of more than the upper limit of normal (ULN) at least twice in the past 6 months were selected on the basis of the following criteria : documented HIV infection, no alcohol abuse (≤ 20g alcohol daily, no history of chronic alcohol consumption), no history of hereditary and autoimmune liver disease, no evidence of hemochromatosis (normal ferritinemia and saturation coefficient), negative HCV RNA, and negative serum HBV DNA. Patients with any contra-indication for liver biopsy were excluded. All selected patients provided an informed consent form.

Data on demographics, drug or alcohol use and history of liver diseases were obtained at first visit. History of HIV infection was obtained by chart review information. A month-by-month documentation of the prescribed drug combination allowed a calculation of each patient's individual cumulative drug exposure. Blood samples were taken from all selected patients for laboratory parameters before liver biopsy to exclude viral, hereditary or autoimmune liver disease.

LIVER BIOPSY

- Slides were evaluated by a single pathologist (F. Charlotte), deemed adequate based on specimen size and number of portal tracts and scored according to the Metavir scoring system: no fibrosis, F0; fibrosis without septae, F1; fibrosis with some septae, F2; fibrosis with numerous septae, F3; cirrhosis, F4.
- Hepatic steatosis was classified on a 4-point scale: 0, none; 1, steatosis involving ≤ 5% of the hepatocytes; 2, 6%-30%; 3, >30%.
- Intra-lobular necrosis was graded 0 (absent) to 3 (1, less than one; 2, one or two; 3, more than two hepatocyte necrosis injuries per lobule). Hepatocyte ballooning was graded 0 (absent) to 2 (1, ballooned hepatocytes in less than 50% of lobules; 2, ballooned hepatocytes in more than 50% of lobules).



NASH was defined by the presence of steatosis > 10% associated with lobular necrosis (grade 1 or more) and/or hepatocyte ballooning (grade 1 or more), with or without fibrosis (grade 1 or more).

STUDY POPULATION

Between July 2003 and March 2007, 33 HIV-positive patients were eligible to undergo liver biopsy.

- Three patients were excluded from further observation :
- In one case, liver biopsy showed zones of centrilobular hepatocytic atrophy with regenerative hepatocytic activity consistent with regenerative nodular hyperplasia.
 - A second patient retrospectively admitted chronic alcohol consumption.
 - The third patient had a history of several cholestatic infections and was found by chart review to have HIV-associated cholangiopathy.

Table 1 - Demographic and clinical characteristics of the 30 included patients

Age, median years [range]	46 [31-67]
Male [No. (%)]	29 (97)
Mean BMI [kg/m ² [range]]	23 [17-29]
Median duration HIV [years [range]]	13 [3-21]
Median CD4-count [x 10 ⁶ /L (range)]	402 [156-921]
Median HIV RNA [copies/mL (range)]	200 [200-94600]
HIV RNA <200 copies/mL [No. (%)]	21 (70%)
Median ALT [U/L [range]]	80 [32-232]
Median AST [U/L [range]]	61 [28-302]
Median GGT [U/L [range]]	111 [22-617]
Median fasting cholesterol [mmol/L [range]]	4.6 [2.0-13.3]
Median fasting triglycerides [mmol/L [range]]	2.4 [0.5-11.7]
Median fasting glycemia [mmol/l [range]]	5.1 [4.7-7.7]
Median HbA1c [mmol/l [range]]	5.4 [1.8-8.8]
Median fasting insulin [µU/L [range]]	14.8 [2.7-140]
Age, median years [range]	46 [31-67]
Exposure to :	
NRTI [No. (%)]	30 (100%)
NNRTI [No. (%)]	30 (100%)
NNRTI [No. (%)]	20 (67%)
IP [No. (%)]	25 (84%)

Figure 1 - Duration of cART

Table 2 - Histologic abnormalities in liver biopsies

Fibrosis	n	%	Steatosis	n	%
F0	12	40	< 5%	12	40
F1	12	40	5-30%	9	30
F2	2	6.7	>30%	9	30
F3	1	3.3	Macro	12	66.6
F4	3	10	Mixed	6	33.3
F1-4	18	60			
Activity			Steatosis and inflammation		
A0	14	46.7	Steatosis and inflammation (NASH)	16	53.3
A1-3	16	53.3	Steatosis without inflammation	2	6.7
			Inflammation without steatosis	8	26.6
			No steatosis/no inflammation	4	13.3

Table 3 - Factors associated with NASH lesions

	No NASH (n=14)	NASH (n=16)	P*
Age, median years [range]	46 [41-49]	49 [43-53]	
Median BMI [kg/m ² [range]]	22 [18-29]	24 [17-28]	
Median duration HIV [years [range]]	15 [8-18]	13 [3-19]	
Median CD4-count [x 10 ⁶ /L [range]]	314 [156-921]	477 [207-745]	
Median HIV RNA [copies/mL [range]]	200 [40-94600]	200 [40-33300]	
Median ALT [U/L [range]]	80 [32-232]	81 [43-221]	
Median AST [U/L [range]]	62 [28-115]	60 [29-302]	
Median GGT [U/L [range]]	177 [22-617]	80 [41-472]	
Median fasting cholesterol [mmol/l [range]]	4.4 [2.2-9.3]	4.7 [2.2-10.3]	
Median fasting triglycerides [mmol/l [range]]	1.6 [0.5-3.3]	2.7 [0.8-11.7]	
Median fasting glycemia [mmol/l [range]]	4.8 [4.5-7.7]	5.2 [4.6-7.7]	p=0.02
Median fasting insulin [mU/L [range]]	11 [2.3-41]	19 [6.3-140]	p=0.008
Median HOMA [range]	2 [0-9]	4 [2-35]	p=0.008

*Mann-Whitney test

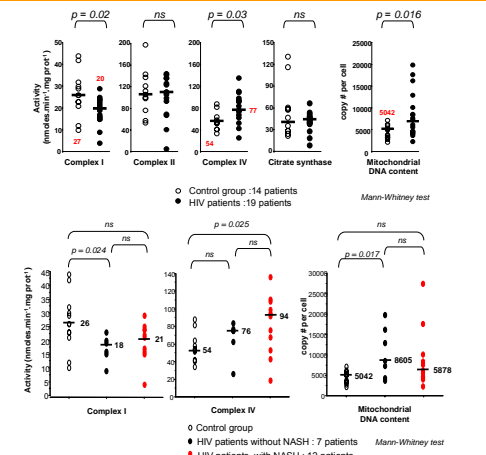
MITOCHONDRIAL ANALYSIS

19 HIV-infected patients were compared to a control group for the mitochondrial analyses.

Controls consisted in a group of patients with no liver disease who underwent hepatic resection for liver metastasis. Normal liver sample was taken during surgery for the mitochondrial analyses and were assessed using the same procedure as in HIV infected patients. All the controls had normal liver histological examination.

A fragment of the liver biopsy was snap frozen in liquid nitrogen and kept at -80°C before use. A part of the fragment was used for spectrophotometric assays of the mitochondrial activity on liver homogenates. They included the activities of respiratory chain complex I (CI) and citrate synthase (CS) and the activities of respiratory chain complexes I and IV (CI and CIV). Another part of the fragment was used for an extraction of total liver DNA using a routine procedure based on SDS-proteinase K digestion, followed by isopropanol precipitation. The amounts of nuclear and mitochondrial DNA were quantified by real time PCR using LightCycler FastStart Reaction System 1 kit (Roche). The amount of nuclear DNA was quantified by amplification of a fragment of the 28S ribosomal gene using as standard serial dilutions of total DNA from control fibroblasts while the amount of mitochondrial DNA was quantified by amplification of a fragment of the 12S ribosomal gene using as standard serial dilutions of a linearized plasmid containing the entire 12S gene as an insert.

Figure 2 - Mitochondrial respiratory chain and mtDNA evaluation



CONCLUSIONS

HIV-positive patients with chronic ALT elevation have liver lesions at a high rate.

The most common histological finding in this study was steatohepatitis consistent with the diagnosis of NASH.

NASH lesions were significantly correlated with hyperglycemia and hyperinsulinemia.

In our study, the evaluation of the mitochondrial respiratory chain differed from the pattern observed in typical mitochondrial toxicity. The activity of the respiratory complex IV and amount of mtDNA as observed by quantitative PCR was significantly increased in HIV-infected patients compared to the control group whereas the activity of complex I was decreased.

This finding seems to remove the hypothesis of the mitochondrial DNA toxicity in the genesis of the observed histologic liver abnormalities.