Non- Viral Liver Disease Burden In HIV Monoinfected Individuals: A Longitudinal Observational Retrospective Cohort Study

Short title: Non-Viral liver Disease And HIV

Total word count: 1529

Conflicts of interest for all authors pertaining to this manuscript: none

Funding source none

Abstract

Recent advances in antiviral therapy have improved outcomes in HIV-positive individuals co-infected with hepatitis B and C virus (HBV/ HCV). Our aim was to assess prevalence and predictors of chronic liver disease (CLD) due to the metabolic syndrome (MS), alcohol and antiretrovirals (ARVs) use in HIV monoinfected individuals. This was a retrospective cohort study (2005-2012). HIV-positive patients with negative HBV/HCV serology and at least two elevated alanine aminotransferase (ALT) levels six months apart were included. Data are presented as mean ±SD or percentage. Despite negative viral serology, 27% (1047/3872) of HIV positive individuals had persistently elevated ALT. Only 243 (23.2%) were investigated (by imaging in the majority, only 58 undergoing liver biopsy/transient elastography). CLD was identified in 66.2%, this being clinically significant in 1 in 4 individuals. Potential CLD risk factors were alcohol (44.2%), hepatotoxic ARVs (74.1%) and MS risk factors (68%) with 68.7% having >1 risk factor. On multivariate logistic regression analysis serum triglyceride (OR 1.482, 95% CI 1.053-2.086, p=0.024) was the only independent predictor of CLD. Overall, 4.3% were referred to Hepatology services. In conclusion less than 6% of HIV monoinfected individuals with persistently elevated ALT undergo objective assessment of hepatic fibrosis. Despite non-stringent criteria, some degree of non-viral CLD is identified in approximately two-thirds of those investigated, risk factors being synonymous with those for the MS. This increasing yet under-recognised non-viral CLD burden warrants timely recognition to prevent long-term morbidity and mortality.

Key words: chronic liver disease, alcohol use, metabolic syndrome, antiretroviral therapy, fatty liver.

Introduction

In HIV positive individuals liver disease [mostly due to hepatitis B and C virus (HBV and HCV)] is the third most common cause of mortality after AIDS related illness and non-AIDS non-viral malignancy (Morlat et al.; 2000). However, the discovery of direct acting antiviral (DAA) drugs has resulted in a paradigm shift in the management of HCV infection (Aghemo & De Francesco, 2013). The future liver disease burden in HIV-positive individuals is likely to be due to alcohol related liver disease (ARLD), non-alcoholic fatty liver disease (NAFLD) and/or antiretrovirals (ARV) related. Our aim therefore was to assess prevalence, aetiology, and predictors of non-viral related chronic liver disease (CLD) in a cohort with HIV mono infection.

Methods

This retrospective longitudinal cohort study was conducted at a teaching hospital in South East England from 2005-2012. The electronic HIV database was screened to identify HBV and HCV negative individuals with at least two alanine aminotransferase (ALT) levels>upper limit of normal (ULN) six months apart who had been investigated with one or more of the following: imaging (ultrasonography (USG), computed tomography (CT) and magnetic resonance imaging (MRI), Transient elastography (TE) and/or a liver biopsy. The interval between persistently elevated ALT and further investigations was not specifically recorded though was less than 12 months in most cases. Those with a positive HBV and HCV serology and or incomplete/ unavailable medical records were excluded

Study definitions

CLD: presence of one or more of the following:> F1 metavir fibrosis on liver biopsy (Bedossa &Poynard, 1996) and or liver stiffness measurement (LSM) > F1 (metavir) on TE; imaging showing any one of the following: fatty liver, heterogenous or irregular liver (Allan, Thoirs & Phillips, 2010);

Clinically significant liver disease as defined as: presence of one or more of the following: ≥F2 fibrosis (metavir) on liver biopsy and or LSM > F2 (metavir) on TE; moderate to severe hepatic steatosis on liver biopsy; portal hypertension (cirrhotic or non-cirrhotic); hepatic decompensation; and liver related mortality

Medical records of individuals fulfilling study criteria were retrospectively reviewed and data collected. Individuals were classified into three groups according to potential CLD risk factors (could be classified into more than one category).

Alcohol-related: ≥ 21units alcohol per week for men and ≥ 14 units alcohol per week for women.

Metabolic syndrome (MS) related: one or more risk factor for the MS [(dyslipidaemia defined as total cholesterol >5mmol/L, HDL <1.0mmol/L, LDL >3.mmol/L, triglycerides >2.0mmol/L); BMI > 30; diabetes mellitus (DM)/ impaired glucose tolerance (defined as a fasting plasma glucose <7 mmol/L and a two-hour glucose tolerance test value of 7.8 -11.1 mmol/L)

ARVs-related (use of ARVs could be past or current) : didanosine, stavudine or nevaripine (use of any duration); efavirenz use > 16 mths (since about 60% of the cohort were efavirenz experienced, we only classified those exposed to efavirenz for longer than the median period (>16 mths) as at risk of hepatotoxicity)

TE was performed using Fibroscan (Echosens Fibroscan® 402 Touch). Steatosis was classified as mild (10%- <30%), moderate (30%-60%) or severe (>60%).

Statistical analysis

Data are presented as mean □standard deviation, median (interquartile range) or number (%). Standard statistical tests were used: Mann-Whitney U, multivariable binary logistic regression. Statistical analyses were undertaken using SPSS Version 20.0 (Chicago, IL, USA).

Results

During the study period (2005-2012) 3872 HIV-positive individuals were identified. Since this was a retrospective study we were unable to determine why HIV physicians elected to investigate some and not other individuals with elevated ALT. Fig 1 shows how the final study cohort (n=222) was selected. Table 1 shows data of the cohort at entry. Of the 147 (66.2%) with CLD, 36 patients (24.5%) were considered to have clinically significant CLD. This included 11 (30.6%) with ≥F2 fibrosis of whom three had cirrhosis; 12 (33.3%) with moderate to severe steatosis, seven (22.2%) with both ≥F2 fibrosis and moderate to severe steatosis and six (16.7%) with non-cirrhotic portal hypertension (NCPHT). Overall 6/36 (16.7%) with clinically significant liver disease developed hepatic decompensation

Table 2a shows results of the univariate analysis in patients with and without CLD. Factors with p value <0.05 on univariate analysis were considered for entry into a multivariate binary logistic regression analysis. Due to collinearity between HIV duration and diagnosis pre-1996, BMI and weight, cholesterol and triglyceride, alcohol use/week at entry and alcohol use/week at last visit Receiver Operator Characteristic

(ROC) curves were performed to determine which variable had the best predictive value for CLD. Hence the following variables were finally entered into the multivariate binary regression analysis: age, HIV duration, BMI, triglyceride, fasting blood glucose, weekly alcohol intake at entry, PI-based therapy, use of didanosine, nevirapine and stavudine. Serum and triglyceride level (OR 1.482, 95% CI 1.053-2.086, p=0.024) was the only independent predictor of CLD (table 2b).

The majority of patients (68.7%) with CLD had more than one risk factor the most common combination being potentially hepatotoxic ARV use and risk factors for the MS (31.3%). Overall one or more risk factor of the MS was seen in approximately two-thirds of patients, potentially hepatotoxic ARV use in approximately three-quarters and alcohol use in 44% of patients.

Discussion

This retrospective cohort study has identified the significant yet under-recognised non-viral CLD burden in HIV monoinfected individuals with an elevated ALT. Despite a negative viral serology, about 30% (1047/3872) of our HIV cohort had a persistently elevated ALT over a six month period. Despite non-stringent CLD criteria, some degree of CLD was identified in two-thirds, this being clinically significant in approximately 1:4 individuals. Potential risk factors or CLD included alcohol use (44.2%), one or more feature of the MS (68%) and ARV use (74.1%), with 68.7% having > 1 risk factor. Serum triglyceride (OR 1.482, 95% CI 1.053-2.086, p=0.024), was the only independent predictor of CLD. The most striking observation however was that only 23% with persistently elevated ALT were investigated further with 58/1047 (5.5%) undergoing objective assessment of hepatic fibrosis and 45/1047 (4.3%) being referred

to Hepatology.

The recently commissioned UK Liver report indicates that mortality from liver disease has increased 500% since 1970, this now constituting the third commonest cause of premature death in the UK. Alcohol causes about 75% of these deaths (Williams et al.; 2014). Additionally with approximately about 25% of UK adults now defined as obese, about 1 in 20 are estimated to have non-alcoholic steatohepatitis (NASH)(Williams et al.; 2014). This is likely to be applicable to HIV positive individuals as well. Studies using CT scanning and ultrasound have in fact detected fatty liver in 13% and 37% of American and European HIV patients respectively (Crum-Cianflone et al.; 2009; Price et al.; 2014), consistent with our 47% prevalence. A recent American study reported that 65% of HIV monoinfected individuals with elevated ALT had clinically significant liver pathology, including 55% with nonalcoholic steatohepatitis and 18% with bridging fibrosis, (Morse CG et al.; 2015)).

Our prevalence of fatty liver was high at 50% despite a young (mean age 47.6 $\pm$10.1 yrs) Caucasian cohort with low MS risk factors (14.9% obese, 12.2% DM and 57.7% elevated triglyceride). Others have reported that HIV-positive individuals with NAFLD have a lower BMI (26.3 $\pm$0.5 vs. 30.2 $\pm$1.0, p= 001) and are more physically active (percentage of fat mass 19.4 $\pm$0.9 vs. 22.7 $\pm$1.2, p = 0.026) compared with their HIV-negative counterparts with NAFLD (Mohammed et al.; 2007). Clearly additional factors contribute to NAFLD in HIV positive individuals particularly ARVs: stavudine and didanosine result in hepatic steatosis (McGovern B et al.; 2006); ritonavir elevates serum triglycerides and didanosine and lopinavir cause insulin resistance (Rao, Lee & Grunfeld, 2006).

Rates of ARLD have risen by almost 50% in the UK over the last seven years (Sheron et al.; 2012) though only limited data exists on alcohol use and ARLD prevalence in HIV-positive individuals. In our cohort ~40% were drinking alcohol above the national recommended levels with 18.9% being binge drinkers. Bonacini et al reported a 37% vs. 25% all cause mortality (p=0.03) in those drinking < and > 50 gms alcohol/day, with lowest mortality rates in those consuming<10gms/day (Bonacini, 2011). "Safe" alcohol consumption is however difficult to define in the HIV monoinfected individuals who often have additional risk factors for CLD. In the current study of the 65 (44.2%) with CLD drinking in excess of recommended guidelines, all but 12 individuals potentially had additional risk factors for CLD.

An important limitation of this study was that since < 25% with persistently elevated ALT were investigated, it is unclear if our results can be extrapolated to the whole cohort. Therefore, we are now prospectively screening the remainder (n=804) for CLD using TE with controlled attenuation parameter - our initial results indicate a 47.5% and 24.3% prevalence of hepatic steatosis and hepatic fibrosis (LSM >7.2kPa) consistent with our retrospective data. Additionally there are no significant differences in ALT and risk factors for chronic liver disease , between those investigated and those not initially investigated (data not shown) (Yishi T et al., 2015)

In conclusion, this retrospective study demonstrates the significant yet under recognised non-viral liver CLD burden in HIV-positive individuals with an elevated ALT and underscores the need for HIV physicians in conjunction with their Hepatology colleagues to implement routine screening strategies and referral protocols.

Figure legends

Fig1. Flow diagram showing how the study cohort was selected

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Table 1. Demographic data at entry in whole cohort (n=222).

Age (yrs)	*47.6 ±10.1*
Males	208 (90.8 %)
Ethnicity White UK	143 (64.3%)
White non-UK	43 (19.4%)
Black non-UK	27 (12.2%)
Other	9 (4.1%)
HIV duration (yrs)	10.0 (6-17)
CD4 at diagnosis (cells/mm^3)	325 (165 - 482)
CD4 nadir(cells/mm^3)	219 (106 - 350)
HIV Viral Load undetectable (<40mmol/L)	202 (91.0%)
HIV diagnosed pre-1996	37 (16.6%)
Weight (kg)	77.7 ±15.5
Body Mass Index (BMI)	26.7 ±5.4
BMI $\geq$30	33 (14.9%)
Cholesterol (≤5mmol/L)	5.4 ±1.2
HDL (≥1mmol/L)	1.2 ±0.4
LDL (≤3mmol/L)	3.0 ±1.0
Triglycerides (<2mmol/L)	2.9 ±2.7
Chol:HDL (≤4mmol/L)	4.5 ±2.7
Fasting blood glucose (4-5.9 mmol/L)	5.6 ±1.7
Diabetes Mellitus	27 (12.2%)
Lipodystrophy	34 (14.8%)
Alcohol intake (units)	
Alcohol (units/wk) at entry	20 (2-38)
Alcohol (units/wk) at last visit	7 (0-21)
Alcohol (units/day)	0 (0-7)
Binge drinking	42 (18.9%)
Drinking > recommended weekly limit	83 (37.4%)
ARV history	
ARV duration (mths)	72 (0-390)
NNRTI-based therapy	167 (75.2%)
PI-based therapy	128 (57.7%)
Didanosine	60 (27%)
Nevirapine	47 (21.2%)
Efavirenz	139 (62.6%)
Ritonavir	113 (50.9%)
Darunavir	46 (20.7%)
Stavudine	45 (20.3%)
Saquinavir	15 (6.8%)
Initial ALT(<40 iu/L)	54 (46-70)
Highest ALT	90 (64-125)
Most recent ALT	35 (25-50)
Further investigations	
Ultrasound	198 (89.2%)
CT Scan	43 (19.4%)
MRI	8 (3.6%)
Transient elastography	16 (7.2%)
Liver biopsy	42 (18.9%)
Radiological findings	
Fatty liver	105 (47.3%)
Heterogeneous/ irregular liver	21 (9.5%)
Varices/ abnormal portal venous flow	11 (5.0%)

Ascites	2 (0.9%)
Liver Biopsy **(n=42)**	
Steatosis : None	18 (42.9%)
Mild	5 (11.9%)
Moderate	13 (31.0%)
Severe	6 (14.3%)
Fibrosis F0	14 (33.3%)
F1	16 (38.1%)
F2	4 (9.5%)
F3-F4	9 (21.4%)
Transient Elastography (n=16)	
F0	8 (50%)
F1	3 (21.4%)
F2	3 (21.4%)
F3-4	2 (14.3%)
Chronic liver disease (CLD)	147 (66.2%)
Clinically significant CLD	36/147 (24.5%)
Non-CLD	75 (33.8%)
Referred to Hepatology	45 (20.3%)

Some patients fulfilled more than one criteria for CLD

DM diabetes mellitus, HDL High density lipoprotein, LDL Low density lipoprotein, CT computerised tomography, MRI magnetic resonance imaging, ARV antiretrovirals, NNRTI non-nucleoside reverse transcriptase inhibitors

Table 2a. Univariate analysis of factors associated with chronic liver disease

Parameter	Chronic Liver Disease n=147 (60.2%)	No Chronic Liver Disease n=75 (30.7%)	OR	95% CI	P value
Age (yrs)	**49.03 ±9.69**	**44.08 ±9.80**	**1.062**	**1.029-1.097**	**<0.001**
Males	140 (95.2%)	68 (90.7%)	0.486	0.164-1.440	0.193
Ethnicity					
White UK	93 (63.3%)	49 (65.3%)	0.949		0.751
White non-UK	32 (21.8%)	11 (14.7%)			
Black non-UK	15 (10.2%)	12 (16%)			
Other	6 (4.1%)	3 (4%)			
Duration of HIV (years)	**12.8 ±8.2**	**8.2 ±4.7**	**1.137**	**1.079-1.199**	**<0.001**
CD4 at diagnosis	290.0 (120-494)	283.0 (175-409)	1.000	0.999-1.002	0.513
CD4 nadir	217 (84-319)	284 (151- 372)	0.999	0.997-1.000	0.112
No. diagnosed pre-1996	**36 (97.3%)**	**1 (2.7%)**	**24.00**	**3.220-178.878**	**0.002**
Body mass index (BMI)	**26.8 ±4.6**	**25.4 ±4.4**	**1.080**	**1.002-1.164**	**0.043**
Weight	**78.2 ±14.4**	**73.4 ±12.2**	**1.022**	**1.001-1.043**	**0.039**
Cholesterol (mmol/L)	**5.5 ±1.3**	**5.2 ±1.0**	**1.284**	**1.011-1.632**	**0.040**
HDL (mmol/L)	1.3 ±0.5	1.2 ±0.4	1.612	0.837-3.103	0.153
LDL (mmol/L)	2.9 ±1.1	3.1 ± 0.9	0.897	0.682-1.181	0.441
Triglycerides(mmol/L)	**2.5 (5-15.5)**	**1.80 (5-9)**	**1.238**	**1.042-1.470**	**0.015**
Chol:HDL (mmol/L)	4.5 ±1.7	4.6 ±1.6	0.963	0.815-1.138	0.668
FBG (mmol/L)	**5.8 ±2.0**	**5.2 ±0.2**	**1.359**	**1.048-1.761**	**0.021**
Alcohol (units/wk) at entry	**21.0 (1.3-57.5)**	**10.0 (2-30)**	**1.011**	**1.002-1.021**	**0.024**
Alcohol(units/wk) at last follow up	**10.0 (0- 24.8)**	**0 (0-18.8)**	**1.023**	**1.003-1.043**	**0.024**
Alcohol (daily units)	2.0 (0-9)	0 (0-5)	1.060	0.999-1.125	0.055
Binge drinking	32 (21.8%)	10 (13.2%)	1.739	0.790-3.829	0.169
HAART	93 (63.7%)	37 (48.7%)		0.090-0.101	0.098
Duration HAART(mths)	**99 (50-150)**	**60 (40-96)**	**1.013**	**1.007-1.019**	**<0.001**
NNRTI-based therapy	109 (74.7%)	58 (76.3%)	0.841	0.437-1.618	0.604
Duration NNRTI	48 (24-106.5)	48 (24-72)	1.007	0.989-1.187	0.084
PI-based therapy	**91 (62.0%)**	**37 (48.7%)**	**1.727**	**0.081-0.091**	**0.049**
Duration PI-based	48 (36-96)	48 (24-67.5)	1.008	-3.788-28.401	0.094
Didanosine	**52 (35.6%)**	**8 (10.5%)**	**4.893**	**2.179-10.989**	**<0.001**
Duration Didanosine	48 (19.5-86.0)	36 (12-36)	1.022	0.993-1.022	0.140

Nevirapine	**37 (25.3%)**	**10 (13.2%)**	**2.312**	**1.077-4.966**	**0.032**
Duration Nevirapine	60 (24-114)	12 (6-12)	1.002	0.989-1.015	0.739
Efavirenz	88 (60.3%)	51 (67.1%)	0.781	0.432-1.414	0.415
Duration Efavirenz	36 (12-72)	36 (12-54)	1.005	0.996-1.014	0.298
Ritonavir	76 (52.1%)	36 (47.4%)	1.324	0.755-2.323	0.328
Duration Ritonavir	36 (12-48)	24 (12-48)	1.086	0.945-1.247	0.244
Darunavir	25 (17.1%)	19 (25%)	0.686	0.352-1.338	0.269
Duration Darunavir	48 (21-60)	36 (21-48)	1.191	0.854-1.661	0.303
Stavudine	**40 (27.4%)**	**5 (6.7%)**	**5.545**	**2.084-14.749**	**<0.001**
Duration Stavudine	36 (12-48)	60 (35.5-90)	0.965	0.930-1.001	0.058
Saquinavir	11 (7.5%)	4 (5.3%)	1.502	0.461-4.890	0.499
Duration Saquinavir	36 (24-82)	36 (6-87)	1.004	0.966-1.044	0.830
Further investigations					
USS	136 (92.5%)	60 (82.2%)	2.592	1.100-6.110	0.029
CT	21 (14.3%)	22 (30.1%)	0.402	0.204-0.791	0.008
MRI	5 (3.4%)	0 (4.3%)	1.168	0.270-5.054	0.835
Fibroscan	16 (11.0%)	0		0.003-0.006	0.003
Biopsy	39 (92.9%)	3 (7.1%)	8.667	2.580-29.109	<0.001
Referred to hepatology	40 (27.4%)	5 (6.6%)	5.234	1.970-13.907	<0.001

BMI = body mass index, total cholesterol (normal ≤5mmol/L), HDL = high density lipoprotein (normal ≥1mmol/L), LDL = low density lipoprotein (normal ≤3mmol/L), Triglycerides (normal <1.69mmol/L), Chol:HDL ratio (normal ≤4mmol/L), FBG = fasting blood glucose (normal <6.1mmol/L, impaired fasting glucose 6.1-6.9mmol/L)

Table 2b. Multivariate analysis of factors associated with chronic liver disease

	P value	Exp (B)	95% CI for Exp (B)	
Age	0.254	1.030	0.979	1.084
Duration of HIV	0.117	1.082	0.980	1.195
BMI	0.056	1.105	0.997	1.224
PI based therapy	0.809	1.115	0.460	2.703
Didanosine therapy	0.797	1.221	0.266	5.599
Nevirapine therapy	0.497	0.658	0.196	2.205
Stavudine therapy	0.157	3.290	0.632	17.126
Triglyceride	**0.024**	**1.482**	**1.053**	**2.086**
Fasting blood glucose	0.866	1.034	0.700	1.528
Weekly intake alcohol	0.101	1.017	0.997	1.037

Fig 1. Flow diagram showing how the study cohort was selected