Poster # 788

Background: Microbial translocation and immune activation occur as HIV disease progresses and has been linked with the development of liver disease. Most of these studies, however, have been done in vitro and in animal models. Our objective was to investigate the pathways of liver fibrogenesis, including microbial translocation, apoptosis, and cytokine activation in HIV mono-infected and HIV-HCV co-infected adults in the Miami Adult Studies on HIV (MASH) Cohort.
Methodology: After obtaining informed consent in a subset of the MASH cohort participants, blood was collected at baseline and 12 months later for indices of liver fibrosis (FIB-4 and APRI), bacterial translocation (plasma LPS), immune activation (soluble CD14), pro-fibrotic cytokine (TGF-β1), and hepatocyte apoptosis (cytokeratin-18 (CK-18)). FIB-4 and APRI were calculated [age (years) x AST (U/L)]/[PLT (109 cells/L) X ALT$^{1/2}$ (U/L)], and [AST (x upper limit of normal range) x 100]/platelet count (10^9 cells/L)], respectively. LPS was determined using the Limulus Amebocyte Lysate kit (Lonza, Wackersville, MD). Soluble CD14 and TFG-β1 were measured using Quantikine ELISA kits (R&D, Minneapolis, MD). Plasma levels of CK-18 were determined by M30 Apoptosense ELISA kit (PEVIVA, Bromma, Sweden). Questionnaires were used for demographics.
Results: Among 80 participants, the mean age was 46.65±7.77, 65% were male, and 75% were black non-Hispanic; 87% were on ART and 25% were HIV/HCV co-infected. Liver fibrosis progressed faster in HIV/HCV co-infected than in HIV mono-infected participants [FIB-4 (2.21±SD 1.61 vs 1.21±SD 0.44, p=0.02), APRI (0.64 ±SD 0.64 vs. 0.29 ±SD 0.11, p=0.03)]. In a linear regression analysis faster progression of liver fibrosis using either FIB-4 or APRI was associated with higher CK-18; for FIB-4 (β=11.027, p=0.038) and sCD14 (β=0.0006, p=0.029); for APRI the association was CK-18 (β=47.45, p=0.005) and sCD14 (β=0.0002, p=0.042). Soluble CD14 was associated with TGF-β1 (β=2.330, p=0.001). Using mixed models to evaluate change over time, increase in sCD14 was predictive of faster liver fibrosis [FIB-4 (β=0.0005, p=0.040), APRI (β=0.0002, p=0.043)]. Faster liver disease progression was associated with higher levels of CK-18 over time for both FIB-4 and APRI (β=16.756, p<0.001, and β=37.940, p<0.001). All analyses were controlled for age, gender and CD4 cell count.
Conclusions: Advancement of liver fibrosis estimated by FIB-4 and APRI was associated with increased bacterial translocation, immune activation, hepatocellular apoptosis, and increased level of a pro-fibrotic cytokine TGF-β-1, over a period of one year. Larger studies are warranted to increase the understanding of liver fibrogenesis in order to identify pro-fibrotic targets.

Microbial Translocation, Immune Activation, Apoptosis and Liver Fibrosis in the MASH Cohort

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BACKGROUND

Liver disease is a leading cause of non-AIDS related mortality in HIV disease (Weber R et al., 2006). Microbial translocation and immune activation occurs as HIV disease progresses and has been linked with the development of liver disease (Chen J et al., 2014; Lin W et al., 2013; Page EE et al., 2011). Most of these studies however, have been done *in vitro* and in animal models. Our objective was to investigate the pathways of liver fibrogenesis, including microbial translocation, apoptosis, and cytokine activation in HIV mono-infected and HIV-HCV co-infected adults in the Miami Adult Studies in the HIV (MASH) Cohort.

METHODS

After obtaining informed consent in a subset of 80 participants of the MASH cohort, blood was collected at baseline and 12 months later for indices of liver fibrosis (FIB-4 and APRI), bacterial translocation (plasma LPS), immune activation (soluble CD14), pro-fibrotic cytokine (TGF-β1), and hepatocyte apoptosis (cytokeratin-18 [CK-18]). FIB-4 and APRI were calculated [age (years) x AST (U/L)]/[PLT (10^9 cells/L) x ALT$^{1/2}$ (U/L)], and [AST (x upper limit of normal range) x 100]/platelet count (10^9 cells/L)], respectively. LPS was determined using the Limulus Amebocyte Lysate kit (Lonza, Wackersville, MD). Soluble CD14 and TGF-β1 were determined with Quantikine ELISA kits (R&D, Minneapolis, MD).

METHODS (Cont.)

CK-18 levels were determined with M30 Apoptosense ELISA kit (PEVIVA, Bromma, Sweden). Questionnaires were used for demographics. Descriptive statistics were used for demographics and linear regression and mixed models for associations with markers of liver disease progression.

RESULTS

Table 1: Population Statistics

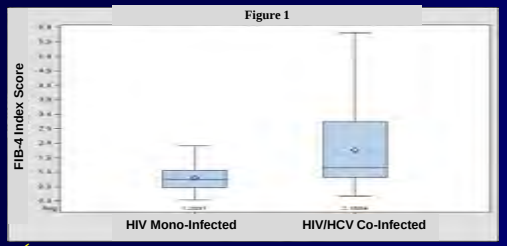
Characteristic	N=80
Age, (Mean in years±SD)	46.65±7.77
Gender % (N)	
Male	65.00 (52)
Female	35.00 (28)
Race/Ethnicity % (N)	
White Non-Hispanic	2.50 (2)
White Hispanic	16.25 (15)
Black Non-Hispanic	75.00 (60)
Black Hispanic	2.50 (2)
Other	3.75 (3)
ART % (N)	87.34 (69)
Laboratory Values	Mean±SD
CD4 Cell Count (cells/μL)	533.54±341.44
HIV Viral Load (log_{10}copies/mL)	2.77±1.32
FIB-4 score	1.44±0.97
APRI score	0.37±0.36
CK-18 U/L	126.85±106.43
Soluble CD14 ng/mL	1803.83±409.56
LPS EU/mL	0.24±0.07
TGF-β1 ng/mL	2194.57±2560.33

✓ Among 80 participants, the mean age was 46.65±7.77, 65% were male, and 75% were black non-Hispanic; 87% were on ART and 25% were HIV/HCV co-infected.

RESULTS (Cont.)

✓The HIV/HCV co-infected participants had more advanced liver fibrosis than those with HIV mono-infection [FIB-4 (2.21±SD 1.61 vs. 1.21±SD 0.44, p=0.02), APRI (0.64 ±SD 0.64 vs. 0.29 ±SD 0.11, p=0.03)] .

Figure 1: Comparison of FIB-4 Index Score between HIV mono-infected and HIV/HCV co-infected



✓In a linear regression analysis, more advanced liver fibrosis (FIB-4 or APRI) was associated with higher CK-18; for FIB-4 (β=11.027, p=0.038) and sCD14 (β=0.0006, p=0.029); for APRI, the association was CK-18 (β=47.449, p=0.005) and sCD14 (β=0.0002, p=0.042). Soluble CD14 was associated with TGF-β1 (β=2.330, p=0.001).

✓Using mixed models to evaluate change over time, increase in sCD14 was predictive of faster liver fibrosis [FIB-4 (β=0.0005, p=0.040), APRI (β=0.0002, p=0.043)]. Faster liver disease progression was associated with higher levels of CK-18 over time for both FIB-4 and APRI (β=16.756, p<0.001, and β=37.940, p<0.001). All analyses were controlled for age, gender and CD4 count.

RESULTS (Cont.)

Figure 2: Linear regression of soluble CD14 levels and FIB-4 index scores

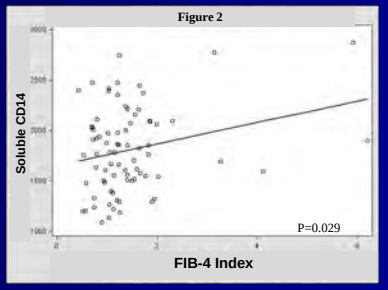
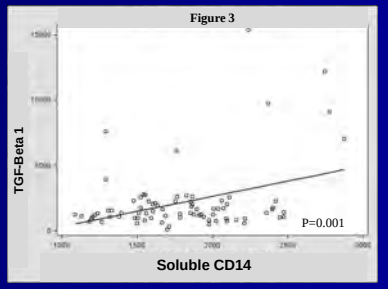


Figure 3: Linear regression of TGF-β1 and soluble CD14 levels



CONCLUSIONS

Advancement of liver fibrosis estimated by FIB-4 and APRI was associated with increased bacterial translocation, immune activation, hepatocellular apoptosis, and increased level of a pro-fibrotic cytokine TGF-β-1, over a period of one year. Larger studies are warranted to increase the understanding of liver fibrogenesis in order to identify pro-fibrotic targets.

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