Cytokines and Liver Fibrosis in HIV/HCV Co-Infection: The HHH Hemophilia Cohort Study

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ABSTRACT

Background: Despite improved survival and immune function with antiretroviral therapy, HIV infection accelerates hepatitis C liver disease, and an increasing proportion of those with co-infection develop fibrosis. We hypothesized that HIV accelerates HCV progression through upregulation of cytokines that promote fibrosis. We, therefore, conducted a prospective, multi-center study to evaluate cytokines in HCV fibrosis by HIV status.

Methods: Among 781 HCV(+) hemophilic men from 34 U.S. hemophilia treatment centers, 220 (28.2%) underwent liver biopsy, of whom 92 (41.8%) were HIV(+) and 128 (58.2%) HIV(-). Biopsies were independently reviewed using Ishak, Metavir, and Knodell systems. Plasma cytokine immunoassays (Il-6, IL-10, IFN-γ, TGF-β) were measured by ELISA assay (Quantikine R&D Systems, Minneapolis, MN). Tissue cytokine mRNA was measured by ribonuclease protection assay (Riboquant, Pharmingen, San Diego, CA), and radiolabeled band densitometry. Cytokine promoter genotype polymorphisms were measured by molecular typing and gel electrophoresis, with ethidium bromide staining. Chi-square tests for association and trend, and Wilcoxon rank sum tests were performed to compare cytokine assays by HIV status.

Results: Of the 220 men who underwent biopsy, 86 (39.1%) had fibrosis (Ishak score 3-6). Fibrosis was more common in HIV/HCV co-infected than in HCV mono-infected, 43/92 (46.7%) vs 41/128 (32.0%), p=0.03, with fibrosis scores, 2.3±0.2, vs. 1.9±0.2, p=0.06. HIV/HCV co-infected had higher AST, 57 vs 42 U/ml, p=0.03; and lower platelets, 184,000 vs 219,000/ul, p<0.001, but similar HCV duration, 38 vs. 36 yr, p=0.07; and similar proportion IFN/RBV treated, 47% vs 41%, p=0.39, than HCV mono-infected men. More co-infected than mono-infected had high TFG-β2 ELISA (>243 pg/ml), 12.5% vs 4.2%, p=0.028, but TGF-β ELISA values, p=0.201, and TGF-β mRNA; p=0.885, were similar. Fewer HIV(+) than HIV(-) had high level IFN-γ ELISA (>5 pg/ml), 11.4% vs 23.7%, p=0.024, with lower IFN-γ ELISA values, p=0.035, and lower IFN-γ mRNA, p=0.030. Cytokine promoter genotypes did not differ by HIV status, all p≥0.388.

Conclusions: Nearly half of HIV/HCV co-infected hemophilic men have Ishak ≥ 3 fibrosis, a 1.5-fold higher prevalence than in HCV mono-infected men. Although a greater proportion of co-infected have high level TFG-β ELISA and low IFNγ ELISA and mRNA levels, mechanisms other than cytokine dysregulation may contribute to higher fibrosis progression in HIV/HCV co-infection.

BACKGROUND

Hepatitis C (HCV) is the leading cause of chronic liver disease and mortality in individuals with hemophilia, of whom 90% were exposed to HCV through clotting factor concentrates. About 40% of HCV(+) were also infected with HIV, with peak seroconversion in 1982, 10+ years after HCV infection. The proportion with fibrosis, now 30+ years from initial HCV infection, and 20 years since initial HIV infection is not known. Before the AIDS epidemic, 20% had biopsy evidence of cirrhosis (Spero, 1979). We and others have shown that HIV accelerates end-stage liver disease at least 4-fold in hemophilic men (Ragni, 2001). Although HAART therapy, which improves HIV survival and immune function, also reduces HCV progression (Ragni, 2008), an increasing proportion of HIV/HCV co-infected hemophilic men are progressing to end-stage liver disease, now, nearly 25 years after HIV infection. The mechanism of HIV-induced HCV progression is not understood, although HIV dysregulates production of cytokines that promote liver fibrosis.

Cytokines and growth factors, which are upregulated in HIV infection, may play a role in preventing or retarding liver decompensation in hepatitis C. In the experimental bile duct ligation model of liver failure, IL-6$^{-/-}$ mice develop liver failure more quickly than IL-6$^{+/+}$ controls, but can be rescued by IL-6 treatment (Ezure, 2000). TFG-β1, also upregulated in HIV infection (Kekow, 1991), however, inhibits the effects of IL-6 (Yokomuro, 2002), potentially limiting its cytoprotective effect in clinical those with HIV/HCV infection, although the mechanism remains unclear. Cytokine gene polymorphisms, including TGF- β1, TNF-a, and IL-10 genotypes, known to influence lung fibrosis and heart and lung rejection in transplant patients, may also influence HCV disease outcome (Powell, 2000). These findings have suggested the potential importance of cytokines in outcome of liver cirrhosis and fibrosis in HIV/HCV co-infection. We had the unique opportunity to study the impact of HIV infection on HCV liver disease progression on a group of HCV-infected hemophilic men, comparing plasma and tissue cytokines, and cytokine promoter genotypes in HIV/HCV co-infected hemophilic men with HCV mono-infected hemophilic men.

METHODS

Subjects: Hemophilia A or B, HCV(+) by ELISA; HIV(+) or HIV(-); age ≥ 18 years old, from 34 U.S. HTCs

Clinical and Laboratory Studies:

1. One-time blood sample: cytokine (IL-6, IL-10, TNF-α, IFN-γ, TGF-β), LFTs, CD4, AFP, ferritin
2. Voluntary liver biopsy for histology by Knodell, Ishak, and Metavir scores, reviewed by independent pathologist
3. Tissue cytokine mRNA (IL-6, TGF-β_1, TGFβ_2), cytokine promoter genotypes, HCV viral RNA
4. Enrollment form: lifestyle, clinical symptoms, viral exposures; liver biopsy safety form

Laboratory Assays

1. Plasma cytokine immunoassays: ELISA assay (Quantikine R&D Systems, Minneapolis, MN)
2. Tissue cytokine mRNA: Ribonuclease protection assay (Riboquant, Pharmingen, San Diego, CA)
3. Cytokine promoter genotype: Molecular typing and gel electrophoresis, with ethidium bromide staining.

Statistical Analysis Descriptive statistics were computed for all variables. Cytokine immunoassays were compared by chi square analysis or Fisher's exact test. Cytokine mRNA values were not normally distributed and were log-transformed and then analyzed by Wilcoxon two-sample tests. Cytokine promoter phenotypes were compared by chi square tests. Chi square analysis was used to test for association and trend. Wilcoxon rank sum tests were used to compare cytokine assays by HIV status. All data analyses were performed using SAS 9.1.3 software package.

RESULTS

HCV+ Hemophilic Men Undergoing Liver Biopsy

	HIV(+) N = 93	HIV(-) N = 127	p value
Age at Enrollment (mean)	42.5	40.1	p=0.068
Race			
Caucasian	80 (86.0%)	113 (89.0%)	p=0.51
Non-Caucasian	13 (14.0%)	14 (11.0%)	
Type			
Hemophilia A	83 (89.3%)	96 (76.2%)	p=0.013*
Hemophilia B	10 (10.7%)	30 (23.8%)	
ETOH			
No. < 2-3 times/week	21 (38.9%)	36 (47.4%)	p=0.34
No. ≥ 2-3 times/week	33 (61.1%)	40 (52.6%)	
Smoker			
No. Current	22 (25.3%)	52 (43.7%)	p=0.007*
No. Never	35 (40.2%)	45 (37.8%)	
No. Quitter		30 (34.5%)	22 (18.5%)
Analgesics			
No. ≥ Weekly	48 (56.5%)	51 (42.2%)	p=0.043*
No. < Weekly	37 (43.5%)	70 (57.8%)	
Alternative Drug			
No. Ever (%)	31 (33.3%)	34 (26.8%)	p=0.29
HCV Genotype			
No. Genotype 1	56 (81.2%)	73 (81.1%)	p=0.99
No. Genotype 2-6	13 (18.8%)	17 (18.9%)	
IFN ± RBV Treatment			
No. Ever	44 (47.8%)	52 (40.9%)	p=0.31
No. Never	48 (52.2%)	75 (59.1%)	
HCV Viral Load			
No. ≥ 600 U/ml	68 (78.2%)	90 (77.6%)	p=0.92
Duration HCV Infection	42.5	40.1	p=0.068
Duration HIV infection (yr)	24.6	-	
Median CD4/ul	495	799	p<.0001*
HIV RNA (No. ≥ 400 copies/ml)	18 (32.1%)	-	
HAART Treatment	75 (80.7%)	-	
Clinical Blood Count (CBC)			
Hemoglobin (gm/dl)	14.9±0.2	15.4±0.1	p=0.064
Platelets (No./uL)	184.3±6.6	219.6±6.5	p=0.0005*
Liver Function Tests			
AST (U/L)	56.6±5.9	41.9±3.6	p=0.002*
ALT (U/L)	62.8±7.4	54.5±4.5	p=0.42
Ferritin (ng/ml)	215.7±28.5	208.6±16.7	p=0.20
Alpha-fetoprotein (ng/ml)	10.0±3.6	6.2±1.7	p=0.061

Liver Fibrosis Score by HIV Status

Biopsy Score	HIV(+) N = 93	HIV(-) N =127	p value
Knodell Score (0-22)			
Mean ± SEM	5.5 ± 0.3	5.1 ± 0.3	p=0.39
Ishak Fibrosis Score (0-6)			
Mean ± SEM	2.3 ± 0.2	1.9 ± 0.2	p=0.065
No. Score ≥ 3-6	43 (46.3%)	41 (32.3%)	p=0.035*
Metavir Fibrosis Score (F0-F4)			
Mean ± SEM	1.6 ± 0.1	1.3 ± 0.1	p=0.045*
No. Score ≥ F2	44 (47/3%)	41 (32/3%)	p=0.024*

Ishak Score 3 | Ishak Score 4 | Ishak Score 5 | Ishak Score 6

Cytokine Promoter Phenotype and Liver Fibrosis by HIV Status

No.(+) / No. Tested	HIV(+) (N = 93) Metavir < F2	HIV(+) (N = 93) Metavir F2-F4	HIV(-) (N = 127) Metavir < F2	HIV(-) (N = 127) Metavir F2-F4
TGF-β phenotype				
Low	1/14 (7.1%)	4/34 (11.8%)	2/21 (9.5%)	3/63 (4.8%)
Intermediate	6/14 (42.9%)	3/34 (8.8%)	5/21 (23.8%)	15/63 (23.8%)
High	7/14 (50.0%)	27/34 (79.4%)	14/21 (66.7%)	45/63 (71.4%)
			*p=0.591	**p=0.120
IL-6 phenotype				
Low	3/14 (21.4%)	7/34 (20.6%)	2/21 (9.5%)	10/63 (15.9%)
High	11/14 (78.6%)	27/34 (79.4%)	19/21 (90.5%)	53/63 (84.1%)
			*p=0.369	**p=0.560
IL-10 phenotype				
Low	2/14 (14.3%)	12/34 (35.3%)	2/21 (9.5%)	17/63 (27.0%)
Intermediate	10/14 (71.4%)	15/34 (44.1%)	13/21 (61.9%)	29/63 (46.0%)
High	2/14 (14.3%)	7/34 (20.6%)	6/21 (28.6%)	17/63 (27.0%)
			*p=0.584	**p=0.640

*p value, comparing Metavir < F2 by HIV status; **p value, comparing Metavir F2-F4 by HIV status.
The overall p value for cytokine phenotypes did not differ by HIV status, p=0.388.

RESULTS

Cytokine Levels By Fibrosis Score and By HIV Status

Cytokine ELISA Immunoassay

No (+)/ No Tested	HIV(+) N = 93	HIV(-) N = 127	p value
TGF-β (≥ 243 pg/ml)			
Metavir < F2	3/16 (18.7%)	0/31 (0%)	p=0.034*
Metavir ≥ F2-F4	7/72 (9.7%)	5/86 (5.8%)	p=0.356
IL-6 (≥ 3 pg/ml)			
Metavir < F2	4/16 (25.0%)	17/31 (54.8%)	p=0.068
Metavir ≥ F2-F4	38/72 (52.8%)	43/86 (50.0%)	p=0.728
IL-10 (≥ 5 pg/ml)			
Metavir < F2	3/16 (18.7%)	4/31 (12.9%)	p=0.676
Metavir ≥ F2-F4	7/72 (9.7%)	9/86 (10.5%)	p=0.877
TNF-α (≥ 10 pg/ml)			
Metavir < F2	3/16 (18.7%)	6/31 (19.3%)	p=1.00
Metavir ≥ F2-F4	17/72 (23.6%)	18/86 (20.9%)	p=0.686
IFN-γ (≥ 5 pg/ml)			
Metavir < F2	1/16 (6.2%)	5/31 (16.1%)	p=0.648
Metavir ≥ F2-F4	8/72 (11.1%)	23/86 (26.7%)	p=0.014*

Cytokine Tissue mRNA

Mean ± SEM	HIV(+) N = 93	HIV(-) N = 127	p value
TGF-β mRNA			
Metavir < F2	3.58±0.34 (5)	3.83±0.26 (7)	p=0.529
Metavir ≥ F2-F4	5.43±1.47 (10)	4.26±0.76 (15)	p=0.934
IL-6 mRNA			
Metavir < F2	7.07±0.26 (5)	6.58±0.42 (7)	p=1.00
Metavir ≥ F2-F4	5.42±1.11 (10)	5.40±0.39 (15)	p=0.245
IL-10 mRNA			
Metavir < F2	-3.17±0.44 (5)	-3.05±0.32 (7)	p=0.751
Metavir ≥ F2-F4	-2.55±1.02 (10)	-3.61±0.27 (15)	p=0.495
TNF-α mRNA			
Metavir < F2	-2.58±0.46 (5)	-2.73±0.29 (7)	p=0.874
Metavir ≥ F2-F4	-2.44±1.05 (10)	-3.21±0.29 (15)	p=0.934
IFN-γ mRNA			
Metavir < F2	0.47±0.80 (5)	1.23±0.30 (7)	p=0.280
Metavir ≥ F2-F4	-0.80±0.46 (10)	1.24±0.60 (15)	p=0.369

CONCLUSIONS

1. Of 781 HCV(+) hemophilic men, 220 (28.3%) underwent liver biopsy. The latter did not differ demographically from 561 un-biopsied men.
2. After 25+ years of HIV infection, the proportion of HIV(+)/HCV(+) hemophilic men with Metavir ≥ F2 fibrosis is significantly higher than in HIV(-), 47.3% vs. 32.3%, p=0.024, with higher mean fibrosis scores, 1.6 vs. 1.3, p=0.045.
3. After controlling for HIV, older age (but not race, ETOH, or HCV treatment) was predictive of fibrosis, p=0.03.
4. A significantly greater proportion of the HIV(+) group had very high TGF-β2 levels (>243 pg/ml), while a significantly smaller proportion had high IFN-γ levels (>5 pg/ml), as compared with HIV(-) negative hemophilic men. Cytokine tissue mRNA and promoter phenotypes did not differ by fibrosis score or HIV status overall.
5. Cytokine dysregulation may contribute to fibrosis progression among those with HIV/HCV co-infection, but other mechanisms likely affect fibrosis score.

The HHH Study

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