Increasing Hepatitis C Virus RNA Levels in Hemophiliacs: Relationship to Human Immunodeficiency Virus Infection and Liver Disease

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for the Multicenter Hemophilia Cohort Study

We have previously observed an increased frequency of liver failure in human immunodeficiency virus (HIV)-infected hemophiliacs. The purpose of this study was to quantitate hepatitis C virus (HCV) RNA levels in serial samples from HIV-seropositive (HIV+) and HIV-seronegative (HIV-) hemophiliacs before and after HIV seroconversion, and to examine the relationship of HCV RNA levels to CD4 cell counts and to hepatic dysfunction over time. HCV RNA levels were measured on serial samples of serum stored frozen from 17 HCV+/HIV+ and 17 HCV+/HIV- subjects matched within 5 years of their birth dates. All were HIV+ before study entry. HCV RNA levels were quantitated by a branched DNA-enhanced label amplification (bDNA) assay. For samples less than the cut off, HCV RNA was measured by the nested polymerase chain reaction. Individual changes over time, clinical groups, and mean values within predetermined time windows were compared with Wilcoxon rank sum tests. Mean HCV RNA levels increased from 2.76 (standard error [SE] 1.33) \times 10^6 to 2.84 (SE 1.39) \times 10^6 eq/mL during the first 2 years after HIV seroconversion (P = .006). Baseline HCV RNA levels in the pre-HIV seroconversion group were not significantly different from the baseline levels in those who remained HIV+ (P = .79). Over the entire period of study, HCV RNA levels increased nearly threefold in those who remained HIV+ (mean 9.47 [SE 4.78] \times 10^6 to 2.81 [SE 1.13] \times 10^9/ml; P = .02). Among those who became HIV+, HCV RNA levels increased 58-fold (mean 2.86 [SE 1.26] \times 10^6 to 1.86 [SE 0.57] \times 10^9 eq/mL; P = .0001). The rate of increase in HCV RNA levels was steeper faster for HIV+ subjects than for subjects who remained HIV+ (P = .008). HCV RNA levels increased twofold higher in 5 subjects who developed liver failure compared with the 12 who did not (P = .43). HCV RNA levels correlated significantly with CD4 counts (R = -.33, P = .01) and serum aspartate aminotransferase levels (AST) (R = -.36, P = .007). We conclude that HCV RNA levels are significantly higher in HIV+ than in HIV- multi-transfused hemophiliacs. HCV load increases over time, is enhanced by HIV, and further increases as immune deficiency progresses. HCV RNA levels are directly associated with high AST levels. These findings suggest that HIV-induced immune deficiency may promote increased HCV replication.

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non–heat-treated blood products; and had at least 3 serum specimens stored frozen over comparable periods of time. After the 17 HCV+ HIV+ and 17 HCV+ HIV− subjects were identified, we tested interval nucleic acid from serum samples is specifically hybridized to synthetic oligonucleotides and captured onto the surface of a 96-well microplate. Multiple copies of a synthetic branched DNA molecule and a conjugated alkaline phosphatase-labeled probe are then annealed to the immobilized complex (signal amplification) and detected with a chemiluminescent substrate. Because the target is not amplified, the signal is proportional to the physiologic level of target nucleic acid, and the quantity of nucleic acid in the sample can be accurately measured. For samples with values less than the cut-off point of 3.5 × 10^5 genome equivalents/mL, HCV was measured by the polymerase chain reaction (PCR), using nested primers from the 5′ noncoding region of the HCV genome as previously described.1 For quantification estimates and statistical comparisons, PCR-positive samples were assigned a value of 1.75 × 10^6 (one-half the cut-off value for positivity by the bDNA assay), and PCR-negative samples were assigned a value of 0. Mean values and standard errors (SE) were calculated within predetermined time windows. Change in HCV RNA level per unit time for each subject was estimated as the slope between the first and the last specimen. Individual changes over time and between groups were compared with Wilcoxon rank sum tests. Spearman rank order correlations were calculated for simultaneous HCV RNA level, AST level, and CD4 lymphocyte count.

RESULTS

The 17 HCV+ HIV+ subjects had a mean age of 28.6 ± 3.9 (SE) years (median, 26; range, 3 to 62), and the 17 HCV+ HIV− subjects had a mean age of 30.5 ± 4.1 years (median, 27; range, 6 to 64) at the time their baseline assays were performed (median year, 1981 for HIV+ and 1982 for HIV− subjects). HCV+ subjects were observed for a mean of 9.2 ± 0.68 and a median of 9.6 years (range, 4.3 to 14.4 years). HCV− subjects were observed for a mean of 8.9 ± 0.68 and a median of 9.2 years (range, 4.3 to 13 years).

Of the HCV+ HIV+ subjects who were tested pre-HIV seroconversion, 7 were HCV RNA− by PCR, 6 were PCR− but bDNA−, and 3 were bDNA−. The pre-HIV seroconversion sample of 1 subject was unsuitable for testing. Mean HCV RNA levels increased from 2.76 (SE 1.33) × 10^6 to 2.84 (SE 1.39) × 10^6 copies/mL during the first 2 years after HIV seroconversion (P = .006; Fig 1). After the initial increase, HCV RNA levels remained stable during the 2 to 5 years after HIV seroconversion (1.48 [SE 0.56] × 10^6 copies/mL). However, by 5 to 13 years postseroconversion, the HCV RNA mean level had increased markedly to 2.24 (SE 0.84) × 10^7/mL (P = .05).

Baseline HCV RNA levels in the pre-HIV seroconversion samples were not significantly different from the baseline levels in those who remained HIV− (P = .79). Over the entire period of the study, HCV RNA levels increased nearly threefold in those who remained HIV− (mean, 9.47 [SE 4.78] × 10^5 to 2.81 [SE 1.13] × 10^6 copies/mL; P = .02; Fig 2A). Among those who became HIV+, HCV RNA levels increased 58-fold (mean 2.85 [SE 1.26] × 10^7 to 1.66 [SE 0.57] × 10^7 copies/mL; P = .0001; Fig 2B). The rate of increase in HCV RNA levels was eightfold faster for HIV+ subjects (mean, 1.58 × 10^6 copies/mL per year) than for subjects who remained HIV− (mean slope, 1.90 × 10^6 copies/mL per year; P = .009). HCV RNA levels increased twofold faster in the 5 subjects who developed liver failure compared with the 12 who did not (mean slope, 2.44 × 10^6 v 1.22 × 10^6 copies/mL per year; P = .43).

There was a significant correlation between HCV RNA levels and CD4 counts obtained on the 56 dates when both were measured (Spearman R = −.33, P = .01). This correlation was entirely driven by values on 23 dates that were at least 2 years post-HIV seroconversion (R = −.56, P = .006). AST levels also were significantly correlated with HCV RNA levels (R = .36, P = .007) for 53 samples drawn on the same dates as those for HCV RNA levels.

DISCUSSION

Virtually all persons with hemophilia who were transfused with clotting factor concentrates before the implementation of viral inactivation procedures in 1984 became infected with HCV,1,5,7 and the majority remain chronically infected.12 Serum levels of HCV RNA are variably associated with the degree of biochemical and histologic liver inflammation.13,14
and chronic active hepatitis or cirrhosis has been reported in 10% to 20% of multitransfused adults with hemophilia who underwent liver biopsies or who had post mortem examinations.\textsuperscript{15,17}

In our cohort of hemophiliacs in Central Pennsylvania, we have previously reported that 8 (9%) of 91 HCV+ HIV+ acquired immunodeficiency syndrome (AIDS)-free persons compared with none of 58 HCV+ HIV+ persons developed liver failure.\textsuperscript{18} In the present study, we used a quantitative branched DNA-enhanced label amplification assay to compare serial measurements of HCV RNA in a subcohort of age-matched HIV- and HIV+ hemophiliacs. Mean baseline levels of HCV RNA in the two groups were similar. Mean HCV levels increased significantly over the next 5 to 12 or more years in both groups, but the increase was eightfold greater in the HIV+ than in the HIV- group during a period of time when viral inactivated concentrates were in widespread use. HCV RNA levels increased twice as fast in those HCV+ HIV+ individuals who developed liver failure, compared with those who did not, but the sample size was too small to reach significance. One with liver failure had micronodular cirrhosis; another had extensive cholestasis (data not presented). We have not yet observed a case of liver failure during 167 person years of observation in our HCV+ HIV- patients. HCV RNA levels were positively correlated with AST levels in both HIV+ and HIV- patients.

A strong negative correlation was shown between HCV RNA levels and CD4 counts, suggesting that HIV-induced immune deficiency may allow increased HCV replication. Very high viral loads of $2 \times 10^7$ to $3 \times 10^8$/mL were seen only in HIV+ individuals. Thus, HCV may be reactivated or inefficiently cleared in the immune deficient host. Alternatively, immune deficient individuals may be less able to respond to the emergence of certain HCV variants, or may be more susceptible to reinfection from plasma concentrates containing virus below the level of detection. In any case, there is good reason to suspect that HIV+ hemophiliacs are at higher risk for the development of HCV-related liver failure as well as for the transmission of HCV to their sexual partners.\textsuperscript{18}

The increase in HCV RNA levels over several years even in HIV- individuals is of great interest. This suggests that HCV propagates more efficiently over time, implying that the immune system is not adequate to control the infection. T-cell deviations including lower CD4+ percentages and counts and CD56+ natural killer subsets have been previously reported in HIV- persons with hemophilia.\textsuperscript{19}

The pathogenesis of hepatocellular damage by HCV is poorly understood. As with chronic hepatitis B infection, evidence is emerging that liver damage may be mediated by the immune reaction to infected hepatocytes, rather than by the virus itself.\textsuperscript{20} However, the demonstration of HCV antigens in liver biopsies and the correlation between levels of viremia and degree of lobular inflammation suggests that HCV may be directly cytopathic to liver cells.\textsuperscript{13,21,22} Our data provide strong circumstantial evidence that cellular immunity is important in controlling HCV infection, and that HIV-induced immune deficiency may permit increased HCV replication. Confirmation of the possible association of high levels of HCV RNA with more severe liver disease will require analysis of data from our larger multicenter cohort study. However, our findings favor the mechanism of direct cytopathicity rather than cellular immune reactivity as the cause of hepatocellular damage in persons with chronic HCV infection.
Recently, Sherman et al\textsuperscript{22} have also reported that HCV RNA levels measured by the same methodology were higher in 13 HIV\textsuperscript{+} than in 30 HIV\textsuperscript{−} individuals. They did not find a correlation between HCV RNA levels (mean, 3.8 \times 10^7 in HIV\textsuperscript{+} and 1.18 \times 10^5 in HIV\textsuperscript{−} patients) and CD4 counts (range, 292 to 1,024 cells/\muL). However, this is not surprising because our strong negative correlation was driven by lower CD4 counts (median, 201; data not shown) more than 2 years after HIV seroconversion.

Using the same quantitative bDNA assay, Lau et al\textsuperscript{13} have reported median HCV RNA levels of 2.70 \times 10^6 eq/mL in recipients of blood transfusions and 6.3 \times 10^6 eq/mL in health care workers and intravenous drug users who were participating in clinical trials with interferon \alpha. Seven of 13 subjects (54\%) who were bDNA\textsuperscript{+} but PCR\textsuperscript{−} had a sustained response to interferon \alpha, compared with only 4 of 33 who were bDNA\textsuperscript{−}. Fourteen of 17 of our HIV\textsuperscript{−} subjects were bDNA\textsuperscript{−}, and mean levels of 10^7 eq/mL were observed in our HIV\textsuperscript{+} subjects. These observations suggest that sustained remissions with interferon \alpha may be difficult to achieve in HCV\textsuperscript{+} hemophiliacs, particularly those who are HIV\textsuperscript{+}.

In summary, we have shown that HCV RNA levels are significantly higher in HIV\textsuperscript{+} than in HIV\textsuperscript{−} multitransfused hemophiliacs. HCV load increases over time, is enhanced by HIV, and further increases as immune deficiency progresses. HCV RNA levels are strongly associated with AST levels. Some HCV\textsuperscript{+} HIV\textsuperscript{+} hemophiliacs with rapidly progressive liver failure have very high levels of HCV viremia. Our findings emphasize the need for clinical trials to assess the benefit of interferon \alpha in HCV\textsuperscript{+}/HIV\textsuperscript{−} as well as HCV\textsuperscript{+}/HIV\textsuperscript{−} hemophiliacs. This should now be possible using quantitative HCV RNA assays to assess response to therapy.

ACKNOWLEDGMENT

The authors thank Susan Wilson (Atlantic Research Corp) for computer programming, and the investigators from 16 institutions comprising the Multicenter Hemophilia Cohort study listed in Eyster et al\textsuperscript{1} for their helpful comments.

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Increasing hepatitis C virus RNA levels in hemophiliacs: relationship to human immunodeficiency virus infection and liver disease. Multicenter Hemophilia Cohort Study

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