STEATOSIS AND HEPATITIS C IN AN ALASKA NATIVE/AMERICAN INDIAN POPULATION

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ABSTRACT

Objectives. To determine the prevalence and characteristics of steatosis in Alaska Natives/American Indians (AN/AI) with chronic hepatitis C virus (HCV) infection.

Study Design. This outcomes study began in 1994, and 988 AN/AI have been enrolled, including 222 study patients with a positive HCV RNA who underwent liver biopsy.

Methods. Study patients were analyzed for sex, age at biopsy, estimated length of infection, body mass index (BMI), genotype, ethanol use, HCV RNA and alanine aminotransferase levels. A pathologist blinded to patient identity and clinical data reviewed all biopsy slides for histologic activity and fibrosis.

Results. Moderate to severe steatosis was found significantly more often in genotype 3 than in genotypes 1 and 2 (p = 0.008). On multivariate analysis, BMI > 30 and Ishak fibrosis score ≥ 2 were significantly associated with steatosis (p = 0.0013 and 0.0002, respectively), but only genotype 3 was associated with presence of moderate to severe steatosis (p = 0.008).

Conclusions. Our findings in a cohort of AN/AI are consistent with results of previous studies in other groups that steatosis is associated with fibrosis in HCV and infection with genotype 3 is associated with more severe steatosis.

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Keywords: hepatitis C, steatosis, genotype, fibrosis
INTRODUCTION

Steatosis occurs in 50% of liver biopsies in patients with chronic hepatitis C virus (HCV) infection. Risk factors for steatosis in HCV infection include genotype 3, alcohol abuse and those found in nonalcoholic fatty liver disease, which include elevated body mass index (BMI), increasing age, insulin resistance and hypertriglyceridemia.

A number of studies have found an association between steatosis and fibrosis stage (1-7), or between steatosis and necroinflammation in chronic HCV infection (8). Others have found an association between genotype 3 and hepatic steatosis (1, 2, 5-7, 9-10). The development of steatosis in genotype 3 has been attributed to a direct steatogenic effect of the virus, whereas steatosis in genotype 1, and possibly genotype 2, has been attributed to metabolic factors such as insulin resistance and obesity (11).

There have been no published studies on HCV steatosis in Native American people. We therefore conducted a population-based study of chronic HCV infection in Alaska Native and American Indian people (AN/AI), in which we determined the prevalence and associated characteristics of steatosis in persons who underwent liver biopsy.

MATERIAL AND METHODS

Patients

This study began in 1994, and 988 AN/AI with chronic HCV infection have been enrolled. Of these, 709 had a positive HCV RNA by polymerase chain reaction within one year of admission into the study. There were 222 patients who underwent at least one percutaneous liver biopsy, usually as part of evaluation for treatment. All 222 had a positive HCV RNA within one year of biopsy and prior to any treatment. Sixteen patients who underwent liver biopsy prior to 1994 were included, as they were consented for the study and had stored sera available for HCV RNA testing. All patients included were negative for hepatitis B surface antigen and patients who were human immunodeficiency virus positive were excluded. Patient characteristics were compared to those of the 487 HCV RNA positive patients in the long-term study who did not have a liver biopsy.

Approval for this study was obtained from the Alaska Area Native Health Services Institutional Review Board (IRB) in Anchorage, the University of Washington Medical Center IRB in Seattle, the Centers for Disease Control and Prevention IRB in Atlanta, GA, and three Alaska Native health corporation boards. All patients signed an IRB-approved informed consent.

Estimated date of infection

The Alaska Area Native Health Services has a serum bank containing over 600,000 sera collected from previous studies over the past 30 years and that is maintained by the Centers for Disease Control Arctic Investigations Program in Anchorage; many HCV long-term study enrollees have sera stored there. When participants were enrolled in the study for HCV follow-up, their consent was also obtained for anti-HCV testing of stored sera. Available sera were tested in chronologic order to identify the earliest anti-HCV positive date. The estimated date of infection was determined using historical risk factor data and the results from tests of stored sera (12).
Determination of HCV RNA level and genotype

Testing for HCV RNA and HCV genotype was performed at the University of Washington. HCV RNA levels were determined by the branched DNA assay version 2.0 (Bayer Corporation, Tarrytown, NY) and by quantitative Reverse Transcription-Polymerase Chain Reaction. The limit of detection of the branched DNA assay is 200,000 genome equivalents/ml. These results were converted to International Units (IU)/ml using the manufacturer’s recommended conversion factor of 5.8 genome equivalents/ml to 1 IU/ml. For samples negative below this limit, an endpoint dilution assay using Roche Amplicor (Roche Diagnostic Systems, Branchburg, NY) was used to characterize further low-level viremia (limit 100 copies/ml). HCV genotype was performed by restriction fragment length polymorphism analysis of the 5′ non-coding region, as described previously (13).

Histologic evaluation of liver biopsies

All liver biopsy slides were reviewed by a pathologist (HD) who was blinded to patient identity and demographic, clinical and biological data. Histologic activity was evaluated using the Knodell system (14) and fibrosis using the Ishak system (15). Steatosis was graded as 0, 1 (< 30%), 2 (30-65%), and 3 (> 65%). The degree of steatosis was graded by the percent fat present, rather than by systems used for fatty liver disease. The latter systems include necro-inflammatory features which could be misleading in persons with chronic HCV infection, since the pathologist might not be able to tell in any given case whether such necro-inflammatory were secondary to hepatitis C, or steatohepatitis (16,17).

Statistical analysis

Patients were analyzed for sex, age at biopsy date, estimated length of infection, body mass index (BMI) at the time of biopsy, HCV genotype, ethanol use at the time of biopsy, HCV RNA within 1 year of biopsy, alanine aminotransferase (ALT) within 30 days of biopsy, and histologic activity and fibrosis found on biopsy, using bivariate and multivariate analyses. We defined significant ethanol consumption as an average of > 10 grams per day at the time of liver biopsy.

In bivariate analysis, the chi-square test or the Cochran-Armitage trend test was used for comparisons of the proportion of persons with steatosis between two or more groups. When adjusting for a third variable, the Cochran-Mantel-Haenszel test was used. Multivariate analyses of the proportion of persons with steatosis were conducted by use of logistic regression. To examine risk factors for presence of any steatosis, we used purposeful backwards selection, starting with all 10 covariates being evaluated. To examine risk factors for presence of moderate to severe steatosis, only variables with a bivariate P-value < 0.25 were considered for the logistic regression, because of limited sample size. Variables were considered confounders and remained in the model if their exclusion changed the value of the coefficient(s) by more than 15%. Exact P-values (2-sided) were used where appropriate. All statistical analyses were performed using the software SAS (version 8.0, Cary, NC) and StatXact (version 4.0, Cytel Corporation, Cambridge, MA).
RESULTS

Characteristics of patients
Median age, sex, residence (urban versus rural) and genotype distribution were similar in patients who were biopsied versus those who were not biopsied (Table I). Those biopsied had significantly higher viral loads and ALT levels ($p < 0.01$).

Genotype distribution, BMI and steatosis
The genotype distribution of the 222 patients who were biopsied was as follows: 137 (62%) genotype 1, 48 (22%) genotype 2 and 37 (17%) genotype 3. Steatosis was found in 117 (53%) of 222 biopsies. Seventy of 137 (51%) persons with genotype 1, 23 of 48 (48%) with genotype 2, and 24 of 37 (65%) with genotype 3

| Characteristic                        | Biopsied patients (n=222) | Patients not biopsied (n=487) | p-value |
|---------------------------------------|---------------------------|-------------------------------|---------|
| Median age $^a$                        | 41.3 years                | 42.2 years                    | 0.19    |
| Sex (% female)                        | 50% (n = 110)             | 50% (n = 242)                 | 0.97    |
| Residence (% urban)                   | 58% (n = 129)             | 64% (n = 311)                 | 0.14    |
| Genotype $^b$                         |                           |                               |         |
| 1                                     | 62% (n = 137)             | 64% (n = 311)                 | 0.71    |
| 2                                     | 21% (n = 48)              | 22% (n = 109)                 |         |
| 3                                     | 17% (n = 37)              | 14% (n = 66)                  |         |
| 4                                     | 0%                        | 0.2% (n = 1)                  |         |
| HCV infection length (years) $^a$     | 15.0                      | 13.0                          | 0.13    |
| Viral load (IU/ml) $^b$                | 850,000                   | 440,000                       | 0.01    |
| ALT levels (% > 40) $^b$               | 94% (205/219)             | 75% (355/475)                 | < 0.01  |

$^a$ Age and estimated length of hepatitis C virus (HCV) infection at admission date into the long-term HCV follow-up study;

$^b$ Genotype, ALT and viral load testing within one year of admission date into the long-term HCV follow-up study.

Figure 1. The percentage of hepatocytes with fat (steatosis) by HCV genotype.

* $p$-value=0.008 for HCV genotype 3 versus genotypes 1 and 2.
had steatosis (p = 0.10). Grade 1 steatosis was found in 93 (42%) of patients, grade 2 in 20 (9%) and grade 3 in 4 (2%). The degree of steatosis by genotype is shown in Figure 1. Steatosis grade 2 or 3 was found less frequently in persons with genotypes 1 (11/137, 8%) and 2 (4/48, 8%) than in those with genotype 3 (9/37, 24%, p = 0.008).

BMI was available in 166/222 (75%) of participants, of whom 74 (45%) had a BMI ≥ 30. BMI ≥ 30 occurred in 49%, 40% and 33% of persons with genotypes 1, 2 and 3, respectively (p = 0.27). After controlling for BMI (Table II), there was a trend toward persons with genotype 3 being more likely to have any steatosis than those with genotypes 1 and 2 combined (p = 0.07). Among persons with BMI < 30, genotype 3 was associated with an increased likelihood of having any steatosis (p = 0.04). Controlling for BMI, persons with genotype 3 also were more likely to have moderate to severe steatosis than those with genotypes 1 and 2 combined (p = 0.02), as well as those with genotype 1 (p = 0.01).

**Association of factors with steatosis**

BMI (p = 0.003) and Ishak fibrosis score (p = 0.0004) were the only factors significantly associated with any steatosis upon bivariate analysis. Upon multivariate analysis, this association remained with BMI > 30 (p = 0.0013, OR 3.1, 95% CI 1.6-6.1) and Ishak fibrosis score ≥ 2 (p = 0.0002, OR 4.1, 95% CI 2.0–8.5). With BMI and Ishak fibrosis score accounted for, genotype was not associated with the presence of any steatosis (p = 0.24). Upon bivariate analysis, only HCV genotype 3 was associated with the presence of moderate to severe steatosis (p = 0.008). This remained the only significant association upon multivariate analysis (OR 3.0, 95% CI 1.4-6.3). Current alcohol use of > 10 grams/day was not significantly associated with steatosis (p = 0.22), nor were other patient characteristics (Table III).

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**Table II.** Percentage of persons with any steatosis and moderate to severe steatosis, by body mass index (BMI) and hepatitis C virus genotype.

| BMI     | Genotype 1          | Genotype 2          | Genotype 3          | p-value   |
|---------|---------------------|---------------------|---------------------|-----------|
|         | Percentage with any steatosis |                     |                      |           |
| < 30    | 36% (19/53)         | 47% (9/19)          | 65% (13/20)        | 0.07      |
| ≥ 30    | 71% (36/51)         | 54% (7/13)          | 70% (7/10)         |           |
|         | Percentage with moderate to severe steatosis |                  |                      |           |
| < 30    | 8% (4/53)           | 5% (1/19)           | 20% (4/20)         | 0.02      |
| ≥ 30    | 12% (6/51)          | 8% (1/13)           | 30% (3/10)         |           |

* a p-value for genotype 1 and 2 combined versus genotype 3;  
* b Moderate to severe steatosis: ≥ 33%;  
* c p-value for genotype 3 versus genotype 1 = 0.01.
This study of AN/AI found a significant association between the presence of any steatosis and genotype 3 in persons with a BMI < 30, but not in the over-all cohort. Upon multivariate analysis, we found that BMI > 30 and Ishak fibrosis score ≥ 2 were significantly associated with the presence of any steatosis. Before and after controlling for BMI, genotype 3 patients were significantly more likely to have moderate or severe steatosis compared to those with genotypes 1 and 2. In fact, upon multivariate analysis, only genotype 3 was associated with the presence of moderate to severe steatosis. One or both of these findings have been reported in other studies (2, 3, 5, 6, 8, 9).

A review article noted that 12 of 14 studies have reported an association between steatosis and genotype 3 in chronic HCV infection (18). Several studies (1, 5, 7, 9, 19-22) have also reported a significant association between genotype 3 and moderate to severe steatosis, as we did. One study found an association between moderate to severe steatosis and genotype 3 in those with BMI < 25 (19). Our finding of an association with genotype 3 and any steatosis only in patients with a BMI < 30 is consistent with the hypothesis that steatosis in genotype 3 is more likely to be a viral effect, rather than metabolic one (2, 11, 23, 24). In the over-all cohort, we found no significant association between genotype 3 and those with any degree of steatosis. Several studies have found
an association between genotype 3 and low serum cholesterol (19, 21). Testing of cholesterol levels was not performed routinely as part of this study, but further investigation into the metabolic status of the cohort might help explain our findings.

Achievement of a sustained virologic response by treatment of hepatitis C with pegylated, or standard interferon and ribavirin has been shown to cause a significant decrease in hepatic steatosis in persons with genotype 3 (25, 26). However, because of ethical concerns, we did not perform post-treatment biopsies on any of our 55 study patients who were treated, since these are not part of current hepatitis C management guidelines from the American Association for the Study of Liver Diseases and National Institutes of Health (27, 28).

We previously estimated a minimum prevalence of chronic HCV infection in the AN/AI population living in Alaska to be 0.82%, with the highest prevalence, 2.63%, found in those aged 40 to 59 years (29). This is less than the 1.8% prevalence reported for all groups in the United States, based on data from the 1999-2002 National Health and Nutrition Examination Survey (30). However, our findings indicate that the prevalence and characteristics of steatosis in AN/AI with chronic HCV infection are similar to those of other groups studied.

In conclusion, our population-based study in AN/AI supports the findings of previous studies indicating that the presence of steatosis in liver biopsy tissue is associated with an increased risk of liver fibrosis. In addition, we confirmed that infection with HCV genotype 3 is associated with the presence of steatosis, albeit only in persons who are not obese, and, when present, steatosis is more likely to be moderate or severe in degree than when found in the liver biopsies of persons infected with HCV genotypes 1 or 2.

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