Alanine aminotransferase flare-up in hepatitis C virus carriers with persistently normal alanine aminotransferase levels in a hyperendemic area of Japan

HIROFUMI UTO1, JOJI KUROGI2, YUKA TAKAHAMA3, KAZUNORI KUSUMOTO2, KATSUHIRO HAYASHI2, AKIO IDO4, MICHIKORI KOHARA5, SHERI O STUVER6,7, AKIHIRO MORUCHI1, SUSUMU HASEGAWA1, MAKOTO OKETANI1, and HIROHITO TSUBOUCHI1,4

1Digestive Disease and Life-style related Disease, Health Research Human and Environmental Sciences, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima 890-0056, Japan
2Gastroenterology and Hematology, Faculty of Medicine, University of Miyazaki, Kiyotake, Japan
3Miyazaki Prefectural Industrial Support Foundation, Miyazaki, Japan
4Department of Experimental Therapeutics, Translational Research Center, Kyoto University Hospital, Kyoto, Japan
5Department of Microbiology and Cell Biology, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan
6Department of Epidemiology, Boston University School of Public Health, Boston, MA, USA
7Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA

Background. The clinical features of hepatitis C virus (HCV) carriers with persistently normal alanine aminotransferase (PNALT) levels (ALT ≤ 34 IU/l) have not been fully elucidated. We investigated clinical factors associated with ALT flare-up in PNALT individuals in a HCV hyperendemic area of Japan.

Methods. We analyzed 101 HCV carriers who had PNALT between 1993 and 2000. The first occurrence of ALT flare-up (ALT ≥ 35 IU/l) between 2001 and 2005 was evaluated by the Kaplan-Meier method. Multivariate analysis of factors predicting ALT flare-up were conducted using Cox proportional hazards models.

Results. The mean follow-up period was 2.8 years, and the 5-year cumulative incidence of ALT flare-up was estimated to be 31.8%. In multivariate analysis, an ALT level of 20–34 IU/l and a high serum ferritin level (≥ 90 ng/ml) in the most recently available data up to the year 2000, as well as H63D heterozygosity in the HFE gene, were independently and strongly associated with the incidence of ALT flare-up (Hazard ratios = 5.6, 3.1, and 4.8, respectively). In addition, HFE H63D heterozygosity was significantly associated with higher serum ferritin levels in subjects with PNALT (153.8 ± 73.3 ng/ml in subjects with the 63HD genotype vs. 89.4 ± 51.3 ng/ml in subjects with the 63HH genotype, P = 0.043).

Conclusions. HCV carriers with PNALT in this population were at risk for ALT flare-up. Basal ALT levels, serum ferritin levels, and HFE polymorphism are potentially important predictors of ALT flare-up.

Key words: hepatitis C virus, persistent normal ALT, community-based population, ferritin, ALT flare-up

Introduction

Persistent hepatitis C virus (HCV) infection is one of the most common causes of chronic liver disease, liver cirrhosis, and hepatocellular carcinoma (HCC).1–3 The progression of HCV infection to hepatic fibrosis and HCC is associated with several factors, including elevated levels of alanine aminotransferase (ALT), duration of infection, age, and sex.4–7 Short-term studies have shown that 20%–30% of patients with persistent HCV infection have persistently normal serum ALT levels and minimal necroinflammatory changes in the liver. Liver damage in these HCV carriers does not appear to progress to severe hepatitis or HCC.8–10 For this reason, HCV-infected patients with persistently normal ALT (PNALT) are typically not treated for infection or examined by liver biopsy.11,12 However, there have also been reports indicating that hepatic fibrosis can progress slowly even when serum ALT levels remain normal,13,14 suggesting that PNALT patients should be treated and biopsied.15 Recently, Tanaka et al.6 reported that individuals with normal ALT levels are still at risk for developing HCC. These contrasting findings may result from differing clinical definitions of ALT abnormality, the time frame for defining persistence, and patient age at the time of infection or liver biopsy. Because of these ambiguities, the clinical features and disease progression in HCV carriers with PNALT remain unclear and warrant investigation.
ALT reactivation can occur many years after infection in some PNALT patients,\textsuperscript{10,16,17} potentially leading to progressive liver damage. Although the efficacy of combination therapy with interferon and ribavirin or interferon monotherapy in PNALT patients may be similar to that in patients with abnormal ALT levels,\textsuperscript{18,19} these therapies are expensive, effective in only 50% of patients, poorly tolerated, and unsuitable for some patient populations, especially older individuals. Because of this variability, it is important to define the clinical features of HCV carriers with PNALT, especially in older patients. This information will help identify HCV carriers at risk for fibrosis and HCC and help determine the best treatment options.

Since 1993, we have been following HCV-seropositive residents in a hyperendemic area of Japan. Our previous studies of this community-based population showed that abnormal ALT levels (≥35 IU/l) were associated with a fourfold increased risk of HCC.\textsuperscript{20} Because of reports that HCV patients with normal ALT levels are also at risk for HCC, we decided to elucidate the clinical and virological features of HCV carriers with PNALT. The present analysis focuses on ALT flare-up in HCV carriers with PNALT. In addition, the average age of subjects in this study was 71.4 years, which is older than the average age of HCV carriers in the United States. Because it is estimated that the age of HCV carriers in the United States and Europe will increase over the next two or three decades, becoming more similar to the situation in Japan,\textsuperscript{21} this seminal study provides important clinical information applicable to other HCV patient populations.

Methods

Study population

Between 1993 and 1995, we examined 1151 residents who tested positive for anti-HCV antibodies in a hyperendemic area (Town C) of Japan.\textsuperscript{22} The overall prevalence of anti-HCV antibodies was higher (20.6%) in this region than in the surrounding area. As part of a collaborative effort between the University of Miyazaki, the local government, and the public health service, an ultrasonography screening program was started in 1994 to detect HCC in HCV seropositive residents of Town C. In 2001, a clinical research study was initiated in conjunction with the liver disease screening program.

Of these residents, 440 HCV carriers with at least four annual ALT measurements between 1993 and 2000 were included in the present analysis. These subjects tested positive for HCV core antigen (HCVcAg) or HCV RNA at least 6 months after their initial anti-HCV screening and were diagnosed as having persistent HCV infection (HCV carriers) in 1995. Although these subjects included HCV carriers who had taken oral or intravenous medical herbs or other palliative therapies, we excluded those subjects who had received interferon therapy or were diagnosed with HCC before 2000. Subjects with normal ALT levels between 1993 and 2000 were considered to have PNALT in 2000.

Serological studies and viral markers

Between 1993 and 1995, HCV-specific antibodies were detected using a second-generation enzyme immunoassay kit (Immunochek F-HCV Ab, International Reagents, Kobe, Japan). Biochemical tests were also performed to measure levels of ALT (normal value, <35 IU/l), aspartate aminotransferase (normal value, <40 IU/l), and γ-glutamyl transpeptidase (normal values: males, <70 IU/l; females, <30 IU/l) annually from 1993 to 2000. ALT levels in HCV-infected patients can be affected by the progression of liver fibrosis, and platelet counts correlate with the progression of liver fibrosis. However, platelet counts were not obtained before 2001 and could not be included in this study. Serum levels of HCVcAg were determined by a fluorescence enzyme immunoassay (Immunochek F-HCV Ag Core, International Reagents),\textsuperscript{23} with a detection threshold of 8 pg/ml of HCVcAg. For anti-HCV antibody-positive residents with HCVcAg levels below 8 pg/ml, HCV RNA was examined in 1995 by a qualitative reverse transcription polymerase chain reaction (PCR) assay (Amplicore HCV, Roche Diagnostics, Tokyo, Japan). The serologically defined genotype (serotype) of HCV was determined using a serological genotyping assay kit (Immunochek F-HCV Grouping, International Reagents). We also examined patient ferritin levels (normal values: males, ≥24 and ≤286 ng/ml; females, ≥7 and ≤110 ng/ml) using serum stored from 1996 to 2000.

Mutational analysis of the HFE gene

Mild to moderate iron overload is associated with liver injury in patients with chronic hepatitis C. HFE mutations could be associated with excess iron loading in patients with chronic hepatitis C. We determined whether HFE mutations were associated with ALT flare-up in subjects with PNALT. The following three major point mutations in HFE have been associated with hereditary hemochromatosis: cysteine to tyrosine at amino acid 282 (C282Y), serine to cysteine at amino acid 65 (S65C), and histidine to aspartic acid at amino acid 63 (H63D). To test for these mutations in PNALT HCV carriers, genomic DNA was extracted using a MagExtractor System MFX-2000 (Toyobo, Osaka, Japan), according to the manufacturer’s protocols.