We evaluated the utility of alanine aminotransferase (ALT) measurements in predicting the incidence of hepatocellular carcinoma (HCC) in a cohort of 667 adults with chronic hepatitis C virus (HCV) infection from a community-based population in Japan, between 1994 and 2003. Cox proportional hazards regression analysis was used to describe the relationship between prediagnostic levels of ALT and the rate of HCC, after adjusting for age and gender; hazard ratios (HRs) and 95% confidence intervals (CIs) were obtained. Over an average of 8 years of follow-up, 52 HCC cases were identified. A significant association between a 20 IU/L difference in higher ALT level and subsequent HCC incidence was observed (HR = 1.2; 95% CI: 1.1, 1.3). An abnormal ALT level (>35 IU/L) increased the HCC rate by 4-fold compared to a normal ALT level (HR = 4.1; 95% CI: 2.1, 8.4). Among 551 subjects with at least 4 repeated measurements of ALT, those with persistently abnormal ALT levels (n = 118) had a strong, significantly increased HCC rate compared to those with persistently normal ALT levels (n = 296) (HR = 23.2; 95% CI: 3.0, 178.5). This study demonstrates that elevated ALT levels, measured on an average of 8 years before HCC diagnosis, predict an increased rate of HCV-associated HCC in a community-based population and that utilizing serial measurements to identify persistent ALT abnormality may be useful in determining HCC risk.

Key words: alanine aminotransferase; chronic hepatitis C virus infection; hepatocellular carcinoma

Hepatitis C virus (HCV) infection has become a major public health concern, with ~170 million persons chronically infected worldwide. Approximately 70% of those acutely infected will become persistently infected with HCV, leading to an increased risk of liver fibrosis, liver cirrhosis and development of hepatocellular carcinoma (HCC). Furthermore, with an increasing global incidence of ~80% over the past 20–30 years, HCC has become the fifth most common cancer and ranks third with respect to cancer-related mortality. In developed countries, including the United States and Japan, HCV infection has been identified as the main risk factor for HCC. Although the exact pathogenesis of HCV-associated HCC is unknown, chronic inflammation related to the immune response to HCV infection, with a resultant increased proliferation of hepatocytes, likely promotes carcinogenesis in the liver. Consequently, this process of persistent injury and regeneration creates a procarcinogenic environment in which frequent genetic mutations and/or instability are common. Recently, Tanioka et al. reported that an elevated serum alanine aminotransferase (ALT) level at blood donation was positively associated with subsequent HCC risk among HCV-infected donors in Japan. ALT is an enzyme present in the liver, which is released into the blood stream with increasing liver tissue damage, and thus represents activated inflammatory necrosis of hepatocytes. Other studies in Japan have shown that sustained elevated ALT levels in HCV-infected liver disease patients lead to increased HCC risk and recurrence of HCC.

Since most individuals infected with HCV remain asymptomatic, studies based on clinic patients may overestimate the true effect of ALT on HCC risk. In contrast, the community-based setting offers a unique advantage, in that it is mostly composed of infected individuals who are asymptomatic, minimizing the bias introduced by using patients who may have more severe liver disease. We conducted a prospective study to examine the utility of ALT measurements in predicting the incidence of HCC in chronically HCV-infected subjects in a community-based population in Japan.

Material and methods

Study Population

Subjects in this study were participants in a community-based cohort study conducted within the adult population of Town C in southwestern Miyazaki Prefecture, Japan; characteristics of the study population have been described previously. Briefly, beginning in 1993, anti-HCV positive residents have been identified in conjunction with the annual government-sponsored general health examinations conducted in Town C. As of 1994, such residents have been invited to take part in a liver disease screening program to monitor HCC development. The screening program involves annual ultrasonographic liver disease examinations, which was augmented with a self-administered questionnaire and collection of blood sample since 2001. Serum markers of liver disease are measured in the blood samples provided (e.g. ALT). Additional measurements of ALT were available, beginning in 1993, for the study subjects who attended the government-sponsored general health examinations.

For the present analysis, the follow-up period started at the date (year) of first ALT measurement (baseline) and ended at the year of diagnosis of HCC, year of death, or December 31, 2003, whichever came first. There were no subjects lost to follow-up. Subjects without evidence of chronic HCV infection were not included in the present analysis. Chronic HCV carriers were defined as persons with at least 1 HCV RNA or HCV core antigen positive result, between 1995 and 2003. However, individuals with a positive RNA or core antigen result followed by 2 consecutive negative results were considered not to be chronic carriers.

A total of 52 incident HCC cases occurring between the years 1994 and 2003 were included in this analysis. Suspected liver cancer cases were identified at the liver disease screenings, and the diagnosis of HCC was subsequently confirmed by their primary physicians. For 40 cases of HCC, the diagnosis was determined on the basis of information collected via biopsy and/or imaging analysis using magnetic resonance imaging, computed tomography scan, angiography or ultrasonographic tomography. An additional 12 HCC cases were identified based on death certificate information, which was obtained from routine searches of vital statistics records that

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are collected and maintained by the Municipal Public Health Department; for these cases, the year of death was used as the year of diagnosis. For 4 HCC cases, HCV viremia status could not be determined; however, since all 4 cases were anti-HCV seropositive, HCV chronicity was assumed.

Laboratory assays

Anti-HCV antibodies were measured by a third-generation enzyme-linked immunosorbent assay (Lumipulse Ortho II; Ortho-Clinical Diagnostics, K.K., Tokyo, Japan). Specimens were tested for HCV RNA using a reverse-transcriptase polymerase chain reaction assay (Amplicore HCV, Nippon Roche, Tokyo, Japan). Between 1995 and 2001, HCV core antigen level was measured by a fluorescent enzyme immunoassay (Immunocheck F-HCV core antigen; Koka-sai Shiyaku, Kobe, Japan); starting in 2002, an immunoradiometric assay replaced the fluorescent enzyme immunoassay to measure HCV core antigen (Ortho HCV Ag IRMA Test; Ortho-Clinical Diagnostic, K.K., Tokyo, Japan).

Statistical analyses

The incidence of HCC was evaluated in the 667 adult residents of Town C who were determined to be chronically infected with HCV. Cox proportional hazards regression was used to describe time-dependent variable based on multiple ALT values of Town C who were determined to be chronically infected with HCV. Cox proportional hazards regression was used to describe the relationship between prediagnostic levels of ALT and the rate of HCC. Hazard ratios (HRs) and associated 95% confidence intervals (95% CI) were computed, while adjusting for age group and gender. The first available ALT measurement was evaluated as both a continuous and a dichotomous (≥ 35 IU/L vs. < 35 IU/L) variable. In addition, a time-dependent variable based on multiple ALT values obtained between 1993 and 2002 was also assessed as both a continuous and dichotomous (≥ 35 IU/L vs. < 35 IU/L) factor. In Instances when ALT values were not available from consecutive years, the last observed value was carried forward until the next available measurement. ALT measurements obtained during the same calendar year as HCC diagnosis were excluded. For the subset of 551 subjects who had at least 4 ALT measurements, 2 mutually-exclusive groups were evaluated: (i) persistently abnormal ALT (all values ≥ 35 IU/L, n = 118), (ii) persistently normal ALT (all values < 35 IU/L, n = 137), and (iii) fluctuating ALT (n = 296). We examined the association between ALT group and HCC incidence following the fourth ALT measurement. The SAS statistical program (v. 8.0) was used in all analyses, the p-values quoted are 2-sided, and the statistical significance was set at p < 0.05.

Results

Among the 667 subjects analyzed, 52 HCC cases occurred over an average of 7.9 years of follow-up, representing a total of 5,292 person-years. The study population had a mean age at baseline of 62.6 years (range: 34–90) and was comprised of 288 (43.2%) men (Table I). Men experienced a rate of HCC incidence that was 3-fold higher than that of women (HR = 3.0; 95% CI: 1.7, 5.4). There was a 20% increase in the HCC incidence rate associated with a 10-year increase in age (HR = 1.2; 95% CI: 0.9, 1.7), which was not statistically significant (p = 0.16). Subsequent Cox models were adjusted for gender and categories of age at first ALT measurement.

After adjusting for categories of age and gender, a 20 IU/L difference in baseline ALT was associated with a statistically significant 20% increase in the rate of HCC incidence (Table II). When baseline ALT was dichotomized, an abnormal ALT level (≥ 35 IU/L) increased the rate of HCC by 4 times compared to a normal ALT level (< 35 IU/L). In comparison, when ALT was examined as a time-varying covariate based on measurements obtained between 1993 and 2002, the same effect for a 20 IU/L increased difference in ALT was observed, and the higher HCC rate associated with abnormal ALT (≥ 35 IU/L) was only slightly attenuated (Table II). When we examined the relationship by length of follow-up, we observed a similar 3- to 4-fold association between an abnormal baseline ALT level and HCC development for regardless of length of follow-up (Table III). A sensitivity analysis was performed to examine the validity of the assumption of chronic HCV infection in the 4 HCC cases from which viremia information was not available. Excluding these 4 HCC cases did not remarkably change the effect estimate of elevated ALT on the hazard of liver cancer (data not shown).

Within the sub-group of 551 subjects with at least 4 ALT measurements, 26 HCC cases occurred over an average of 5.3 years of follow-up. The characteristics of these subjects were the same as those of the total cohort studied (data not shown); of note, subjects with persistently abnormal ALT were more likely to be younger and to be men compared to subjects with persistently normal ALT (p < 0.05 and p < 0.0001, respectively). There was a strong significant age- and gender-adjusted increased rate of HCC associated with persistently abnormal ALT compared to those with persistently normal ALT (Table IV). Subjects with fluctuating ALT experienced a three-fold greater age- and gender-adjusted rate of HCC incidence compared to subjects with normal ALT, although the association was not statistically significant (p = 0.3) and very unstable as evidenced by the wide 95% CI.

**TABLE I – BASELINE CHARACTERISTICS OF HCV CHRONICALLY INFECTED PARTICIPANTS**

| Characteristic | Total n (%) |
|---------------|-------------|
| Age (years)   |             |
| <50           | 78 (11.7)   |
| 50–59         | 124 (18.6)  |
| 60–69         | 305 (45.7)  |
| ≥70           | 160 (24.0)  |
| Male          | 288 (43.2)  |
| Mean ALT (SD) | 45.5 (41.5) |
| <35 IU/L      | 349 (52.3)  |
| ≥35 IU/L      | 318 (47.7)  |
| Mean years of follow-up (SD) | 7.9 (2.9) |

n = 667; Town C HCV Study, 1994–2003; SD, standard deviation.

**TABLE II – HAZARD RATIO ESTIMATES FOR ALT AS CATEGORIZED AND HCC INCIDENCE AMONG SUBJECTS WHO ARE HCV CHRONICALLY INFECTED**

|                      | HR1 | 95% CI          |
|----------------------|-----|-----------------|
| ALT level (20 IU/L increase) |     |                 |
| Baseline             | 1.2 | (1.2–1.3)       |
| Time-varying         | 1.2 | (1.1–1.2)       |
| Abnormal ALT (≥ 35 IU/L) |     |                 |
| Baseline             | 4.0 | (2.1–7.9)       |
| Time-varying         | 3.7 | (1.9–7.3)       |

ALT, alanine aminotransferases; HR, hazard ratio; CI, confidence interval.

Town C HCV Study, 1994–2003.

1Adjusted for age groups and gender.

**TABLE III – HAZARD RATIO ESTIMATES FOR ABNORMAL BASELINE ALT (≥35 IU/L), STRATIFIED BY LENGTH OF FOLLOW-UP**

|                      | HR1 | 95% CI | n (HCC) |
|----------------------|-----|--------|---------|
| Follow-up ≥1         | 4.2 | (2.1–8.1) | 667 (52) |
| Follow-up > 1        | 3.7 | (1.8–7.2) | 647 (47) |
| Follow-up ≥2         | 3.1 | (1.5–6.2) | 617 (41) |
| Follow-up > 3        | 3.4 | (1.6–7.2) | 598 (35) |
| Follow-up > 4        | 4.5 | (1.8–11.2) | 576 (29) |
| Follow-up > 5        | 4.3 | (1.6–11.7) | 552 (23) |
| Follow-up > 6        | 3.1 | (1.0–10.0) | 519 (15) |
| Follow-up > 7        | 2.5 | (0.6–10.0) | 483 (9)   |
| Follow-up > 8        | 3.2 | (0.3–31.2) | 423 (4)   |

HR, hazard ratio; CI, confidence interval; HCC, hepatocellular carcinoma.

1Adjusted for age groups and gender.
ALT, alanine aminotransferases; HR, hazard ratio; CI, confidence interval.

| ALT classification | HR  | 95% CI     |
|--------------------|-----|------------|
| Persistently normal | 1.0 |            |
| Fluctuating         | 2.9 | (0.4–23.7) |
| Persistently abnormal | 19.8 | (2.6–152.6) |

**Discussion**

In this large, prospective, community-based study, elevated ALT level predicted an increased rate of HCC-associatted incident HCC during a mean follow-up of almost 8 years. The present findings are consistent with the hypothesis that the relative risk of HCC increases with severity of liver damage, as indexed by elevated ALT. Similar results were reported by Tanaka et al. for anti-HCV seropositive blood donors, aged between 16 and 64 years, in Japan. In that study, the investigators found that, compared to subjects with an ALT of ≤29 Karumun Units, subjects with a higher ALT experienced a significantly greater risk of developing HCC over a mean follow-up of 8 years. An association of higher ALT level and HCC incidence was observed even within the normal range of values utilized in the study by Tanaka et al. (HR = 6.23; 95% CI: 2.7–13.5). Although direct comparisons with the results presented by Tanaka et al. were not possible due to differences in the normal cut-off values used, a significantly increased rate of HCC associated with ALT levels between 20 and 34 IU/L compared to lower levels was not observed in the present study (HR = 1.5; 95% CI: 0.3–7.0).

Chronic injury of hepatocytes in subjects infected with HCV is largely a result of both virus-specific and virus-nonspecific immune responses. This chronic inflammation contributes to the procarcinogenic environment by causing ongoing regeneration and proliferation of hepatocytes, which invariably increases genetic instability. However, for hepatocarcinogenesis to occur, these accumulating genetic alterations must lead to a malignant transformation. The resulting activated necrosis of hepatocytes can be measured by serum ALT. Of note, the predictive capacity of abnormal ALT increased almost 6-fold when serial measurements of ALT were used to identify subjects with consistently elevated ALT, relative to using one-time, baseline ALT measurement only.

It is known that ALT levels begin to decrease with greater liver injury as the damaged hepatocytes become unable to produce ALT. Thus, those subjects who developed HCC after a relatively shorter follow-up time might be misclassified as having normal ALT related to underlying liver damage. As a result, the estimate of the effect of elevated ALT on HCC incidence could have been underestimated in the present study. However, when the analysis was stratified by length of follow-up, an abnormal baseline ALT level was consistently associated with a 3- to 4-fold increased rate of HCC development regardless of length of follow-up. Therefore, it is unlikely that the estimate of association observed was attenuated as a result of more severe liver injury with concomitant lower ALT among the HCC cases.

There were several limitations to the present analysis. We were unable to adjust for interferon treatment, since this information was not available for all subjects. It is possible that the association of ALT with the rate of HCC development may differ by interferon treatment status. Several studies have shown that interferon treatment reduces HCC risk among subjects infected with HCV and, in some instances, independent of its effect on HCV RNA clearance. Ikeda et al. reported that, regardless of HCV RNA clearance among subjects with persistently normal ALT, the rate of HCC development was significantly lower among patients treated with interferon therapy compared to untreated patients. Thus, by ignoring treatment history, the estimate of the association between ALT levels and HCC incidence may have been overestimated in the present study if study subjects with normal ALT were more likely to have been previously treated with interferon than subjects with abnormal ALT. However, among subjects in the present study with information on interferon treatment (n = 405), normal baseline ALT was associated with a lower likelihood of being ever treated with interferon compared to abnormal baseline ALT (data not shown). We also did not have information on history of heavy alcohol consumption at baseline, which is known to be associated with an increased risk for the development of HCC. However, since alcohol does not appear to increase the risk for HCC through mechanisms other than ones involving liver injury, not adjusting for this risk factor is unlikely to have biased the observed effect estimates.

ALT levels are known to be associated with HCC; however, the relationship has not been described prospectively in a community-based setting. The present study demonstrates that elevated ALT levels, measured on average 8 years before cancer diagnosis, were associated with an increased rate of HCC among subjects chronically infected with HCV. Furthermore, utilizing serial measurements to identify persistent ALT abnormality revealed a stronger association with HCC risk. The results of this study demonstrate the utility and effectiveness of clinically available data in HCV endemic regions to minimize HCC-associated morbidity and mortality.

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