

Long-Term Risk of Hepatocellular Carcinoma in Patients with Chronic Hepatitis B with Normal Alanine Aminotransferase

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Abstract

Background: The Alanine Aminotransferase (ALT) level is considered as a risk factor for the progression to liver cirrhosis and Hepatocellular Carcinoma (HCC) in patients infected with Hepatitis B Virus (HBV) and remains a subject of debate.

Methods: We prospectively compared the incidence of HCC between HBV infected patients with normal ALT and those with elevated ALT.

Result: A total of 378 HBV-infected patients (102 with normal ALT and 276 with elevated ALT) were included. The median follow-up period was 8.2 years. The incidence rates of HCC development were significantly lower in the normal ALT patients than in patients with elevated ALT (0.55 vs 2.20 per 100 person-years, $P = 0.021$, while the incidence rates of hepatic decompensation (0.43 vs 1.23 per 100 person-years) and survival (53.8% vs 47.4% at 10 years) did not significantly differ between the two groups (Kaplan-Meier analysis). The main causes of death were on-hepatic diseases in patients with normal ALT. Multivariate Cox analyses model revealed that the risk of HCC was lower in patients with normal ALT than in patients with elevated ALT (hazard ratio (HR), 0.28, Confidence intervals (CI) (0.14-0.78)), while the risk of hepatic decompensation and mortality was the same in the two groups of patients.

Conclusion: The risk for HCC and liver decompensation normal ALT was markedly reduced in HBV-patients with normal ALT. Aged patients with HBV with normal ALT should therefore maintain long-term surveillance for HCC. Future studies aimed to better identify those with remaining long-term risk for HCC are needed.

Hepatitis B Virus (HBV) infection is a major risk factor for the progression to cirrhosis and Hepatocellular Carcinoma (HCC) worldwide [1]. Patients with liver cirrhosis have a higher risk of developing HCC than patients with less advanced fibrosis [2,3]. Patients with chronic HBV infection are at risk of developing adverse outcomes, including cirrhosis and (HCC), with an estimated lifetime risk of 25%-40% [4-6].

Several studies have identified HBV-related factors as key predictors of HCC development [7]. Hepatitis B Virus e Antigen (HBeAg) seropositivity [8,9], high viral load [10,11], and genotype C [12,13] are independent predictors of HCC development. In addition, hepatitis B viral load was found to be correlated with the risk of progression to cirrhosis [14]. Results from cohort studies have shown that a higher HBV DNA level is associated with a higher HCC risk [15]. However, in patients with an HBV DNA level <2000 IU/mL (low viral load), further categorized viral loads play an insignificant role in predicting HCC, and the HBsAg level becomes the only predictive biomarker [14,16,17]. More specifically, a higher HBsAg level (≥ 1000 IU/mL) is associated with a greater risk of HCC in HBV e antigen (HBeAg)-negative patients with low viral loads [18].

Since 1950s, serum Alanine Aminotransferase (ALT) levels were used as a surrogate marker for non-A, non-B hepatitis among blood donors before identifying Hepatitis C Virus (HCV) [19]. The importance of ALT levels in the progression of Hepatitis B Virus (HBV) infection remains not well investigated. However, Serum alanine aminotransferase level is a valid and sensitive indicator of liver-cell damage [20,21]. ALT is an important parameter for screening, diagnosis and follow-up of liver diseases [22,23]. The aim of this study was to prospectively evaluate the long term effect of ALT on the risk of developing HCC and liver decompensation in a cohort of 378 HBV patients.

Patients and Methods

Patients

The study population comprised naïve patients with compensated HBV infection who visited

our hospital between January 2000 and December 2006. Cirrhosis was diagnosed on the basis of the results of histological examination or the combined results of clinical and imaging examinations. Patients should meet the following inclusion criteria: (i) positive serological test for HBsAg; (ii) absence of HCC at the time of presentation; (iii) naïve of HBV treatment and (iv) a follow up for more than 6 months after the liver biopsy or non-invasive liver tests. At the presentation, information on alcohol consumption habits was obtained through an interview conducted by the physicians.

The exclusion criteria included other coexistent causes of chronic liver disease such as cirrhosis, positive anti-HCV, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, Wilson's disease, hemochromatosis, and Budd-Chiari syndrome; presence of a habit of alcohol consumption together with HBV infection; Habitual drinking was defined as an average daily consumption of an amount equivalent to 40 g/day of pure ethanol for women and 60 g/day for men over a period of more than 4 years. a history of interferon therapy for HBV infection; and presence or history of HIV infection. Patients were enrolled at the time of diagnosis of presentation to care.

Here we used the HEPALIST cohort, a large population of patients admitted for care in Beaujon Hospital (Paris area, France). We extracted 617 treatment-naïve patients with HBV infection and evaluated the effect of normal ALT or elevated ALT on the occurrence of HCC and hepatic decompensation. The study protocol conformed to the ethical guidelines of the 1964 Declaration of Helsinki revised at 2013 and French legislation and informed consent was obtained from each patient at the presentation.

Serological tests and serums quantification

Serum samples at enrollment were routinely tested for viral markers including HBsAg, HBeAg, anti-HBc, anti-HBs and anti-HBe using standard procedures (AXSYM system, Abbott, France). Serum ALT measurements were performed on a Hitachi 911 automat using Boehringer–Mannheim reagents. The threshold of 40 IU/l was used as the upper normal range. This threshold, given by the manufacturer, was calculated as the mean \pm 2 SD value obtained from a control population.

The HBV DNA in the serum was routinely determined with the Versant HBV DNA Assay (bDNA) (Bayer, France) with a sensitivity of 8×10^5 HBV copies/ml for patients enrolled before 2001. After 2001, serum HBV DNA quantification was performed by PCR according to the manufacturer's instructions (CobasAmplicor HBV MONITOR) (Roche Diagnostics Systems, Meylan, France). The assay is based on amplification of a known standard amount added to each test tube. The sensitivity of the assay is 200 copies/ml.

Surveillance

The standard surveillance was made for all patients including laboratory tests every 1-3 months, imaging examinations with abdominal ultrasonography using a high-resolution and real-time scanner for HCC surveillance, and upper endoscopy every 6-12 months or at the time of upper digestive hemorrhage for patient with advanced disease. All patients were tested for serological markers (HBsAg, HBeAg, anti-HBe, antibodies against HCV [anti-HCV], and antibodies against HDV [anti-HDV]) and had liver function

tests performed and α -Fetoprotein (AFP) levels measured at baseline. Throughout the follow-up period, if the Alanine Aminotransferase (ALT) levels remained within normal limits, liver enzyme and AFP levels were assayed every 6 months and, if the ALT levels were elevated, at least every 3 months. Serum samples collected at each visit were stored at -20°C until analysis.

Treatments

Patients were considered for treatment when they had HBV DNA levels above 2000 IU/ml, serum ALT levels above the Upper Limit of Normal (ULN) and advanced liver disease assessed by liver biopsy (or two non-invasive markers once validated in HBV infected patients) showing moderate to severe active necroinflammation and/or at least moderate fibrosis according Metavir Score. However, in patients who fulfill the above criteria for HBV DNA and histological of advanced liver disease, treatment may be initiated even the ALT levels are persistently normal and HBVDNA levels ≥ 5.0 log copies/ml. Hepatocellular carcinoma was treated by surgical resection, percutaneous radiofrequency ablation, percutaneous ethanol injection, or transcatheterarterial chemoembolization, depending upon the stage of HCC and liver function.

Prognostic factors

The following variables were assessed as potential predictors of HCC, hepatic decompensation, and mortality: age, sex, aspartate aminotransferase level, albumin level, total bilirubin level, total cholesterol level, platelet count, α -fetoprotein level, diabetes mellitus (presence or absence), HBV-DNA level and Prothrombin time. Diabetes mellitus was diagnosed on the basis of the history of medical treatments for the disease or fasting blood glucose levels (126 mg/dL or more).

Diagnosis of HCC, Follow-up, and Endpoints of the Study

Hepatocellular carcinoma was diagnosed on the basis of the results of histological examinations or typical findings of imaging examinations conducted using contrast agent early enhancement during the arterial phase and washout during the delayed phase in combination with alpha-fetoprotein serum. Hepatic decompensation was defined as one or more of the following manifestations: ascites, jaundice (serum bilirubin level, ≥ 3 mg/dL), hepatic encephalopathy, or rupture of gastro esophageal varices. Ascites was diagnosed by physical and imaging examinations. Hepatic encephalopathy was diagnosed on the basis of clinical examination and the results of the physical and laboratory examinations. Rupture of gastro esophageal varices was confirmed by endoscopy. For patients who were no longer being followed up, complementary data on HCC, hospitalizations due to liver-related causes, or death, were retrieved from registries concerning follow-up information or by contacting the patient himself or his primary care physician.

Statistical analyses

Continuous variables are presented as mean (SD) or median (range) and categorical variables as frequencies (percentages). Two tests were used: Student t test and χ^2 test. The effect of age (50-59 vs <50 years or >60 vs <50 years), albumin level, diabetes (presence or absence), gender (male vs female), HBV genotype (genotype D vs genotype non D), total bilirubin level, total cholesterol level, platelet

Table 1: Baseline Characteristics of 378 patients according ALT serum level.

Characteristics	Normal ALT	Elevated ALT		P-value
	All (n=378)	n=102	n=276	
Men	249	70 (69%)	179 (65%)	0.328
Age (Median)	51 [43-63]	49 [43-57]	54 [45-63]	0.022
BMI (kg/m ²)	25	25 [23-27]	25 [22-29]	0.105
History Alcohol	58 (15%)	14 (14%)	46 (24%)	0.072
Diabetes	38 (10%)	8 (8%)	30 (11%)	0.469
Albumin (g/l)	42 [38-45]	42 [39-44]	41 [38-45]	0.071
AFP(μg/l)	7 [4-12]	8 [5-12]	7 [4-12]	0.239
Bilirubin (μmol/l)	16 [10-16]	15 [10-18]	16 [12-20]	0.056
Platelets (x10 ⁹ /L)	157 [134-207]	159 [145-207]	156 [134-187]	0.043
Prothrombin (%)	82 [69-92]	83 [78-92]	81 [69-94]	0.065
HBV-DNA (log copies/ml)	3,9 [3-5]	3,3 (3-5)	4,2 (2,7-6)	0.122
Genotype D	170 (45%)	68 (67%)	144 (52%)	0.030
Genotype non-D	208 (55%)	34 (33%)	132 (48%)	
F1-2	309 (82%)	86 (84%)	219 (79%)	0,085
F3-4	73 (19%)	16 (16%)	57 (21%)	
Total cholesterol (g/l)	1,7 [1,2-2,9]	1,6 [1,1-2,2]	1,7 [1,0-2,9]	0.044

Data are presented as No. (%) unless otherwise specified. Abbreviations: IQR: Interquartile Range; BMI: Body Mass Index was calculated as weight divided by height squared (kg/m²); SD: Standard Deviation; Statistically significant (P < .05).

count, α-fetoprotein level, HBV-DNA level (≥5 vs ≤5 log copies/ml) and Prothrombin time on incidence of HCC or any hepatic decompensation (ascites, hepatic encephalopathy, or rupture of gastro esophageal varices and jaundice) was tested by Cox regression. The incidence of HCC and hepatic decompensation relation with persistent normal ALT status was estimated as the number of events occurring during normal ALT time and elevated ALT time divided by the corresponding person-time at risk in the groups. The effect of persistent normal ALT was analyzed by Cox regression. The time scale used was calendar time since 1 January 2000 and antiviral therapy was considered as a time-dependent covariate. Models were also tested with adjustment for age, sex, and diabetes, HBV DNA levels and HBV genotype. Results are presented as Hazard Ratios (HRs) together with 95% confidence intervals, estimated using the profile likelihood method. Survival curves with respect to normal ALT status were estimated from the cumulative hazard functions for normal ALT and elevated ALT follow-up time obtained from the Cox regression models, and significant differences were assessed by testing

Table 2: Incidence Rates of HCC and hepatic decompensation and overall death per 100 Person-Years for patient with normal ALT and for those with elevated ALT Person-Time.

Endpoints	Normal ALT			Elevated ALT			(Normal ALT vs Elevated ALT)	
	Event	PY	Rate	Events	PY	Rate	HR (CI95%)	P value
Any events	7	734	0.91	31	1017	3.04	0.42 (0.16-0.98)	0.001
HCC	4	721	0.55	19	867	2.2	0.28 (0.14-0.78)	0.021
HD	3	692	0.43	13	1003	1.29	0.38 (0.11-0.88)	0.037
Death/LT	4	734	0.52	22	1017	2.16	0.33 (0.18-0.68)	0.002

HD: Hepatic Decompensation; PY: Person-Year; LT: Liver Transplantation and CI_{95%}: Confidence Intervals; Hazard ration for normal ALT vs elevated ALT serum.

HR=1 using Wald tests. All tests were 2-sided and a P value of <.05 was considered statistically significant. Data analysis was performed with SAS 9.3 software (SAS Institute, Cary, North Carolina).

Results

A total of 378 patients with HBV infection were enrolled and followed up for up to 8.4 years (mean, 6 [SD, 2.8] years), consisting of 102 patients with persistent normal ALT and 276 patients with elevated ALT level. The occurrence of HCC was 4 (3.9%) and 19 (6.9%), respectively. Characteristics and data from the 378 patients are summarized in Table 1.

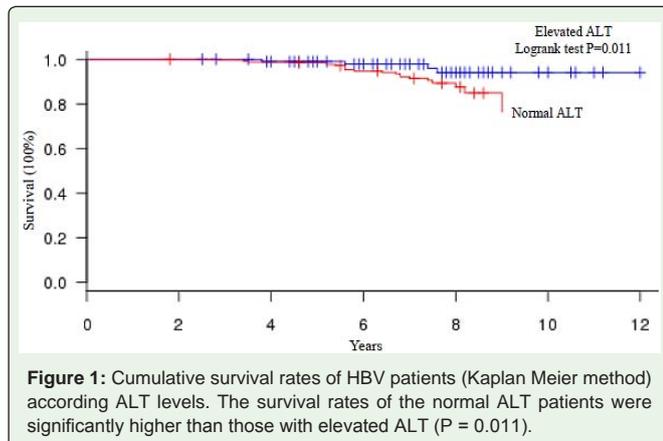
Incidence of HCC

Four (3,9%) of the 102 patients with persistent normal ALT developed HCC during follow-up (after mean 6.4 [SD, 2.3] years of follow-up), corresponding to an incidence of 0.55 per 100 person-years (PY; Table 2). HCC was diagnosed in 1 patient within 2 year after enrollment (19 months), and the other 3 at 5.6, 7.4, and 7.6 years in patients with normal ALT. ALT serum level was tested at the diagnosis of HCC in 3 of these patients, and they were all under the limit of detection. Among the 4 patients with HCC who had normal ALT, all were male, 3 had diabetes mellitus, and 1 had history of alcohol abuse. Three patients had genotype D and 1 had non-genotype D; the incidence rate for HCC was significantly higher in elevated ALT person-time with 2.20 per 100 PY as compared to normal ALT time (0.55 per 100 PY). Only age and sex were found to be baseline factors significantly affecting the incidence of HCC (age 50-59 vs <50 years: HR, 2.45 [95% confidence interval {CI}, 1.16-5.61], P =0.027; age >60 vs <50 years: HR, 3.32 [95% CI, 1.48-7.90], P = 0.007; male vs female: HR, 2.09 [95% CI, 1.06-4.62], P = 0.014).

Hepatic decompensation

In patients with normal ALT, 3 (2.9%) developed ascites, none hepatic encephalopathy, and none variceal bleeding during follow-up. Ascites was diagnosed 5.3, 6.1 and 7.2 years following the enrollment. One of these patients developed HCC, all were males, and none had diabetes mellitus. However, all these patients had advanced liver disease at the time of inclusion. The risk of developing any hepatic decompensation was significantly lower in normal ALT time than in elevated ALT person-time HR, 0.50 [95% CI, 0.11-0.88], P = 0.037.

The Figure 1 shows the cumulative risk of any event (HCC and any hepatic decompensation). The incidence rate for normal ALT time and elevated ALT person-time was 0.91 and 3.04 per 100 PY, respectively. The risk for any event was significantly lower in normal ALT person-time compared to elevated ALT person-time (P = 0.012).



Overall deaths

Three (3%) patient with normal ALT died during follow-up, one died from liver-related cause with HCC. The cause of death for the other 2 patients underwent liver transplantation. Twenty-two (8%) patient's died in the group of patients with elevated ALT. The causes of liver complications related were reported in 7 patients and other causes for 5 patients. Thus, 14 patients (4%) underwent liver transplant. The incidence rate for overall death was 0.52 per 100 PY in patients with normal ALT patients. Significantly lower incidence rate for overall death was seen in normal ALT with 0.52 per 100 PY, compared to 2.16 per 100 PY in elevated ALT person-time ($P = 0.002$).

During the follow-up period, 4 (3.9%) patients with normal ALT and 44 (7.9%) patients with elevated ALT died due to all causes. The cumulative survival rates were significantly different between the two groups (Figure 1) : 97.8% and 92.8% in patients with normal ALT and 98.4% and 78.4% in patients with elevated ALT at 5 and 10 years, respectively (Figure1).

Discussion

In this study, a total of 378 HBV-infected patients were followed for a median of 8.2 years. We evaluate the impact of normal ALT on the risk to develop HCC, hepatic decompensation and overall death. This prospective cohort is to our knowledge the largest of HBV-infected patients with or without normal ALT in European countries. We found that normal ALT reduced the risk of HCC around 28% per year and this risk remained over 5 years, but on a lower level. This highlights the fact that surveillance for HCC needs to be maintained during long-term, also in patients with or without normal ALT [22]. This is further strengthened by the fact that all patients who died from liver-related causes in normal ALT group after had developed HCC. On the other hand, the frequency and method of surveillance remain debated. Furthermore, the long-term effectiveness and cost-effectiveness of surveillance for HCC in these patients requires further studies [22].

Longitudinal studies of untreated patients with chronic HBV indicate that, after diagnosis, the 5-year cumulative incidence of developing cirrhosis ranges from 8% to 20%. The 5-year cumulative incidence of hepatic decompensation is approximately 20% for untreated patients with compensated cirrhosis [6,24]. The risk of liver complications and HCC may differ in cirrhotic and non-cirrhotic

patients [25]. Probably cirrhotic (F4) patients more often develop HCC than F3 or F0-F2 patients [11,26]. Actual data indicates that the HCC incidence is at least 0.2% per year in non-cirrhosis patients [27]. Whereas HCC incidence ranges from 0.1% to 0.4%/year in Western European patients with chronic HBV infection [28].

Previous studies on the impact of normal ALT have often suffered from a retrospective design, included relatively few cirrhotic patients with normal ALT and suffered from short follow-up periods and/or significant loss of patients during follow-up [29,30]. The effect that normal ALT has on HCC has therefore yielded diverging results on the reduction of the risk of developing HCC [31]. Only one study has shown a positive association between the aminotransferase concentration, even within normal range (35-40 IU/l), and mortality from liver disease. The risk of liver cancer and liver related death were 2.9 (2.4 to 3.5) in men and 3.8 (1.9 to 7.7) in women for ALT concentration around 20 IU/l [21].

In one retrospective study, including Asian-American patients with both F3 and F4 patients with a mean follow-up time of 3.5 years, the proportions of those with significant histology were 0, 22, and 45% for age $< \text{or} = 35$, 36-50, and > 50 years, respectively ($n=11$, $n=27$, $n=19$; $P=0.033$). In patients who had fluctuating ALT levels, the corresponding proportions were 22, 42, and 69% ($n=9$, $n=22$, $n=13$; $P=0.091$) [32]. Proportions of patients with advanced liver disease in this study seem close to our finding. However, this study did not evaluate the same endpoints such HCC and hepatic decompensation.

A significantly lower incidence of liver-related complications and liver-related deaths in patient with persistent normal ALT has been noted in several studies [6,33], similar to our study. In meta-analyses with pooled data from both Asian and Western European studies, a reduced risk of HCC, liver-related morbidity, and mortality has been seen in patients with normal ALT [31,34]. The risk for overall death was also significantly reduced in patients with normal ALT in our study, with a majority of non-liver related deaths.

A common problem in studies with long follow-up is the frequent loss of patients during follow-up. In the present study, dropouts were very few (0.7%) and it was the reason why that information according death was not reported for all patients with elevated ALT serum. In this study, we do not have sequential measurement of Hepatitis B virus e antigen (HBe-Ag) at the occurrence of HCC. The importance of HBe-Ag in fibrosis progression with vanishing cirrhosis was not assessed in our study [35,36]. In addition, the correlation between hepatitis B viral load with the risk of progression to cirrhosis [3,37] and the remaining long-term risk for HCC was not analyzed.

Conclusion

To conclude, we found a reduced but persistent long-term risk of developing HCC in HBV patients with normal ALT. This risk persisted during at least 7 years of follow-up in some patients. This indicates that continued surveillance for HCC should be maintained during several years of follow-up.

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