Original

Fibrosis and HBV-DNA Determine the Prognosis of Chronic Hepatitis B Patients : Long-Term Follow-up

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Abstract : The aim of this study was to assess the long-term prognosis of chronic hepatitis B infection, focusing on progression to cirrhosis and hepatocellular carcinoma (HCC) and survival. A total of 88 Japanese patients who were positive for hepatitis B surface antigen (HBsAg) were followed for 6 to 247 months. All were diagnosed as having chronic hepatitis by needle biopsy. Of the 67 patients (76.1%) who were positive for hepatitis B e antigen (HBeAg), 50 received interferon (IFN) therapy of which 45 seroconverted from HBeAg to HBe antibody (anti-HBe). There was a significant difference in the rate of seroconversion between IFN-treated and untreated patients ($p=0.039$). During the follow-up, progression to cirrhosis occurred in 12, liver failure in 5, HCC in 6 and five patients died (4 from hepatic causes and 1 from a non-hepatic cause). Multivariate analysis indicated that both high HBVDNA polymerase (DNA-p) activity ($p=0.036$) and advanced hepatic fibrosis ($p=0.033$) contribute to cirrhosis, with advanced hepatic fibrosis ($p=0.023$) contributing to HCC, and high DNA-p activity being associated with liver failure ($p=0.048$) and death ($p=0.042$). In the present study, we determined that the disappearance of HBeAg was frequently observed, but in sharp contrast disappearance of HBsAg was rarely found. The long-term prognosis of chronic hepatitis B could be independently predicted by advanced hepatic fibrosis and high levels of DNA-p activity. A poor prognosis was linked to the presence of cirrhosis and persistent HBV replication leading to an increased risk of death.

Key words : hepatitis B virus, seroconversion, survival, hepatocellular carcinoma, interferon therapy

Introduction

Chronic hepatitis B virus (HBV) infection is one of the biggest health problems in the world as 350 million people or 5 percent of the world's population are affected by this infection^{1,2)}. HBV infection is characterized by its variation in clinical manifestations ranging from asymptomatic stages of chronic hepatitis, cirrhosis, hepatocellular carcinoma

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(HCC) as well as death. Published estimates on the probability of progression and prognosis are contradictory, suggesting that the proposed Markovian mathematical model³⁾ of the long-term outcome of chronic hepatitis B is somewhat unreliable. Interferon (IFN) with its antiviral and immunomodulatory effects is now in widespread use as the first-line therapy for chronic hepatitis B⁴⁾. However, long-term prospective cohort studies to evaluate the true endpoints, *i.e.*, prevention of cirrhosis, liver failure, HCC and death, are difficult to perform because of the prolonged and slow progress of this protracted disease. In Western countries, the long-term benefit of IFN therapy has been confirmed^{3,5)} in patients with chronic hepatitis positive for hepatitis Be antigen (HBeAg); however, these results may not always be translatable to Asian patients⁶⁾. Since 1981, we have followed a cohort of patients with biopsy-proven chronic hepatitis B to assess the long-term prognosis of the disease. This study not only focused on viral markers (*e.g.*, seroconversion from HBeAg to HBe antibody [anti-HBe] and the disappearance of hepatitis B surface antigen [HBsAg]), but also on true endpoints such as the progression to cirrhosis, liver failure, HCC and death. Concomitantly, we tried to elucidate whether IFN therapy would alter the long-term course of the disease.

Patients and Methods

Patients

Between January 1981 and February 2000, 122 patients with chronic HBV infection were recruited from the following two institutions: Second Department of Internal Medicine, Showa University Hospital and the Department of Gastroenterology, Kanto Medical Center, Nippon Telegraph and Telephone East Corporation. The patients who participated in this study were all newly diagnosed; either self-referred or referred by attending physicians, positive for HBsAg and had elevated serum ALT for more than six months. Informed consent was obtained, and a needle biopsy was done on all patients at enrollment. The findings obtained by liver biopsy were used as the baseline data for this study.

Reasons for exclusion from the study included evidence of HCC on ultrasonography or computed tomography; extrahepatic malignant disorders; pathological cirrhosis or normal liver on liver biopsy; inadequate liver sample for histological assessment; human immunodeficiency virus infection or a past history of either renal dialysis, organ transplantation or an autoimmune liver disease. Out of the 122 patients, 34 cases were excluded, thereby leaving 88 eligible patients in the study population.

At enrollment, patients underwent complete physical examination, serological and biochemical screening including standard liver-function tests and HBV markers. HBsAg, HBeAg, anti-HBe and HBVDNA polymerase (DNA-p) were determined as first-line HBV markers by conventional methods (AxSYM Dinapack: Dainabot, Tokyo, Japan); however, HBVDNA using dot-blot hybridization or branched DNA assay was not investigated in all the patients as the DNA-p assay was only available at the time of this study. When patients were negative for DNA-p, the sera taken at the time of biopsy were stored at -80°C and later assayed for HBVDNA by dot-blot hybridization according to the standard methods⁷⁾, with a sensitivity level of above 0.5 pg HBVDNA/mL. Patients were individually interviewed to obtain their medical history; including family history of HBV related chronic hepatitis, cirrhosis, HCC and alcohol ingestion. Heavy alcohol abuse was defined as habitual intake of more than 60 g/day for at least 5 years.

Liver histology

Liver biopsies were performed with a Tru-cut needle (14 G, $\times 11.4$ cm, Baxter Co, Japan). Only samples measuring at least 18 mm in length after fixation were considered suitable for diagnosis. Each liver sample was analyzed by an external pathologist (MN) who was blinded to clinical information about the patients. All specimens were classified according to the New Inuyama Classification⁸⁾ which defines the degree of necroinflammatory activity from A0 to A3, and the degree of hepatic fibrosis from F0 to F4. The most severe status of activity was classified as A4 and the most severe cirrhosis as F4. Normal liver corresponds to A0/F0.

Follow-up

Monthly viral and biochemical tests as well as clinical assessment were carried out on all patients with additional ultrasonographic scanning every 6 months. Cirrhosis diagnosis was based on liver pathology and/or liver imaging with ultrasonography and computed tomography. The diagnosis of liver failure was verified by the presence of jaundice, ascites and hepatic encephalopathy with HCC being diagnosed by internationally accepted criteria⁹⁾. Additional information about each liver tumor was obtained by ultrasonography, computed tomography and arteriography.

Statistical analysis

The surrogate endpoint for HBeAg-positive patients was the seroconversion to anti-HBe. The endpoints for the whole patient included disappearance of HBsAg, progression to cirrhosis, liver failure, HCC and death. The reference date was June 30, 2002. Of the 88 patients, 27 patients discontinued visiting our hospitals and were censored on the day of the last visit whereas the remaining patients were censored at the reference date.

The Kaplan-Meier method was used for analysis of the occurrence of each endpoint and occurrence rates in groups defined by baseline variables were compared by means of the log-rank test. Cox's proportional-hazards model¹⁰⁾ was employed to assess the following parameters (Table 1). Significant variables were identified by the stepwise procedure forward selection and backward elimination. All p values were two-tailed and all confidence intervals (CI) were 95%. P values less than 0.05 were regarded as significant. Data were analyzed with the SPSS computer program (version 11.0, SPSS Inc, Chicago, IL, USA).

Results

Study group profile

Eighty-eight Japanese patients were followed for a mean period of 73.2 ± 53.6 months (Table 2). Patients were postulated to be infected with HBV during early years of life. None had a past history of acute hepatitis B during their adult periods. Forty-two had a family history of HBV-related chronic hepatitis, cirrhosis or HCC.

At enrollment, 67 patients (75.0%) were positive for HBeAg and 66 patients (75.0%) were positive for DNA-p. Among the 22 patients negative for DNA-p, 15 (68.2%) were positive for HBVDNA which was determined by dot-blot hybridization. The median follow-up period was not significantly different between HBeAg-positive and HBeAg-negative patients. Mean age of patients positive and negative for HBeAg was 33.8 ± 11.7

Table 1. Parameters analyzed by Cox's proportional-hazard model

End point	parameters
Seroconversion	age (years)
HBsAg disappearance	gender (male or female),
Cirrhosis	alcohol ingestion (none or mild or heavy),
Liver failure	family history (yes or no),
HCC	albumin (g/dl),
Death	AST (IU/L),
	ALT (IU/L),
	̑-GTP (IU/L),
	ALP ratio (degree of elevation above upper normal limits),
	prothrombin time (%),
	platelets ($\times 10^4/\text{mm}^3$),
	̑-fetoprotein (ng/ml),
	log DNA-p (cpm),
	anti-HCV (positive or negative),
	HCVRNA (positive or negative),
	IFN therapy (treated or untreated),
	corticosteroid withdrawal therapy (treated or untreated),
	non-antiviral, liver protective drugs (treated or untreated),
	histologic activity (A : 0/1/2/3),
	hepatic fibrosis (F : 0/1/2/3/4)

and 42.2 ± 11.1 years, respectively (not significant).

HBeAg-negative patients had more advanced hepatic fibrosis compared with HBeAg-positive patients (Table 3), which was statistically significant ($p=0.099$, Pearson).

Patients with active virus replication (*i.e.* positive for HBeAg and HBVDNA-p) and active liver disease (*i.e.* elevated serum ALT concentration and chronic active hepatitis on biopsy) were regarded as appropriate cases for IFN therapy; however, prior informed consent was obtained after presentation of information on the possible side effects to the patients. Not all patients in this cohort were given informed consent but 50 patients received IFN treatment. The duration of IFN therapy ranged from 28 days to 6 months. Out of the 50 IFN-treated patients, 42 (84%) were treated for 28 days while the others were treated for a period of time from 2 months to 6 months.

Analysis for HBV viral markers

Among the 67 patients who were found to be positive for HBeAg at enrollment, seroconversion from HBeAg to anti-HBe was observed in 45 patients (67.2%), during a mean follow-up period of 74.8 ± 54.5 months (range, 6 to 247 months). The overall cumulative 5-year probability of seroconversion was 69.5%, *i.e.* the annual probability of seroconversion accounting for 13.9% (Fig. 1). The cumulative 5-year probability of seroconversion for IFN-treated patients and IFN-untreated patients accounted for 80.8% and 46.0%, respectively ($p=0.039$, calculated by the log-rank test) (Fig. 2). Annual seroconversion rates for IFN-treated patients and IFN-untreated patients were calculated to be 16.2% and 9.2%, respectively.

Table 2. Patient profiles and laboratory data at the time of enrollment

Patient Characteristics	
Gender (male/female)	71/17
Age (mean $\pm$ SD, range, years)	36.8 $\pm$ 12.6, 18–69
Alcohol abuse (none/mild/heavy)	23/61/4
Family history of liver disease (positive)	42 (47%)
Histologic stage	
Activity :	
A1	56 (63.6%)
A2	27 (30.7%)
A3	5 (3.4%)
Fibrosis :	
F1	56 (63.6%)
F2	20 (22.7%)
F3	12 (13.6%)
F4	0
Laboratory data (mean $\pm$ SD)	
Albumin (g/dl)	4.1 $\pm$ 0.4
T-Bilirubin (mg/dl)	1.19 $\pm$ 1.55
AST (IU/L)	155 $\pm$ 261
ALT (IU/L)	293 $\pm$ 379
̑-GTP (IU/L)	80.2 $\pm$ 83.7
ALP ratio*	0.95 $\pm$ 0.98
Prothrombin time (%)	85.9 $\pm$ 17.8
Platelet ($\times 10^4/\text{mm}^3$)	18.8 $\pm$ 5.5
̑-fetoprotein (ng/ml)	27.9 $\pm$ 55.8
DNA-p (cpm)	2,790 $\pm$ 8,660
Log DNA-p	6.23 $\pm$ 2.49
HBeAg (positive)	67 (76.1%)
Anti-HCV (positive)	3 (3.4%)
HCVRNA (positive)	1 (1.1%)
Anti-HDV (positive)	0
Follow-up period (mean $\pm$ SD, range, month)	
HBeAg positive cases	74.8 $\pm$ 54.5, 6–247
HBeAg negative cases	68.0 $\pm$ 51.9, 6–209
IFN therapy (treated)	50 (56.8%)

*ALP ratio; we divided levels of ALP by the upper normal limit of ALP

The cumulative 5-year probability of seroconversion in patients with histological hepatic activity A1, A2 and A3 was 32.8%, 75.4%, and 100%, respectively. There was a significant difference in the rate of seroconversion among the three categories of pathological activity ($p=0.024$). Multivariate analysis with a Cox's proportional-hazards model revealed that high ALT levels (hazard ratio=1.003; $p=0.047$) and low DNA-p activity (hazard ratio=0.883; $p=0.023$) were significant contributing factors for seroconversion (Table 4). With the stepwise procedure, other covariates including IFN therapy or liver

Table 3. Correlation between HBeAg status and histologic fibrosis
HBeAg-negative patients had more advanced hepatic fibrosis compared
with HBeAg-positive patients ($p=0.099$)

Fibrosis	HBeAg		Total
	+	-	
F1	45 (67)	11 (52)	56
F2	16 (24)	4 (19)	20
F3	6 (9)	6 (29)	12
Total	67	21	88

(): %

Fig. 1. Cumulative probability of no seroconversion to anti-HBe (Kaplan-Meier method)

histology were not selected as significant independent factors.

Spontaneous HBsAg disappearance was observed in seven patients (8%). The cumulative 10-year disappearance of HBsAg was 9.5%, *i.e.* the annual probability of HBsAg disappearance was 1%. All patients who cleared HBsAg showed good prognosis. Of the 67 patients positive for HBeAg at enrollment, 6 (9.0%) cleared HBsAg during follow-up, while among the 21 patients negative for HBeAg, 1 (4.8%) cleared HBsAg. No difference was evident in terms of the cumulative incidence of HBsAg disappearance between HBeAg-positive and negative patients ($p=0.573$, calculated by the log-rank test). Given all this, it is conceivable that there was no significant predictive factor associated with HBsAg disappearance.

Fig. 2. Cumulative probability of no seroconversion for IFN-treated and IFN-untreated patients (Kaplan-Meier method)
Seroconversion rate for IFN-treated patients was higher than that for IFN-untreated patients ($P=0.039$).
Solid Line=IFN-treated patients, Dotted Line=IFN-untreated patients

Table 4. Hazard ratios of seroconversion, cirrhosis, liver failure, HCC and death (multivariate analysis)

Outcomes	Factors	Hazard ratio (95% confidence interval)	P
Seroconversion to anti-HBe	ALT	1.003 (1.000– 1.005)	0.023
	DNA-p	0.883 (0.780– 0.999)	0.047
Cirrhosis	Log DNA-p*	1.385 (1.021– 1.877)	0.036
	Fibrosis (F1/F2/F3)	3.256 (1.102– 9.621)	0.033
Liver failure	Log DNA-p*	1.626 (1.005– 2.632)	0.048
HCC	Fibrosis (F1, 2/F3)	9.824 (1.373–70.300)	0.023
Death	Log DNA-p*	1.971 (1.024– 3.793)	0.042

* Exponential of correlation coefficient: relative risk should be calculated as an exponential of this value.

Survival analysis

During the follow-up period, progression to cirrhosis occurred in 12 patients (13.6%), liver failure in 5 patients (5.7%), development of HCC in 6 (6.8%), while death was observed in 5 patients (5.7%) (Fig. 3 and 4). The cumulative 10-year probability of cirrhosis, liver failure, HCC, and death was 18.0%, 11.8%, 10.7%, and 8.8%, respectively. Hence, annual probability accounted for 1.8% for cirrhosis, 1.2% for liver failure, 1.1% for

Fig. 3. Cumulative probability of no cirrhosis or liver failure (Kaplan-Meier method)
Solid Line=cirrhosis, Dotted Line=liver failure

Fig. 4. Cumulative probability of no HCC or death (Kaplan-Meier method)
Solid Line=HCC, Dotted Line=death

HCC and 0.9% for death. Incidentally, none of the patients in our study group received a liver transplant and death was attributed to hepatic failure associated with HCC in four patients and to a non-hepatic cause in one patient.

Although HBeAg-negative patients had more advanced hepatic fibrosis than HBeAg-positive patients at enrollment, hepatic failure or death was considered to occur rarely in HBeAg-negative patients relative to those in HBeAg-positive patients. Regarding cirrhosis, out of the 67 patients positive for HBeAg, 8 (12%) developed cirrhosis while of the 21 patients negative for HBeAg, 4 (19%) developed cirrhosis. The cumulative 10-year probability of cirrhosis was 14.1% in HBeAg-positive patients and 30.5% in HBeAg-negative patients ($p=0.300$, calculated by the log-rank test). Examination of hepatic failure and death revealed that among the 67 patients positive for HBeAg at baseline, 5 (7.5%) developed hepatic failure and 5 (7.5%) died. In HBeAg-positive patients, the cumulative 10-year probability of hepatic failure and death was found to be 15.2% and 10.6%, respectively; however, of the 21 patients negative for HBeAg, none developed hepatic failure or died. Concerning HCC, of the 67 patients positive for HBeAg, 5 (7.5%) developed HCC and of the 21 patients negative for HBeAg, 1 (4.8%) developed HCC. The cumulative 10-year probability of HCC was 11.4% in HBeAg-positive patients and 9.1% in HBeAg-negative patients ($p=0.909$, calculated by the log-rank test).

There was no significant difference in the cumulative incidence of cirrhosis, liver failure, HCC and death between IFN-treated and IFN-untreated patients. In more detail, of the 50 patients on IFN therapy, 7 (14.0%) progressed to cirrhosis, while of the 38 patients who received no IFN therapy, 5 (13.2%) progressed to cirrhosis. The cumulative 10-year probability of cirrhosis accounted for 23.2% in IFN-treated patients and 13.7% in IFN-untreated patients ($p=0.567$, calculated by the log-rank test). Of the 50 patients who received IFN therapy, 3 (6.0%) showed onset of hepatic failure, and of the 38 patients who received no IFN therapy, 2 (5.3%) developed hepatic failure. The cumulative 10-year probability of hepatic failure was 13.9% in IFN-treated patients and 11.1% in IFN-untreated patients ($p=0.480$, calculated by the log-rank test). In regard to HCC, of the 50 patients on IFN therapy, three (6.0%) developed HCC, and of the 38 patients who received no IFN therapy, three (7.9%) developed HCC. The cumulative 10-year probability of HCC was 11.2% in IFN-treated patients and 10.3% in IFN-untreated patients ($p=0.885$, calculated by the log-rank test). Concerning fatalities, of the 50 patients who received IFN therapy, 3 (6.0%) died, and of the 38 patients who received no IFN therapy, 2 (5.3%) died. The cumulative 10-year probability of death accounted for 17.5% in IFN-treated patients and 16.3% in IFN-untreated patients ($p=0.301$, calculated by the log-rank test).

In multivariate analysis, high DNA-p activity (hazard ratio=1.385; $p=0.036$) and advanced hepatic fibrosis (hazard ratio=3.256; $p=0.033$) were found to play a role as significant independent risk factors for cirrhosis. Advanced liver fibrosis (hazard ratio=9.824; $p=0.023$) was determined to be a significant independent risk factor for HCC, while high DNA-p activity was verified as a significant independent risk factor for liver failure (hazard ratio=1.626; $p=0.048$) and death (hazard ratio=1.971; $p=0.042$) (Table 4). The cumulative probability of HCC in patients with hepatic fibrosis F1, F2 or F3 is shown in Fig. 5. There were significant differences in the incidence of HCC among fibrosis stages 1, 2 and 3 ($p=0.0052$). Other parameters including age, gender, HBeAg status, blood test data, IFN therapy, corticosteroid withdrawal therapy, stronger Neo-Minophagen C therapy and UDCA therapy did not show significant associations with prognosis of the patients enrolled in the present study (data not shown).

Fig. 5. Cumulative probability of no HCC (Kaplan-Meier method)
 Solid Line=hepatic fibrosis F1, F2, Dotted Line=hepatic fibrosis F3
 $P=0.0052$ (calculated by the log-rank test)

Discussion

In this study, we evaluated the long-term clinical outcome in a cohort of patients with biopsy-proven chronic hepatitis B. We excluded patients with cirrhosis and those in an immune tolerance phase, *i.e.* asymptomatic carriers with virus replication not accompanied by active liver disease, because we intended to determine the actual clinical course of chronic hepatitis B. All the enrolled patients were Japanese, characteristically infected with HBV during the early years of life by mother-to-infant transmission¹¹⁾, with low coinfection with hepatitis delta virus (HDV)¹²⁾.

Accordingly, our cohort represents a special population, indicating that the results obtained in the present study may not translate to populations featuring different modes of HBV transmission and different immunogenetics in the area, such as the Mediterranean where HDV coinfection is common¹³⁾, as well as Central Europe and North America where perinatal transmission is not common.

The natural history of chronic hepatitis B infection is not well known. The majority of previous studies have reported that during the immune disappearance phase, spontaneous HBeAg clearance showed an increasing trend with annual rates amounting from 9 % to 14 %^{5, 14-16)}. By the Markovian mathematical model, the annual probability of disappearance of HBeAg was estimated to be 10% in interferon-alpha untreated patients³⁾. A seroconversion rate during 10-year follow-up period was described as 70% in a population-based study of Alaskan natives¹⁷⁾. In sharp contrast, however, our data showed that overall annual probability of seroconversion accounted for 13.9% while the counterpart in the sub-population, *i.e.* IFN-untreated patients was 9.2%, suggesting that our findings were similar to those of the previously published data.

In our study, IFN therapy was found to exert a significant effect on seroconversion from HBeAg to anti-HBe but failed to provide any benefit to the survival rates. The annual seroconversion rate for IFN-treated patients was significantly higher than that of IFN-untreated patients (16.2% vs. 9.2%, $p=0.039$). However, IFN therapy was not associated with a decrease in the risk of developing cirrhosis, liver failure, HCC or death, which was confirmed by log-rank tests and multivariate analysis. Recent studies suggest that a sustained response following IFN therapy can lead to decreasing risks of cirrhosis and HCC, and contribute to an improvement in the survival rate^{18,19}. We do not know why IFN therapy failed to show benefits on survival rate in our study; however, it is postulated that such unfavorable outcomes might be attributable to the following conditions of our study including limited duration of IFN therapy, limited number of cases, and a non-randomized study mode. The duration of IFN therapy in our group was relatively short, for example, 28 days in 42 patients (84%) and more than 2 months in only eight patients (16%). Recently, IFN therapy for four to six consecutive months has been recommended for treatment of chronic hepatitis B²⁰.

It has been demonstrated that for sustained responders to IFN treatment, long duration of therapy exhibited significant improvement for patients with chronic HBV infection.²¹ Several factors conducive to a favorable response to IFN therapy^{22,23} have been identified. They include the following, a high pretreatment serum ALT level, a low pretreatment serum HBV DNA level, adult acquired HBV infection, active liver histology, female and no coinfection with HIV and/or HDV. In the present study, high pretreatment serum ALT levels and low pretreatment DNA-p levels were verified as significant predictors of seroconversion after IFN therapy among HBeAg-positive patients.

The delayed disappearance of serum HBsAg in the natural history of chronic HBV-infected patients was considered to be an unusual event throughout the world. The annual rate of delayed HBsAg disappearance has been estimated to be 0.4% to 2% in Western countries^{14,24}, where HBV infection usually occurs in adults, and to be 0.1% to 0.8% in Asian countries²⁵, where HBV infection usually occurs perinatally. Patients who cleared HBsAg were supposed to attain an improvement in liver function, thereby enjoying a better outcome²⁶. However, some reports suggested that the onset of cirrhosis was sometimes observed in spite of HBsAg disappearance, followed by further progression to HCC in some patients²⁷. In our study, seven patients cleared HBsAg during the follow-up period, with the annual rate of delayed disappearance of HBsAg accounting for 0.95%. All seven patients showed alleviation in their liver function after HBsAg disappearance; furthermore, none progressed to cirrhosis or HCC. It was previously reported that superinfection of HCV in HBsAg carriers over 35 years of age is associated with the clearance of HBsAg²⁸; however, no patients who cleared HBsAg in our study group were superinfected with HCV and we could not identify any predictive factors associated with delayed HBsAg disappearance.

It is documented that the annual probability of developing cirrhosis in chronic HBV-infected patients ranges from 0.4% to 14.2%^{2,29}. By mathematical modeling, the annual probability of developing cirrhosis is estimated to be 1.0%³. Our data suggests that the annual probability of developing cirrhosis is 1.8%, while both high DNA-p levels and advanced hepatic fibrosis were found to be significant risk factors for cirrhosis, with high DNA-p levels being identified as a significant risk factor for liver failure and death.

Several studies have evaluated factors influencing survival in patients with chronic HBV infection³⁰⁾. Patients presenting with prolonged HBV replication and recurrent episodes of hepatitis have a trend toward worsening prognosis primarily due to complicated onset of cirrhosis or HCC³¹⁾. In this regard, Fattovich *et al* reported that patients with high HBVDNA levels had an odds ratio of 13.1 for developing cirrhosis³²⁾.

According to the previous reports, the annual probability of developing HCC among patients with chronic hepatitis B and among patients with cirrhosis was reported to range from 0.2% to 0.7%^{18, 29, 33)}, and from 1 to 7.8%³⁴⁾, respectively. On the other hand, our findings indicate that the annual probability of developing HCC among our patients accounted for 1.1% and furthermore advanced hepatic fibrosis is a significant independent risk factor for HCC. Several studies reported contradictory results concerning HBeAg and the risk of HCC in patients with chronic HBV infection³⁵⁾; however, our data suggests that the presence of HBeAg does not increase the risk of HCC.

Chronic alcohol abuse and coinfection with HDV or HCV have been reported to accelerate liver injury, resulting in an elevated risk of cirrhosis and HCC, and finally evolving into reducing survival^{36, 37)}. In our study, these factors were not selected as independent risk factors responsible for worsening prognosis. We had only 4 patients (5 %) of heavy alcohol abuse, 3 patients (3 %) positive for anti-HCV, and no patients positive for anti-HDV. In the present study, the number of patients presenting with these factors was too small to reach a significant point for statistical analysis.

In conclusion, our study suggests that the presence of advanced hepatic fibrosis and active replication of HBV increases the risk of poor prognosis and death. It is conceivable that discontinuance of HBV replication before progression to an irreversible state of liver damage is essential to avoid unfortunate outcomes. First, we recommend that liver biopsy should be done in patients with chronic hepatitis B with elevated serum ALT levels. Secondary, if the hepatic fibrosis is advanced, it is advisable to administer anti-viral drugs to suppress HBV replication as soon as possible.

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