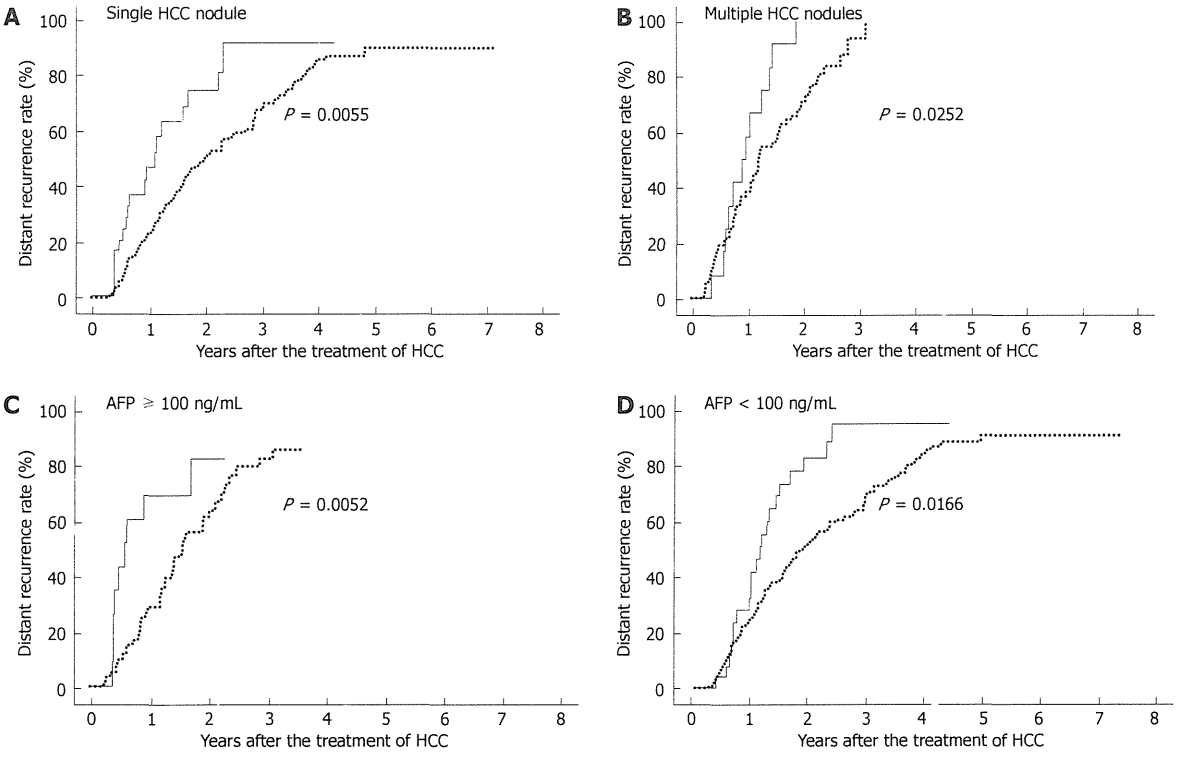


Figure 2 Diabetic patients with inadequate maintenance of blood glucose have higher rate of hepatocellular carcinoma recurrence after stratification by other risk factors. A: $P = 0.006$ for single hepatocellular carcinoma (HCC) nodule; B: $P = 0.025$ for multiple HCC nodules; C: $P = 0.005$ for AFP ≥ 100 ng/mL; D: $P = 0.017$ for α-fetoprotein (AFP) < 100 ng/mL. The cumulative incidence of the recurrence of HCC was significantly higher in diabetic patients with inadequate maintenance of blood glucose (solid line) than in the others (dotted line). after stratification by number of HCC nodules and by initial level of AFP.

Table 3 Multivariable analysis of factors associated with survival

Factors	Odds ratio (95%CI)	*P*-value
Inadequate maintenance of blood glucose	2.77 (1.38-5.57)	0.0046
Alcohol drinking ≥ 60 g/d	6.34 (1.35-29.7)	0.019
Child Pugh grade B	2.24 (1.12-4.46)	0.022
AFP ≥ 100 ng/mL	3.40 (1.88-6.18)	< 0.0001

Inadequate maintenance of blood glucose was defined as an average of casual blood glucose of ≥ 200 mg/dL. AFP: α-fetoprotein.

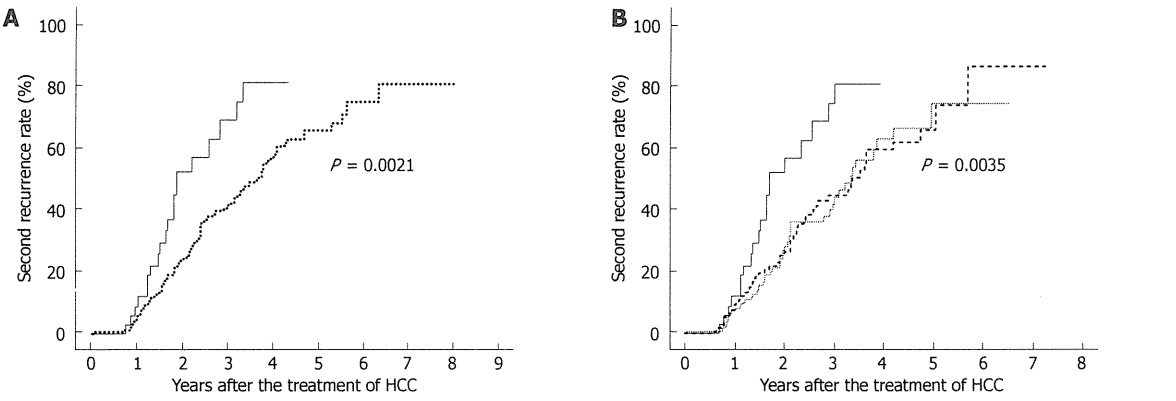
($P = 0.11$). of the survival rate was compared among the three groups, i.e., the diabetes with inadequate maintenance of blood glucose group, the diabetes with adequate maintenance of blood glucose group, and the non-diabetes group. The survival rate was significantly poorer in the diabetes with inadequate maintenance of blood glucose group than in the other two groups ($P = 0.0003$) (Figure 4B), while it did not differ between the diabetes with adequate maintenance of blood glucose group and the non-diabetes group.

The number of HCC nodules, which was a significant factor for HCC recurrence, was not related to survival ($P = 0.34$). Patients with excessive alcohol drinking had poor survival prognosis compared to those with non-excessive or no alcohol drinking ($P = 0.046$). Survival was better in patients in Child-Pugh A class than in patients in Child-Pugh B class ($P = 0.0082$). AFP ≥ 100 ng/mL was associated with poor survival compared with AFP < 100 ng/mL ($P < 0.0001$).

On multivariate analysis, inadequate maintenance of blood glucose was a significant predictor of poor survival [2.77 (95%CI, 1.38-5.57), $P = 0.0046$] independent of excessive alcohol drinking [6.34 (95%CI, 1.35-29.7), $P = 0.019$], initial level of serum AFP ≥ 100 ng/mL [3.40 (95%CI, 1.88-6.18), $P < 0.0001$] and Child-Pugh classification grade B [2.24 (95%CI, 1.12-4.46), $P = 0.022$] (Table 3).

DISCUSSION

The impact of metabolic factors, such as hyperglycemia, diabetes and obesity, on distant recurrence and survival after curative RFA therapy for HCC was analyzed retrospectively. We identified that inadequate maintenance of blood glucose in diabetic patients was a significant and independent risk factor for early recurrence of HCC and a risk factor for poor survival, whereas obesity and diabetes were not. Diabetic patients with inadequate maintenance of blood glucose had a higher rate of HCC recurrence and poorer survival compared with diabetic patients with adequate maintenance of blood glucose and non-diabetic patients. In other words, even in patients with diabetes, if



Second recurrence rate at	1-year	2-year	3-year
Inadequate maintenance of blood glucose	9.0%	53.1%	69.6%
Others	5.9%	24.3%	42.3%

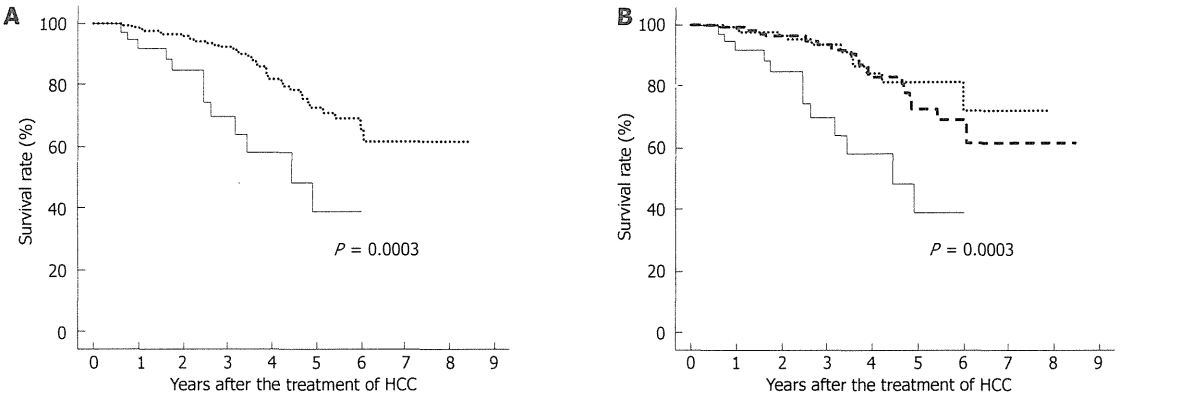
Second recurrence rate at	1-year	2-year	3-year
Diabetes with inadequate maintenance of blood glucose	9.0%	53.1%	69.6%
Diabetes with adequate maintenance of blood glucose	5.9%	22.1%	43.7%
Non-diabetes	5.6%	21.5%	36.5%

Figure 3 Kaplan-Meier curves showing a higher rate of second recurrence of hepatocellular carcinoma in diabetic patients with inadequate maintenance of blood glucose. A: The cumulative incidence of the second recurrence of hepatocellular carcinoma (HCC) was significantly higher in diabetic patients with inadequate maintenance of blood glucose (blood glucose ≥ 200 mg/dL solid line) than in the others (dotted line) (P = 0.002); B: The rate of second recurrence of HCC was significantly higher in diabetic patients with inadequate maintenance of blood glucose (solid line) than in diabetic patients with adequate maintenance of blood glucose (blood glucose < 200 mg/dL, broken line) or non-diabetic patients (dotted line) (P = 0.004). There was no significant difference in the rate of second recurrence of HCC between diabetic patients with adequate maintenance of blood glucose and non-diabetic patients.

the blood glucose was adequately maintained, the HCC recurrence rate and survival did not differ significantly compared with those in non-diabetic patients. These results indicate the possibility that adequate management of hyperglycemia may lead to reduction in the risk of HCC recurrence and improvement of overall survival.

The contribution of diabetes to the development of HCC has been confirmed in several reports[27-30]. The impact of diabetes on the recurrence of HCC after treatment has also been discussed, but with conflicting results[20-22]. A recent study from Taiwan demonstrated that diabetes may not affect the intra-hepatic HCC recurrence and survival after RFA[23]. The results of the present study also indicated that diabetes itself is not a significant risk factor if the level of blood glucose is adequately managed. Rather, hyperglycemia was a significant risk factor for the recurrence of HCC. There may be several mechanisms involved in the relationship between hyperglycemia and HCC recurrence. Hyperglycemia promotes cancer cell proliferation in pancreatic cancer cells and breast cancer cells[31-33] through accelerated cell cycle progression or through the production of reactive oxygen species, leading to activation of protein kinase C and increased DNA synthesis in cancer cells[34]. A previous study in hepatitis C patients indicated that hyperglycemia after challenge with 75-g oral glucose tolerance test was associated with the risk for HCC while hyperglycemia at fasting was not[35]. A possible reason for this result may be that patients with post-challenge hyperglycemia may have higher fluctuations in daily glucose levels that lead to oxidative stress[35], because it was reported that acute fluctuations in blood glucose levels cause greater oxidative stress than sustained chronic hyperglycemia[36]. Taken together, a possible mechanism for the relationship between higher level of casual blood glucose and development of HCC in the present study may be that daily fluctuations in serum glucose levels caused greater oxidative stress. Alternatively, hyper-insulinemia or increased level of insulin-like growth factor, which are caused by hyperglycemia, may be related to carcinogenesis[37-39]. Insulin levels were not measured in our study; therefore, the effects of insulin could not be identified.

Discussions are now taking place on methods of treating diabetes from the standpoint of cancer prevention. Control of hyperglycemia could reduce cancer incidence, which means that hyperglycemia could directly contribute to the development of cancer[39]. The results of our study also showed that adequate management of hyperglycemia may lead to reduction in the risk of HCC recurrence and improvement of overall survival. Improvement in insulin resistance is undoubtedly the most important factor for the treatment of diabetes, but glycemic control is often difficult to achieve with dietary therapy, exercise, or insulin resistance-improving drugs alone. It was reported that metformin may be associated with a lower risk of cancer[38] and there is a theoretical concern that exogenous insulin may be associated with an increased risk of cancer[40]. In fact, a recent study reported that insulin therapy in patients with HCV infection is linked with the development of HCC[41]. On the other hand, with insulin treatment, concomitant use of metformin has been reported to offset the carcinogenic risk of insulin[42]. Whether glycemic control should be a priority, or whether avoiding hyper-insulinemia because



Second recurrence rate at	1-year	2-year	3-year
Inadequate maintenance of blood glucose	91.7%	84.6%	69.7%
Others	98.7%	95.9%	92.4%

Second recurrence rate at	1-year	2-year	3-year
Diabetes with inadequate maintenance of blood glucose	91.7%	84.6%	69.7%
Diabetes with adequate maintenance of blood glucose	99.2%	96.3%	93.7%
Non-diabetes	99.2%	96.4%	93.3%

Figure 4 Patients with inadequate maintenance of blood glucose have a lower survival rate. A: The survival rate after curative local ablation therapy for hepatocellular carcinoma (HCC) was significantly lower in diabetic patients with inadequate maintenance of blood glucose (blood glucose ≥ 200 mg/dL solid line) than in the others (dotted line) (P = 0.0003); B: The survival rate was significantly lower in diabetic patients with inadequate maintenance of blood glucose (solid line) than in diabetic patients with adequate maintenance of blood glucose (blood glucose < 200 mg/dL, broken line) or non-diabetic patients (dotted line) (P = 0.0003). There was no significant difference in survival rate between diabetic patients with adequate maintenance of blood glucose and non-diabetic patients.

of therapy should be a priority, is an issue for future investigation.

In terms of survival of HCC patients, associations with liver function and tumor factors have been reported[1,19], but conflicting results have been reported for the relationship with diabetes[20,21]. These two studies involved heterogeneous groups of HCC patients treated with various therapies, including surgery, local ablation therapy and transcatheter arterial embolization. This heterogeneity may have led to the conflicting results, because the survival of HCC patients may be strongly affected by the initial treatment. Our study involved a homogeneous patient population, i.e., all patients were initially treated curatively by RFA. The results of our study suggest that glycemic control in diabetic patients, more so than diabetes itself, plays a role in survival. The mechanism by which glycemic control and survival are related is unknown, but frequent recurrence of HCC in hyperglycemic patients and the accumulation of damage in liver function because of repeated treatment intervention for HCC may lead to worsening survival.

In conclusion, inadequate maintenance of blood glucose in diabetic patients was a significant and independent risk factor for early recurrence of HCC and for poor survival. Adequate management of hyperglycemia in diabetic patients may lead to reduction in the risk of HCC recurrence and improvement in overall survival.

COMMENTS

Background

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. Radiofrequency ablation (RFA) therapy is an efficient curative therapy for HCC, but long-term survival is limited because of the high rate of distant recurrence of approximately 80% within 5 years. Identification of factors related to recurrence of HCC and therapeutic intervention targeting these factors may lead to prevention of frequent recurrence of HCC and improved survival.

Research frontiers

Metabolic factors, such as obesity and diabetes, have been identified as risk factors for several types of cancer, such as cancer of the liver, pancreas, kidney, and colon. These metabolic factors may be related to recurrence of HCC. The impact of diabetes on the recurrence of HCC after treatment has been discussed, but with conflicting results.

Innovations and breakthroughs

The authors identified that inadequate maintenance of blood glucose in diabetic patients was a significant and independent risk factor for early recurrence of HCC and a risk factor for poor survival, whereas diabetes was not. In other words, even in patients with diabetes, if the blood glucose was adequately maintained, then the HCC recurrence rate and survival did not differ significantly from those in non-diabetic patients.

Applications

The results of the present study indicate the possibility that adequate management of hyperglycemia in diabetic patients may lead to reduction in the risk of HCC recurrence and improvement of overall survival.

Peer review

This is an important study in which the effect of inadequate maintenance of blood glucose in diabetes has been shown as a significant risk factor for distant recurrence of hepatocellular carcinoma and poor survival after curative radiofrequency ablation therapy.

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Noninvasive estimation of fibrosis progression overtime using the FIB-4 index in chronic hepatitis C

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SUMMARY. The FIB-4 index is a simple formula to predict liver fibrosis based on the standard biochemical values (AST, ALT and platelet count) and age. We here investigated the utility of the index for noninvasive prediction of progression in liver fibrosis. The time-course alteration in the liver fibrosis stage between paired liver biopsies and the FIB-4 index was examined in 314 patients with chronic hepatitis C. The average interval between liver biopsies was 4.9 years. The cases that showed a time-course improvement in the fibrosis stage exhibited a decrease in the FIB-4 index, and those that showed deterioration in the fibrosis stage exhibited an increase in the FIB-4 index with a significant correlation ($P < 0.001$). Increase in the ΔFIB-4 index per year was an independent predictive factor for the progression in liver fibrosis with an odds ratio of 3.90 ($P = 0.03$). The area under the receiver operating characteristic curve of the ΔFIB-4 index/year for the prediction of advancement to cirrhosis was 0.910. Using a cut-off value of the ΔFIB-4 index/year <0.4 or ≥0.4, the cumulative incidence of fibrosis progression to cirrhosis at 5 and 10 years was 34% and 59%, respectively in patients with the ΔFIB-4 index/year ≥0.4, whereas it was 0% and 3% in those with the ΔFIB-4 index/year <0.4 ($P < 0.001$). In conclusion, measurement of the time-course changes in the FIB-4 index is useful for the noninvasive and real-time estimation of the progression in liver fibrosis.

Keywords: FIB-4, fibrosis, HCV, noninvasive.

INTRODUCTION

Advanced stage of liver fibrosis in chronic hepatitis C is associated with failure of interferon therapy or development of major concomitant disease such as variceal bleeding, liver failure and hepatocellular carcinoma [1–3]. Therefore, evaluation of the stage of liver fibrosis is essential in clinical practice. Liver biopsy is the gold standard for diagnosis of liver fibrosis [4,5], but inaccuracy in evaluation of fibrosis because of sampling errors [6–8] or by the inter-observer variation has been reported [9]. Real-time assessment of liver fibrosis may be clinically useful, but the invasiveness of liver biopsy precludes repeated examinations.

A variety of noninvasive methods to diagnose liver fibrosis have been proposed. Recently, transient elastography [10–13] and real-time tissue elastography [14] using ultrasonography have been developed, but these modalities are not widely available. For blood tests, the aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio [15], the AST/platelet ratio index (APRI) [16,17] and the Fibrotest [18,19] have been reported to be useful. The FIB-4 index is another prediction value of liver fibrosis in chronic hepatitis C based on the standard biochemical values and age. The FIB-4 index has been reported to be markedly useful for the prediction of advanced liver fibrosis [20,21]. Given its noninvasiveness and simplicity, the FIB-4 index has the advantage of an easy follow-up of the time-course changes by repeated measurements.

In the present study, we investigated the utility of the real-time assessment of the FIB-4 index for the prediction of time-course progression in liver fibrosis.

Abbreviations: ALT, alanine aminotransferase; AST, aspirate aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

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PATIENTS AND METHODS

Patients

A total of 421 patients with chronic hepatitis C who had repeated liver biopsies between 1991 and 2010 at the Musashino Red Cross hospital were consecutively investigated. All patients received interferon therapy after the first biopsy and had nonsustained virological response. A second

biopsy was performed at least 6 months after the completion of interferon therapy. Exclusion criteria were as follows: (i) co-infection with HBV or HIV (n = 1), (ii) alcohol abuse (intake of alcohol equivalent to pure alcohol 40 g/day or more) (n = 8), (iii) the presence of nonalcoholic steatohepatitis (n = 14), (iv) the presence of hepatocellular carcinoma (n = 15), (v) interval between paired biopsies was <1.5 years (n = 41) and (vi) length of biopsy sample <15 mm (n = 28). The demographic characteristics of the 314 patients enrolled are shown in Table 1.

Assessment of liver fibrosis stage

Liver biopsy was carried out under laparoscopic or ultrasonographic guidance. A sample 15 mm or larger was collected and evaluated. The fibrosis stage was categorized according to the METAVIR score: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Two pathologists examined all samples and determined the fibrosis stage. When staging was inconsistent between the two pathologists, an appropriate stage was determined by discussion between the two.

Calculation of FIB-4 index

The FIB-4 index at the time of each liver biopsy was calculated based on the blood test results within 1 month before liver biopsy according to the following formula: The FIB-4 index = (age [years] × AST [IU/L])/(platelet count [10^9/L] × (ALT [IU/L])$^{1/2}$). Change in the FIB-4 index per year (ΔFIB-4 index/year) was calculated by the following formula: ΔFIB-4 index/year = (the FIB-4 index at the second liver biopsy − the FIB-4 index at the first liver biopsy)/interval between paired biopsies (years). Change in AST, ALT, platelet counts per year (ΔAST/year, ΔALT/year, ΔPlatelet counts/year) and the degree of changes in the fibrosis stage per year were calculated similarly.

Table 1 Clinical background of patients

	First biopsy	Second biopsy
Age (years)	53.7 ± 9.8	58.7 ± 9.4
Gender (male/female)	149/165	
AST (IU/L)	64.5 ± 36.7	58.5 ± 37.7
ALT (IU/L)	87.7 ± 58.9	69.9 ± 53.9
Platelet counts (× 10^9/L)	165 ± 48	159 ± 48
Histological findings		
Activity: 0/1/2/3	38/143/117/16	10/147/131/26
Fibrosis: 0–1/2/3/4	139/107/61/7	134/101/63/16
Interval of between biopsies (years)	4.9 ± 2.9	–

AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Statistical analysis

The SPSS software package 15.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. Categorical data were analysed using Fisher's exact test. Continuous variables were compared with Student's t-test. Factors associated with the progression in liver fibrosis were analysed by multivariate logistic regression analysis. Association between progression in fibrosis stage and changes in the FIB-4 was analysed by Spearman's rank correlation test. Kaplan–Meier method and log-rank test were used to analyse time to occurrence of fibrosis progression to cirrhosis. A P-value of < 0.05 was considered statistically significant.

RESULTS

Changes in liver fibrosis stage overtime

The clinical backgrounds of patients at the first and second biopsies are shown in Table 1. The average interval was 4.9 years between the two liver biopsies. The fibrosis stage progressed over time in 23%, regressed in 17% and remained unchanged in 60%. Changes of fibrosis stage stratified by the fibrosis stage at the first liver biopsy are shown in Table 2.

Comparison of FIB-4 index and liver fibrosis stage

For the prediction of advanced liver fibrosis (F3–4), a FIB-4 index <1.45 had a negative predictive value of 97%, whereas a FIB-4 > 3.25 had a positive predictive value of 49% at first biopsy. Similarly, a FIB-4 < 1.45 had a negative predictive value of 98%, and a FIB-4 > 3.25 had a positive predictive value of 54% at second biopsy (Fig. 1).

Table 2 Changes of fibrosis stage over time

	Fibrosis stage at second biopsy				
Fibrosis stage at first biopsy	F0–1 (%)	F2 (%)	F3 (%)	F4 (%)	Total
F0–1	98 (71)	33 (24)	8 (5)	–	139
F2	33 (31)	50 (47)	21 (20)	3 (2)	107
F3	3 (5)	18 (29)	33 (55)	7 (11)	61
F4	–	–	1 (14)	6 (86)	7

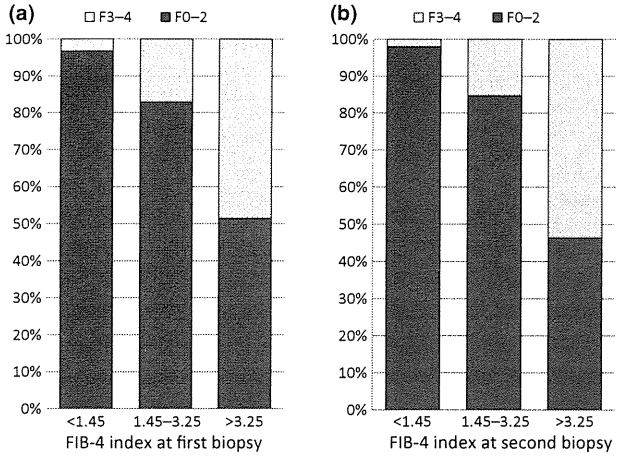

Fig. 1 Comparison of the FIB-4 index and liver fibrosis stage. Patients were categorized into three groups according to the FIB-4 index using cut-off values of < 1.45, 1.45–3.25, > 3.25 at liver biopsy. The lower bar chart (dark grey) indicates patients with F0–2, while the upper bar chart (light grey) indicates patients with F3–4. (a) comparison of the FIB-4 index and liver fibrosis stage at first biopsy and (b) at second biopsy.

Predictive factors for the progression of fibrosis

Higher level of ΔAST/year, lower level of ΔALT/year, lower level of ΔPlatelet counts/year and higher level of the ΔFIB-4/year were significantly associated with the progression of fibrosis overtime (Table 3). Multivariate analysis demonstrated that only the ΔFIB-4 index/year was an independent predictive factor for the progression of fibrosis stage ($P = 0.03$) with an odds ratio of 3.70 (95% CI:1.07–12.5).

Correlation between the degree of changes in the fibrosis stage and the ΔFIB-4 index per year

When the patients were categorized into five groups according to the degree of changes in the fibrosis stage per year (< −0.2, −0.2 – < 0, 0, > 0 – 0.2 and > 0.2), median value of the ΔFIB-4 index/year was −0.29, −0.02, 0.04, 0.16 and 0.47, respectively. The FIB-4 index reduced along the regression of the fibrosis stage, while the FIB-4 index increased along the progression of the fibrosis stage, which showed a significant correlation ($P < 0.001$) (Fig. 2).

Prediction of progression to cirrhosis by the changes in the FIB-4 index per year

The area under the receiver operating characteristic curve of the ΔFIB-4 index/year for the prediction of advancement to cirrhosis was 0.910. By the ΔFIB-4 index/year of 0.4, the sensitivity and specificity for the prediction of advancement to cirrhosis was 80% and 91%. The cumulative incidence of fibrosis progression to cirrhosis, at 5 and 10 years, was 34% and 59%, respectively, in patients with the ΔFIB-4 index/year ≥0.4, whereas it was 0% and 3% in those with the ΔFIB-4 index/year <0.4 ($P < 0.001$) (Fig. 3).

DISCUSSION

Recently, noninvasive markers of liver fibrosis have been used as a predictive factor of liver-related outcome such as

Table 3 Factors associated with the progression of liver fibrosis

	Progression of Liver fibrosis	Nonprogression of Liver fibrosis	*P*-value
Gender (male/female)	31/42	118/123	0.33
Age at first biopsy (years)	54.4 ± 8.7	53.5 ± 10.2	0.50
AST at first biopsy (IU/L)	63.9 ± 35.0	64.8 ± 37.3	0.85
ALT at first biopsy (IU/L)	86.5 ± 58.4	88.1 ± 59.2	0.84
Platelet counts at first biopsy (10^9/L)	15.8 ± 4.6	16.7 ± 4.8	0.16
Change between biopsies			
ΔAST (IU/L)/year	3.8 ± 19.5	−4.1 ± 14.8	<0.001
ΔALT (IU/L)/year	−1.9 ± 28.4	7.2 ± 22.6	0.005
ΔPlatelet counts (10^9/L)/year	−4.1 ± 9.5	−0.002 ± 9.5	0.001
ΔFIB-4 index/year	0.31 ± 0.52	−0.005 ± 0.37	<0.001

ΔAST/year: (AST at the second liver biopsy – AST at the first liver biopsy) /interval between paired biopsies (years); ΔALT/year: (ALT at the second liver biopsy – ALT at the first liver biopsy) /interval between paired biopsies (years); ΔPlatelet counts/year: (platelet counts at the second liver biopsy –platelet counts at the first liver biopsy) /interval between paired biopsies (years); ΔFIB-4 index /year: (the FIB-4 index at the second liver biopsy – the FIB-4 index at the first liver biopsy) /interval between paired biopsies (years).

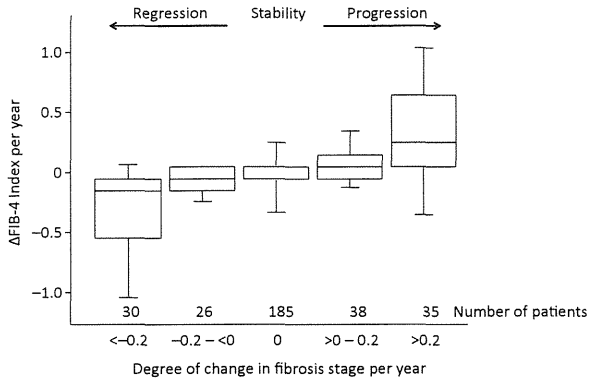

Fig. 2 Correlation between the degree of changes in the fibrosis stage and the ΔFIB-4 index per year. Boxplot of the ΔFIB-4 index/year is shown according to the degree of changes in the fibrosis stage per year. The bottom and top of each box represent the 25 and 75th percentiles, giving the interquartile range. The line through the box indicates the median value, and the error bar indicates the 5 and 95th percentiles.

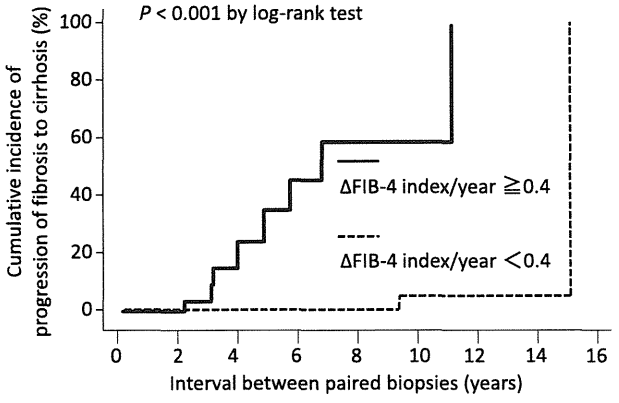

Fig. 3 Cumulative incidence of fibrosis progression to cirrhosis. Patients were categorized into two groups according to the ΔFIB-4 index/year using cut-off value of < 0.4 or ≥ 0.4.

mortality [22–24] or HCC development [24–26] in patients with chronic liver disease. There have been few studies that investigated the association between changes of noninvasive markers and liver-related outcome [27–29]. However, it is still unclear whether there is a relation between the time-course changes in the value of noninvasive markers and progression of liver fibrosis.

The aim of the study was to evaluate the utility of the real-time assessment of the FIB-4 index for the prediction of time-course progression in liver fibrosis. We have shown that the FIB-4 index reduced along the regression of the fibrosis stage, while the FIB-4 index increased along the progression of the fibrosis stage. These results indicate that the measurement of the time-course changes in the FIB-4 index may be useful for the noninvasive and real-time estimation of the progression in liver fibrosis overtime.

Although the gold standard for diagnosis of liver fibrosis is liver biopsy, there are a variety of problems including invasiveness and sampling errors [6]. Diagnostic methods of liver fibrosis by measurement of elasticity of the liver by ultrasonography [10–14] have been developed, but these modalities are not widely available.

The FIB-4 index has an advantage among these noninvasive liver fibrosis diagnostic methods. Firstly, it is quite easily calculated. The parameters required for calculation are only age, AST, ALT and platelet counts, which are measured at the routine examination of patients with liver disease. Therefore, additional blood collection is unnecessary, and the index can be calculated at no cost. Secondly, because of its simple calculation, it is possible to evaluate the clinical conditions in a real-time manner. Repeated measurements of the FIB-4 index make it possible to predict deterioration in liver fibrosis continuously over time. Because no special equipment or system is necessary, and objective data on the clinical conditions are provided in a real-time manner, the FIB-4 index is simple and convenient compared with other noninvasive liver fibrosis diagnostic methods.

It is widely known that a decrease in platelet counts is useful for the prediction of the progression of fibrosis stage [30]. We have reported that elevated AST or ALT is also associated with the progression of liver fibrosis [31]. However, the results of this study showed that a change in the FIB-4 index over time was a more useful factor for the prediction of the progression of fibrosis stage than AST, ALT and changes in platelet counts.

Liver biopsy is still an important examination as the gold standard for diagnosis of liver fibrosis, but time-course changes cannot be readily observed by repeated biopsies because of its invasiveness. On the other hand, it is possible to estimate the progression of liver fibrosis by repeated measurement of the FIB-4 index. Therefore, two examinations should be combined: liver biopsy may be utilized to determine the baseline of fibrosis stage, and the serial measurement of the FIB-4 index may be utilized to predict changes of fibrosis stages overtime in a real-time manner.

In conclusion, we believe that measurement of the time-course changes in the FIB-4 index is useful for the noninvasive and real-time estimation of the progression in liver fibrosis.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

No conflicts of interest exist for all authors.

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厚生労働科学研究費補助金（難病・がん等の疾患分野の医療の実用化研究事業（肝炎関係研究分野））
分担研究報告書

慢性肝炎・肝硬変・肝癌の病態解明と各病態および都市形態別で求められる医療を考慮したクリティカルパスモデル開発のための研究

－B型慢性肝疾患に対する核酸アナログ治療における有効性と肝発癌についての共同研究－

研究分担者名　名古屋第二赤十字病院　消化器内科部長　折戸悦朗

研究要旨：B型慢性肝疾患に対し核酸アナログ治療が行われているが、HBV DNAが十分抑制されている患者においても、肝発癌がみられる場合があり、核酸アナログの治療有効性と肝発癌との関連性は、未だ不明な点がある。今回はHBsAg量にも着目し、日本赤十字病院肝疾患ネットワークのデータを集積して、核酸アナログ治療症例を検討した。多変量解析の結果、治療効果に寄与する因子は、治療前HBV DNA量であり、治療中の肝発癌に寄与する因子は、治療前の病態、すなわち慢性肝炎症例に比べ肝硬変例ではその治療効果に関係なく有意に肝発癌を引き起こすことが示された。慢性肝炎例ではHBV DNA陰性かつHBsAg<100IUではまったく発癌が見られないことが判明した。また一部の治療前保存血清を用いた検討では、治療前のHBsAg量が高い例が有意にその後の治療効果に関連することが示された。

A．研究目的

B型慢性肝疾患患者に対する核酸アナログ治療における、HBs抗原量を含めた各種因子と治療効果および治療中の肝発癌との関係について、全国日赤病院肝疾患ネットワークにおける多施設共同研究。

B．研究方法

日本赤十字病院肝疾患ネットワークの各施設から、核酸アナログ治療中の症例についての、治療前、治療中の患者データ311例を集積し解析した。治療効果については、最終時点でHBV DNAが陰性化しかつHBsAg<100 IU/Lのものを有効例と判定した。また肝発癌については、治療前および治療開始後1年以内に発癌した例は除外した。それぞれ患者データやウイルスマーカーなどとの関連性について、単変量および多変量解析を行った。

C．研究結果

治療中の肝発癌に関与する因子の解析では、単変量では病態、核酸アナログ治療期間、血小板数が有意となったが、多変量解析では、病態のみが有意となった。すなわち治療前の病態が肝硬変である場合は、その治療効果にかかわらず慢性肝炎例に比べて有意に発癌していた。逆に慢性肝炎においては、ウイルスが十分抑制され、HBV DNAが陰性化しかつHBsAg<100 IU/Lであれば発癌は見られないことも明らかとなった。

また、治療効果については、著効と考えられるHBV DNA陰性化かつHBsAg<100 IU/Lを達成する治療前因子の多変量解析では、治療前HBV DNA量低値のみが有意な因子として抽出された。

さらに一部の症例の治療前の保存血清を用いてHBsAg量を希釈定量して検討した結果では、治療前HBsAg量が低い例で有意に治療効果が良好となることも判明した。

D．考察

今回、全国多数の日本赤十字病院肝疾患ネットワークの協力を得て、核酸アナログ治療における治療有効性および肝発癌に関連する因子を多施設共同研究で解析した。その結果、治療有効性には治療前HBV DNA量が、また肝発癌に対しては治療前の病態が大きく関与することが判明した。

核酸アナログ治療の最終目標は、ただ単に血中のHBV DNAを陰性化させるだけではなく、HBsAgをも陰性化させ、究極的には肝発癌を完全に抑制していくことであると考えられる。そのためには、どのような症例が核酸アナログ治療の対象としてふさわしいのか、またそういった症例を治療するにあたり、どの指標を目安にして治療を継続させていくのが適切かといった問題が重要である。今後さらに検討を進めて、これらの問題点を解明していく予定である。

Ｅ．結論

多施設共同研究の結果、慢性肝炎に対する核酸アナログ治療の有用性、肝発癌抑制効果が示されたが、既に肝硬変まで進展してしまっている症例に対しては必ずしも有用であるとは言えないため、さらなる対策が必要であると考えた。

Ｇ．研究発表

1．論文発表

Quantitation of HBsAg predicts response to entecavir therapy in HBV genotype C patients. Orito E, Fujiwara K, Kanie H, Ban T, Yamada T, Hayashi K. World Journal of Gastroenterology 2012; 18(39): 5570-5575.

2．学会発表

Entecavir初回治療例での治療効果予測および効果判定における定量的HBs抗原測定の有用性の検討. 折戸悦朗、藤原圭、日下部篤宣、青木美帆、岩崎弘靖、堀寧、野村智史、梅村修一郎、蟹江浩、坂哲臣、山田智則、林克巳. 第48回日本肝臓学会総会

ワークショップ：C型慢性肝炎における臨床背景の違いと治療法選択の現状と展開；IP-10値を含めたPEG-IFN/RBV療法における治療予測因子の検討. 松浦健太郎、田中靖人、飯尾悦子、日下部篤宣、新海登、宮木知克、野尻俊輔、渡辺綱正、藤原圭、折戸悦朗、城卓志、溝上雅史第48回日本肝臓学会総会

ペグインターフェロン・リバビリンによる2次併用療法非治癒例のうち新規3剤併用療法の適応となる症例数の検討. 藤原圭、日下部篤宣、林克巳、山田智則、坂哲臣、蟹江浩、金本高明、梅村修一郎、野村智史、岩崎弘靖、堀寧、青木美帆、野尻俊輔、城卓志、折戸悦朗. 第16回日本肝臓学会大会

Quantitation of serum HBsAg level predicts response to naïve entecavir therapy in chronic hepatitis B patients with HBV genotype C. E. Orito, A. Kusakabe, K. Fujiwara, H. Kanie, T. Ban, T. Yamada, K. Hayashi. The Liver Meeting 2012, Boston, USA, 2012.

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Ｈ．知的財産権の出願・登録状況（予定を含む。）

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BRIEF ARTICLE

Quantitation of HBsAg predicts response to entecavir therapy in HBV genotype C patients

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Abstract

AIM: To analysis the factors that predict the response to entecavir therapy in chronic hepatitis patients with hepatitis B virus (HBV) genotype C.

METHODS: Fifty patients [hepatitis B e antigen (HBeAg)-negative:HBeAg-positive = 26:24] with HBV genotype C, who received naïve entecavir therapy for > 2 years, were analyzed. Patients who showed HBV DNA levels ≥ 3.0 log viral copies/mL after 2 years of entecavir therapy were designated as slow-responders, while those that showed < 3.0 log copies/mL were termed rapid-responders. Quantitative hepatitis B surface antigen (HBsAg) levels (qHBsAg) were determined by the Architect HBsAg QT immunoassay. Hepatitis B core-related antigen was detected by enzyme immunoassay. Pre-C and Core promoter mutations were determined using by polymerase chain reaction (PCR). Drug-resistance mutations were detected by the PCR-Invader method.

RESULTS: At year 2, HBV DNA levels in all patients in the HBeAg-negative group were < 3.0 log copies/mL. In contrast, in the HBeAg-positive group, 41.7% were slow-responders, while 58.3% were rapid-responders. No entecavir-resistant mutants were detected in the slow-responders. When the pretreatment factors were compared between the slow- and rapid-responders; the median qHBsAg in the slow-responders was 4.57 log IU/mL, compared with 3.63 log IU/mL in the rapid-responders ($P < 0.01$). When the pretreatment factors predictive of HBV DNA-negative status at year 2 in all 50 patients were analyzed, HBeAg-negative status, low HBV DNA levels, and low qHBsAg levels were significant ($P < 0.01$). Multivariate analysis revealed that the low qHBsAg level was the most significant predictive factor ($P = 0.03$).

CONCLUSION: Quantitation of HBsAg could be a useful indicator to predict response to entecavir therapy.

Key words: Chronic hepatitis B; Quantitation of hepatitis B surface antigen; Entecavir; Hepatitis B virus genotype C; Slow-responders; Hepatitis B core-related antigen; Core promoter mutation; Pre-C mutation

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INTRODUCTION

Hepatitis B virus (HBV) is a major causative agent of chronic liver diseases[1]. Various strains of HBV have been isolated all over the world, and have been classified as HBV genotypes from A to J[2]. In Japan, about 85% of patients have HBV genotype C, and about 12% have HBV genotype B[3]. Worldwide, HBV genotypes show specific geographical distributions. HBV genotypes A and D are prevalent in the United States and Europe, while HBV genotypes B and C are prevalent in Asia[4]. Disease progression and prevalence of hepatitis B e antigen (HBeAg)-positive status are often associated with HBV genotypes[5]. Therefore, we analyzed the clinical and virological features of patients with HBV genotype C and homogenous backgrounds, because HBV genotype C is the predominant type in Japan.

Entecavir is widely used as a first-choice nucleot(s)ide analog (NA) for chronic hepatitis B patients, because less than 1% of entecavir-naive patients developed resistant mutants after 5 years of therapy[6-8]. However, in HBeAg-positive patients, the response rate to entecavir therapy is less favorable compared with HBeAg-negative patients[6,7]. In addition, some patients show a slow-response, which indicates that serum HBV DNA levels remain high after long-term entecavir therapy. However, it is unclear which patients become slow-responders. Therefore, the aim of this study is to clarify the virological and clinical characteristics of the slow-responders before and during long-term entecavir therapy for HBV genotype C.

MATERIALS AND METHODS

Patient population

From July 2007, 102 consecutive hepatitis B surface antigen (HBsAg)-positive patients with chronic liver disease were enrolled in a naïve entecavir therapy in our hospital. Ten patients dropped out, 15 patients discontinued therapy, 10 patients received immunosuppressive therapy during entecavir therapy, and 10 patients received entecavir for less than 2 years. Thus, 57 patients were analyzed in this prospective, single center study. The institutional review board of the hospital approved the study. Serum samples were drawn from the patients after obtaining written informed consent.

All the patients received 0.5 mg of entecavir daily. Patients with poor adherence were excluded from the study.

All patients were positive for HBsAg for more than 6 mo, had serum HBV DNA of $\geq$ 3 log viral copies/mL, were negative for anti-HCV, and were negative for anti-human immunodeficiency virus before entecavir therapy. Patients with decompensated cirrhosis, acute hepatitis, or acute exacerbation were excluded. Liver biopsy was not performed in some patients; therefore, the liver disease status was diagnosed by the clinical, laboratory, and imaging tests.

HBeAg-positive patients whose serum HBV DNA levels remained $\geq$ 3.0 log copies/mL after 2 years of entecavir therapy were considered to be slow-responders, while patients with < 3.0 log copies/mL were designated as rapid-responders.

Table 1 Baseline characteristics of the patients with hepatitis B virus genotype C

	HBeAg-negative group	HBeAg-positive group	*P* value
No.	26	24	NS
Age (yr)	57.2 (35-80)	44.2 (35-71)	< 0.01
Gender, M:F	15:16	16:11	NS
Diseases, CH:LC/HCC	21:5	23:1	NS
ALT (IU/mL)	38 (13-950)	102 (812-602)	< 0.01
Platelet counts ($\times 10^4$/mL)	18.6 (3.4-4.9)	18.0 (8.4-26.8)	NS
Albumin (mg/dL)	4.3 (3.4-4.9)	4.2 (2.3-5.0)	NS
Serum HBV DNA level (log copies/mL)	5.1 (3.9-8.8)	7.6 (5.6-8.8)	< 0.01

All data are shown by median (range). HBeAg: Hepatitis B e antigen; HBV: Hepatitis B virus; ALT: Alanine aminotransferase; CH: Chronic hepatitis; LC: Liver cirrhosis; HCC: Hepatocellular carcinoma; NS: Not significant.

Laboratory tests

Quantitation of HBsAg (qHBsAg) was performed using the Architect HBsAg QT immunoassay (Abott Japan, Tokyo, Japan), in accordance with the manufacturer's instructions[9]. The detection range was 0.05 to 250 IU/mL. If HBsAg levels were found to be higher than 250 IU/mL, samples were diluted to 1:500 to 1:20 000. In this study, the results of the quantitative HBsAg levels are shown as the logarithmic value. HBV genotypes were detected by enzyme immunoassay (EIA) (Institute of Immunology, Tokyo, Japan)[10]. The HBV core-related antigen (HBcrAg) was detected by a chemilimunescent EIA method (Fujirebio Inc., Tokyo, Japan)[11]. Pre-C mutation and Core promoter mutations were detected by polymerase chain reaction (PCR) (HBV DNA precore/core promoter mutation decision kit; Roche Diagnostics Japan, Tokyo, Japan). Drug-resistant mutations in HBV against nucleotide analogs (NAs; lamivudine, adefovir and entecavir) were detected by the PCR-Invader method (BML Inc., Tokyo, Japan)[12].

RESULTS

Of the 57 patients, 50 patients were genotype C, three patients were HBV genotype A, one was genotype D, and three were of indeterminate genotype on EIA. Thus, the 50 patients with HBV genotype C were analyzed. Baseline characteristics of the 50 patients are shown in Table 1. The median age of the HBeAg-negative group age was significantly higher, the median alanine aminotransferase (ALT) level was significantly lower, and the median HBV DNA level was significantly lower than those in the HBeAg-positive group.

After 2 years of entecavir therapy, the rates of normalization (< 40 IU/L) of ALT levels were 87.0% in the HBeAg-negative group and 92.5% in the HBeAg-positive group (P = Not significant).

In contrast, at year 2, the rates of reduction in HBV

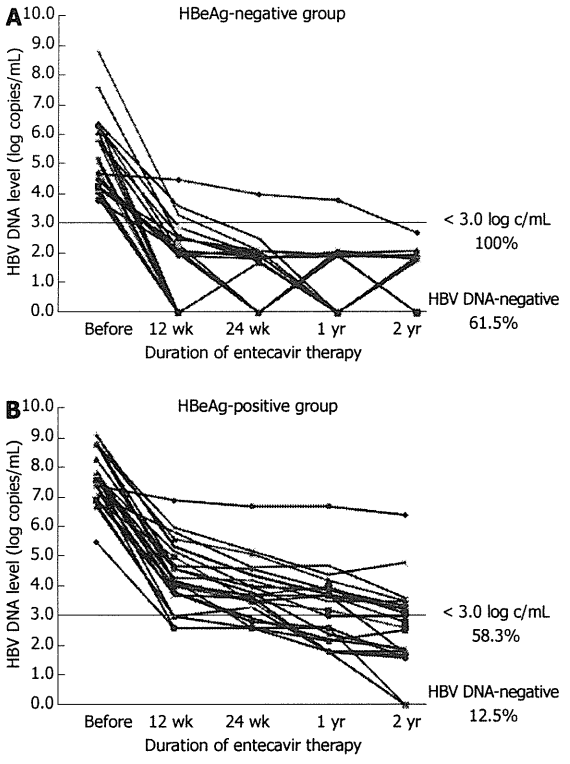

Figure 1 Hepatitis B virus DNA levels before and during entecavir therapy. A: In the hepatitis B e antigen (HBeAg)-negative group, hepatitis B virus (HBV) DNA levels in all patients decreased to < 3.0 log copies/mL at year 2. Of these patients, 61.5% shown to be negative for HBV DNA by the real-time polymerase chain reaction method; B: In the HBeAg-positive group, 58.3% of patients (rapid-responders) showed < 3.0 log copies/mL at year 2, while 41.7% of patients (slow-responders) showed ≥ 3.0 log copies/mL. In addition, at year 2, only 12.5% of the patients were negative for HBV DNA.

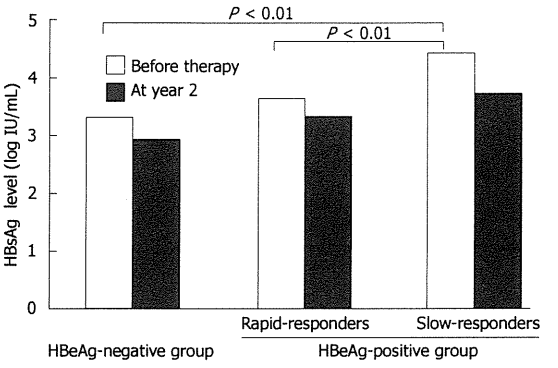

Figure 2 Median quantitative hepatitis B surface antigen levels among patients receiving entecavir therapy. Quantitative hepatitis B surface antigen (HBsAg) levels (qHBsAg) levels in slow-responders in the HBeAg-positive group were significantly higher than those in rapid-responders and the hepatitis B e antigen (HBeAg)-negative group. The median qHBsAg level at year 2 in the slow-responders remained higher than in other groups.

DNA to < 3.0 log copies/mL were 100% in the HBeAg-negative group and 58.3% in the HBeAg-positive group ($P < 0.01$). Thus, in the HBeAg-positive group, 58.3% of patients were designated as rapid-responders, and 41.7% were designated as slow-responders (HBV DNA levels ≥ 3.0 log copies/mL at year 2) (Figure 1). In addition, in the HBeAg-negative group, real-time PCR indicated that 61.5% of the patients were negative for HBV DNA, compared to 12.5% of the HBeAg-positive patients ($P < 0.01$).

Baseline data

When pre-treatment factors were compared between the rapid- and slow-responders (Table 2), age, gender, disease, platelet counts, and albumin were not significantly different. The median ALT level in the rapid-responders group was 131 IU/L compared with 31 IU/L in the slow-responders ($P = 0.02$). The pre-treatment median HBV DNA levels were 7.4 log copies/mL in the rapid-responders, and 8.3 in the slow-responders ($P = 0.06$). The was no difference in the rate of Pre-C and Core promoter mutations between the responder groups. In contrast, the rate of Pre-C mutations in the HBeAg-negative group was 83.3%, compared with 0% in the HBeAg-positive group. Pre-treatment HBcrAg levels did not differ among the three groups. In contrast, the pre-treatment median qHBsAg level was 3.63 log IU/mL in the rapid-responders, compared with 4.57 log IU/mL in the slow-responders ($P < 0.01$).

Data at year 2

At year 2 of therapy, the median qHBsAg level in the rapid-responders was 3.25 log IU/mL, compared with 4.12 log IU/mL in the slow-responders ($P = 0.01$). The median HBcrAg level in the rapid-responders was 5.85 log U/mL, compared with > 6.8 (the upper limit of the detection range) in the slow-responders ($P < 0.01$). In Figure 2, qHBsAg levels before treatment and at year 2 are shown for the HBeAg-negative group, and the rapid-responder and slow-responders in the HBeAg-positive group.

Among all the slow-responders, no entecavir-resistant mutations were found, although three patients showed M204I lamivudine-resistant mutations (Table 3).

Comparison between HBV DNA-negative and -positive patients at year 2 during entecavir therapy in all the patients

At year 2 of entecavir therapy, among 50 patients, real-time PCR showed that 19 (38.0%) were negative for HBV DNA, compared with 31 (62.0%) who were still positive for HBV DNA (Table 4). The pretreatment clinical and virological characteristics between the HBV DNA-negative and -positive groups were compared by univariate analysis. In the HBV DNA-negative group, the median ALT level was significantly lower, the rate of HBeAg-negative status was significantly higher, the median HBV DNA level was lower, and the median qHBsAg level was lower, than those in the HBV DNA-positive group.

However, when multivariate analysis using logistic regression analysis was performed, the median qHBsAg level was the only significant factor that predicted the negative HBV DNA status at year 2 of entecavir therapy

Table 2 Clinical and virological results among the hepatitis B e antigen-negative group, the rapid-responders, and the slow-responders in the hepatitis B e antigen-positive group during 2 years of entecavir therapy

Characteristics	HBeAg-negative group	HBeAg-positive group		*P* value
	(*n* = 26)	RR (*n* = 14)	SR (*n* = 10)	RR *vs* SR
<Baseline data>				
Age	58 (35-80)	45 (34-68)	43 (31-71)	NS
Gender (male:female)	13:13	9:5	6:4	NS
Disease (CH:LC/HCC)	21:5	13:1	6:4	NS
ALT (IU/L)	38 (13-950)	131 (12-602)	31 (13-108)	0.02
Platelet count ($\times 10^4$/mL)	18.6 (3.4-35.1)	17.1 (8.4-22.4)	20.0 (11.0-26.8)	NS
Albumin (mg/dL)	4.3 (3.4-4.9)	4.0 (2.3-5.0)	4.4 (3.7-4.6)	NS
HBV genotype C	100%	100%	100%	NS
HBV DNA (log copies/mL)	5.1 (3.9-8.8)	7.4 (5.6-8.8)	8.3 (7.1-8.8)	NS
qHBsAg (log IU/mL)	3.17 (0.70-4.58)	3.63 (1.68-4.34)	4.57 (4.35-4.76)	< 0.01
HBcrAg (log U/mL)	3.6 (3.0-> 6.8)	> 6.8 (6-> 6.8)	> 6.8 (> 6.8-> 6.8)	NS
Pre-C mutation (%)	83.3	0	0	NS
Core promoter mutation (%)	58.3	57.1	50.0	NS
<At year 2 during therapy>				
HBV DNA (log copies/mL)	0.0 (0.0-2.7)	2.1 (0.0-2.1)	3.5 (3.1-6.9)	-
ALT (IU/L)	18 (9-75)	17.5 (10-31)	23 (13-37)	NS
HBeAg seroconversion	-	23.50%	0%	NS
HBsAg seroclearance	0%	0%	0%	NS
qHBsAg (log IU/mL)	2.91 (0.62-3.9)	3.25 (1.70-3.92)	4.12 (3.23-4.47)	0.01
HBcrAg (log U/mL)	3.0 (3.0-5.4)	5.9 (4.0-> 6.8)	> 6.8 (5.2-> 6.8)	< 0.01
Resistant mutations against entecavir	UDL	UDL	0%	-

HBeAg: Hepatitis B e antigen; ALT: Alanine aminotransferase; CH: Chronic hepatitis; LC: Liver cirrhosis; HCC: Hepatocellular carcinoma; UDL: Under the detection limit; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; qHBsAg: Quantitation of HBsAg level; HBcrAg: HBV core-related antigen; NS: Not significant; RR: Rapid-responder; SR: Slow-responder.

Table 3 Drug resistant mutations in the slow-responders at year 2

Patient	Age (yr)	Gender	Previous therapy	HBV genotype	Drug resistant mutations against						
					Lam L180	Lam M204	Lam/Ade A181	Ade N236	Ent T184	Ent S202	Ent M205
1	52	Male	No	C	Wild	Wild	Wild	Wild	Wild	Wild	Wild
2	35	Male	No	C	Wild	Wild	Wild	Wild	Wild	Wild	Wild
3	68	Male	No	C	Wild	Wild	Wild	Wild	Wild	Wild	Wild
4	56	Female	No	C	Wild	M204I	Wild	Wild	Wild	Wild	Wild
5	36	Female	No	C	Wild	M204I	Wild	Wild	Wild	Wild	Wild
6	45	Male	No	C	Wild	M204I	Wild	Wild	Wild	Wild	Wild
7	35	Male	No	C	Wild	Wild	Wild	Wild	Wild	Wild	Wild
8	67	Female	No	C	Wild	Wild	Wild	Wild	Wild	Wild	Wild
9	39	Male	No	C	Wild	Wild	Wild	Wild	Wild	Wild	Wild
10	44	Female	No	C	Wild	Wild	Wild	Wild	Wild	Wild	Wild

Lam: Lamivudine; Ade: Adefovir; Ent: Entecavir; HBV: Hepatitis B virus.

(odds ratio 8.16, 95% CI: 1.28-52.18, $P = 0.03$).

DISCUSSION

In this study, the clinical and virological features of patients with HBV genotype C who received naïve-entecavir therapy were analyzed. After 2 years of entecavir therapy, about 42% of the HBeAg-positive patients showed HBV DNA levels ≥ 3 log copies/mL, while all of the HBeAg-negative patients showed < 3 log copies/mL. Therefore, the factors associated with the slow response to entecavir therapy among the HBeAg-positive group were studied initially. In addition, among the 50 patients, 38% showed HBV DNA-negative status at year 2. Thus, the pretreatment factors that predict the loss of HBV DNA were analyzed in all 50 patients. According to the multivariate analysis, qHBsAg levels are the most important factor for predicting the response to entecavir therapy in patients with HBV genotype C.

In Japan, HBV genotype C is the most prevalent[3]. The response rates to interferon or NA therapy in patients with HBV genotype C, as well as D, are poor when compared to those with HBV genotype B or A[13]. Thus, in this study, only subjects with HBV genotype C were studied.

Recently, a decline in HBsAg levels during PEG-interferon therapy was reported to be significant in the evaluation of the response to therapy[14-17]. In these reports, HBsAg levels were found to be one of the best viral markers for predicting response to anti-viral therapy and viral

Table 4 Pretreatment clinical and virological characteristics between hepatitis B virus DNA-negative and -positive group at year 2 during entecavir therapy

Characteristics	HBV DNA-negative group (n = 19)	HBV DNA-positive group (n = 31)	P value	
			Univariate analysis	Multivariate analysis
Age	51 (31-73)	52 (32-80)	NS	
Gender (male:female)	12:7	20:11	NS	
Disease (CH:LC/HCC)	17:2	27:4	NS	
ALT (IU/L)	36 (12-366)	108 (13-602)	0.03	NS
Platelet counts ($\times 10^4$/mL)	19.0 (8.8-35.1)	17.8 (3.4-26.8)	NS	
Albumin (mg/dL)	4.35 (3.84-4.85)	4.14 (2.28-4.72)	NS	
HBV genotype (B:C:others)	0:19:0	0:31:0	NS	
HBeAg status (positive:negative)	3:16	21:10	< 0.01	NS
HBV DNA (log copies/mL)	5.1 (3.1-7.4)	7.6 (3.7-8.8)	< 0.01	NS
qHBsAg level (log IU/mL)	3.31 (1.90-4.08)	4.20 (3.06-4.87)	< 0.01	0.03
HBcrAg level (log U/mL)	3.45 (3.0-> 6.8)	> 6.8 (3.0-> 6.8)	NS	
Pre-C mutation (%)	75.0	43.3	NS	
Core promoter mutation (%)	37.5	60.0	NS	

CH: Chronic hepatitis; LC: Liver cirrhosis; HCC: Hepatocellular carcinoma; ALT: Alanine aminotransferase; HBV: Hepatitis B virus; HBeAg: Hepatitis B e antigen; qHBsAg: Quantitation of hepatitis B surface antigen level; HBcrAg: HBV core-related antigen; NS: Not significant.

activity levels in hepatocytes, correlating with cccDNA levels[14,18]. Although qHBsAg is a predictor of response to entecavir therapy[19], there have been no reports in a homogeneous HBV genotype setting. In this study, qHBsAg was demonstrated to be the most significant predictor of entecavir therapy in patients with HBV genotype C.

HBV DNA levels are also considered an important factor associated with response to anti-viral therapy[17]. In this study, there was a tendency for higher HBV DNA levels in the slow-responders as compared to rapid-responders. The association between HBV DNA levels and response to therapy may be clarified further in a larger number of patients.

HBcrAg levels indicate the serum HB core antigen levels plus HBeAg levels[11]. HBcrAg levels are reported to be associated with cccDNA levels in hepatocytes[20]. However, this study showed no association between HBcrAg levels and slow response. This may be explained by the narrow quantitation range of HBcrAg levels, because HBcrAg levels in most patients in the HBeAg-positive group were greater than the upper level of the detection range of the assay.

The association between Core promoter mutation and response rate to NAs is also interesting, because the replication level of HBV is thought to be high in patients with Core promoter mutations. We reported previously that, in patients with HBV genotype C, the rates of HBeAg-positive status and Core promoter mutations are higher than those in patients with HBV genotype B[5]. In this study, a higher rate of Core promoter mutations was observed in the HBeAg-positive patients with HBV genotype C. In addition, a higher rate of Pre-C mutations was observed in the HBeAg-negative group. However, no association between the mutation rate and response rate to therapy was demonstrated in this study.

Higher ALT levels are considered an important factor in predicting good response to PEG-interferon therapy[21]. However, low ALT levels were observed in the HBV DNA-negative group at year 2 of therapy, because a high proportion of the HBeAg-negative patients had low ALT levels, compared to the HBeAg-positive patients. Thus, we consider that, during entecavir therapy, ALT levels are not associated with treatment response.

In this study, no resistant mutations against entecavir were found during 2 years therapy. As reported previously, resistant mutations against entecavir are rarely developed during 5 years of entecavir therapy[8]. Therefore, slow response was not caused by entecavir-resistant mutants.

In conclusion, we suggest that qHBsAg is a significant and convenient indicator for predicting response to entecavir therapy.

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COMMENTS

Background

Entecavir is a nucleot(s)ide analog that is widely used for the treatment of chronic hepatitis B patients. The efficacy of entecavir is very good for hepatitis B e antigen (HBeAg)-negative patients, but not so good for HBeAg-positive patients. The prognosis and response to anti-viral therapies depend on hepatitis B virus (HBV) genotype. The factors that affect the efficacy of entecavir therapy are still unclear, especially in patients with HBV genotype C.

Research frontiers

As quantitation assay of serum hepatitis B surface antigen (HBsAg) has been recently developed, allowing the serum level of HBsAg to be determined over a very wide range. The upper range of HBsAg levels could be detected to 6.7 log IU/mL by the Architect HBsAg QT immunoassay when samples were diluted to 1:20 000. Thus, the authors could analyze the relationship between the efficacy of entecavir therapy and various HBV markers.

Innovations and breakthroughs

This study showed that the quantitative HBsAg level is a significant factor for predicting the efficacy of entecavir therapy in patients with HBV genotype C. Patients with low levels of HBsAg before entecavir therapy often show HBV DNA levels < 3.0 log copies/mL or are negative for HBV DNA at year 2 during therapy.

Applications

Using the quantitation of HBsAg, the efficacy of various anti-viral therapies can be predicted before treatment. The quantitation of HBsAg could be a useful tool for determining the treatment schedule for chronic hepatitis B patients.

Peer review

Although small in patient numbers, the subject matter is interesting and original. This study adds to the emerging data suggesting HBsAg decline is a predictor of response.

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