Assays of HBV markers

Serum hepatitis B surface antigen was measured using radioimmunoassay (Dainabot, Tokyo, Japan) and reversed passive hemagglutination (Institute of Immunology, Tokyo, Japan) using commercial assay kits. hepatitis B e antigen (HBeAg) and antibody to HBeAg were determined using ELISA (Institute of Immunology) with commercial kits. Anti-hepatitis C virus antibody (third-generation anti-HCV) was assessed using ELISA kits (Dainabot).

HBV-DNA was assayed using frozen sera stored at −80°C, and quantified using transcription-mediated amplification and hybridization protection assay (Chugai Diagnostics Science, Tokyo, Japan), as described by Kamisango *et al.*[24] A HBV-DNA value of <3.7 LGE/mL (equivalent to $10^{3.7}$ copies/mL or 5000 copies/mL) was considered to be a low value. For all serial sera from the diagnosis of cirrhosis to the end of the observation period in each patient, the DNA quantification was simultaneously carried out using identical measurement kits.

Statistical analysis

Standard statistical measures and procedures were used. The Mann–Whitney *U*-test and χ^2 tests were employed for the examination of background characteristics between the groups with and without HBV-DNA elimination. Fisher's exact test was also used to analyze the relation of HBV markers to carcinogenesis. Rates of cumulative HBV-DNA disappearance, carcinogenesis and survival were calculated using Kaplan–Meier analysis,[25] and the differences between the analyzed groups were assessed using a log-rank test. A *P*-value of <0.05 using a two-tailed test was considered to be significant. Data analysis was carried out using the computer program SPSS version 11.[26]

RESULTS

HBV-DNA in clinical courses

HBV-DNA was positive in all patients at the initiation of IFN therapy (3.9–8.7 LGE/mL). HBV-DNA became negative (<3.7 LGE/mL) in 25 of 57 patients (43.9%) during the observation period, with a median of 13.6 years. The remaining 32 patients did not show a sustained negative HBV-DNA after the therapy, although nine patients did show transient negative values for a limited period during the therapy.

Clinical courses of HBV-DNA were classified into the four categories mentioned above. Nine patients (15.8%) lost HBV-DNA during and after IFN therapy (type A), 16 patients (28.1%) lost HBV-DNA after cessation of the therapy (type B). The other nine patients (15.8%) showed a transient loss of HBV-DNA (type C), and the remaining 23 (40.4%) retained persistently positive HBV-DNA (type D).

The cumulative rate of HBV-DNA disappearance was calculated using Kaplan–Meier analysis (Fig. 1).

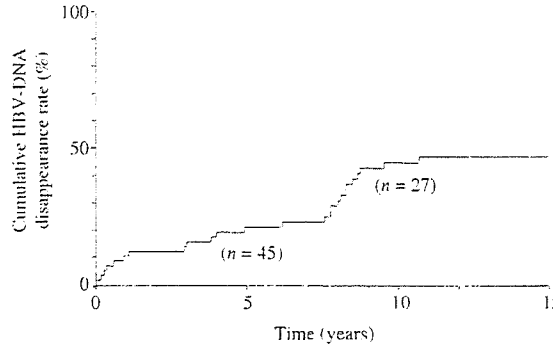

Figure 1 Cumulative hepatitis B virus (HBV)-DNA disappearance rate in the 57 cirrhotic patients with interferon therapy.

DNA became negative in 10.5% at the end of the first year after initiation of IFN therapy, in 12.3% at the third year, 21.0% at the fifth year, 43.7% at the tenth year, and 46.7% at the fifteenth year, respectively.

Hepatocellular carcinogenesis and serial concentration of HBV-DNA

A total of 13 patients developed HCC during the observation period.

The relationship between carcinogenesis and serial concentration of HBV-DNA was analyzed (Fig. 2). None of the nine patients in the type A group developed HCC. Two (12.5%) of 16 patients in the type B group developed HCC: HCC were detected 1.2 years after the disappearance of HBV-DNA in one patient, and 3.6 years after the disappearance of HBV-DNA in the other patient. Three (33.3%) of nine patients in the type C group showed carcinogenesis, and eight (34.8%) of 23 patients in the type D group developed HCC during the observation. Hepatocellular carcinogenesis was significantly associated with persistent positive HBV-DNA after initiation of IFN (2/25 *vs* 11/32; $P = 0.019$ using the $\chi 2$ test, $P = 0.026$ using Fisher's exact test).

Cumulative carcinogenesis rates were analyzed according to the ultimate course of the serial assay of HBV-DNA (Fig. 3). Fifth-year hepatocellular carcinogenesis rates were 0% in patients with HBV-DNA loss, and 9.4% in patients without HBV-DNA elimination; 10-year rates were 8.0% and 22.5%; and 15-year rates were 8% and 44.0%, respectively. The carcinogenesis rate in patients with HBV-DNA elimination was significantly lower than in those without DNA elimination ($P = 0.011$, using a log-rank test).

Hepatocellular carcinogenesis and HBeAg and aminotransferase

The relationship between carcinogenesis and HBeAg positivity during the clinical course was assessed.

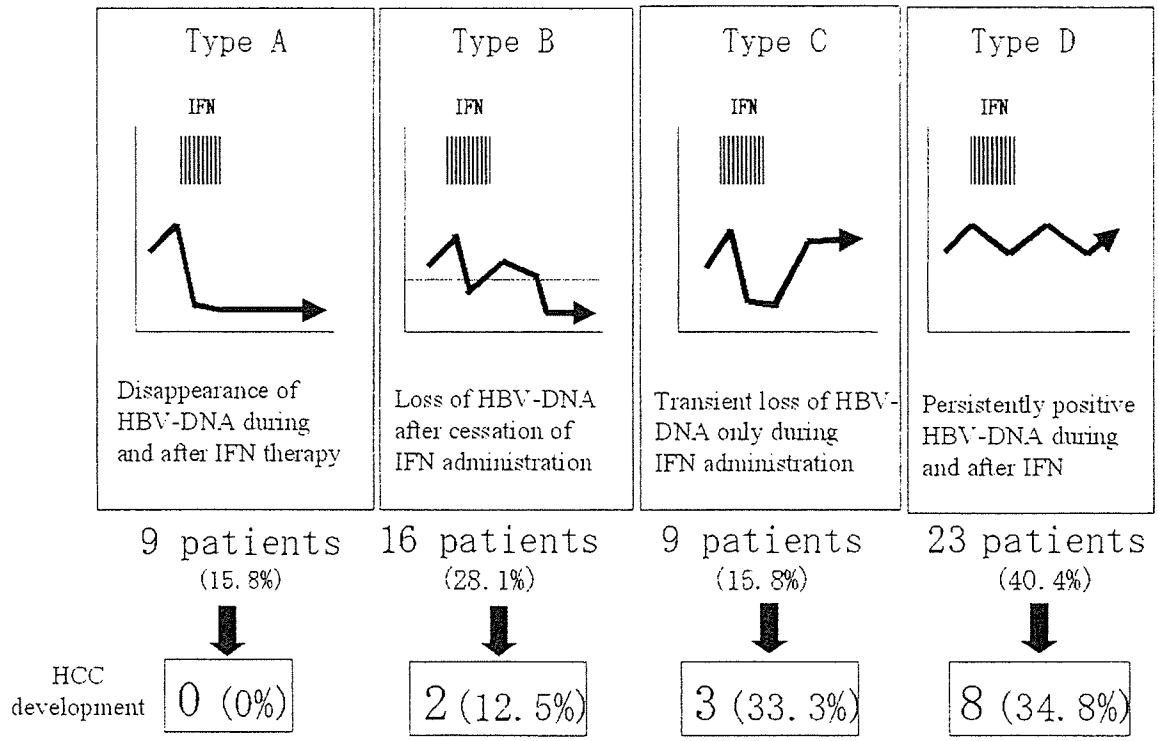

Figure 2 Relation between types of serial hepatitis B virus (HBV)-DNA concentration and carcinogenesis. HCC, hepatocellular carcinoma; IFN, interferon.

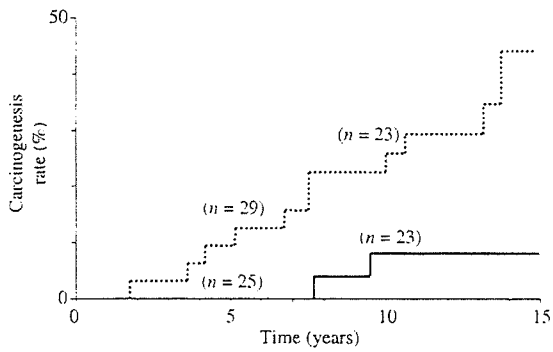

Figure 3 Cumulative hepatocellular carcinogenesis rates in patients (—; $n = 25$) with and (---; $n = 32$) without eventual hepatitis B virus (HBV)-DNA clearance.

HBeAg was positive in 41 patients (71.9%) and negative in 16 (28.1%) at the initiation of IFN therapy. Twenty-eight (68.3%) of the 41 patients showed continuous loss of HBeAg after IFN therapy. HCC developed in four (25.0%) of the 16 patients without HBeAg from the beginning, four (14.3%) of the 28 patients with HBeAg clearance, and five (38.5%) of 13 patients with persistent HBeAg positivity. HBeAg clearance did not significantly decrease the incidence of carcinogenesis risk ($P = 0.12$ using the χ^2 test with Yates' correction).

The relationship between carcinogenesis and a longitudinal course of ALT after IFN therapy was also analyzed. Four (18.2%) of 22 patients with normalization of ALT after IFN therapy developed HCC; nine (25.8%) of 35 patients with persistently abnormal ALT levels developed HCC. The serial values of ALT were not significantly associated with carcinogenesis risk ($P = 0.075$ using the χ^2 test with Yates' correction).

The cumulative HBeAg disappearance rate, HBV-DNA disappearance rate, and ALT normalization rate were calculated in those patients with positive HBeAg at the beginning of IFN treatment (Fig. 4). The HBeAg disappearance rate and DNA disappearance rates were 55.4% and 14.6% at the end of the fifth year, and 55.4% and 40.1% at the tenth year, respectively. The ALT normalization rate at the fifth year was 25.4% and the tenth year rate was 41.2%. Although the incidence of virological and biochemical improvement gradually increased after therapy, the rates evidently differed between virological and biochemical responses.

Influence of the length of interferon therapy on HBV-DNA loss

The influence of the length of the therapy on virological response was assessed.

Although 25 (43.8%) of 57 patients cleared HBV-DNA on overall analysis, 21 (46.6%) of 45 patients who received IFN for more than 6 months and 20 (50%) of 40 patients who received IFN for more than 12 months lost HBV-DNA. Similarly, the HBV-DNA disappearance rate slightly increased correlating with the length of IFN administration: 55.5% in patients who were treated for more than 18 months, 56.0% with more than 24 months' treatment, 64.7% in more than 36 months' treatment, 58.3% in more than 48 months' treatment, and 71.4% in more than 60 months' treatment (Fig. 5). The longer the IFN therapy was carried out, the higher the rate of HBV-DNA disappearance.

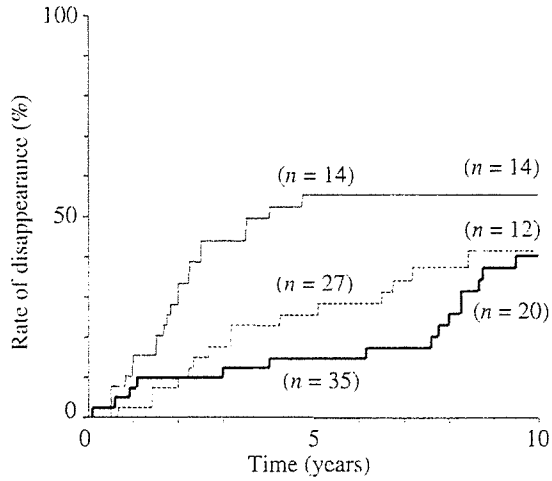

Figure 4 Cumulative (--) hepatitis B e antigen (HBeAg) disappearance rate, (—) hepatitis B virus (HBV)-DNA disappearance rate, and (---) alanine transaminase normalization rate in 41 patients with positive HBeAg at the initiation of interferon therapy.

Prediction of future HBV-DNA elimination

We assessed the relation between an early HBV-DNA response and a future HBV-DNA loss. When the HBV-DNA concentration decreased by ≥2 LGE/mL (decrease to 1/100) during the first 6 months, 15 (60.0%) of 25 patients eventually lost HBV-DNA. In contrast, when the HBV-DNA decrease was <2 LGE/mL during the period, HBV-DNA loss was found in 10 (31.3%) of 32 patients ($P = 0.036$, χ^2 test). Similarly, future HBV-DNA loss was estimated from a decrease in concentration of HBV-DNA at the end of 12 months: HBV-DNA eventually became negative in 15 (62.5%) of 24 patients with a larger DNA decrease of ≥2 LGE/mL at the end of 12 months, eventual DNA loss was found in only 10 (30.3%) of 33 patients with a smaller DNA decrease by <2 LGE/mL. The 12-month decrease of HBV-DNA was significantly associated with future DNA loss ($P = 0.030$, χ^2 test).

The early response of HBV-DNA and the length of IFN therapy were analyzed together for the prediction of eventual HBV-DNA loss. Of 25 patients with a HBV-DNA decrease of ≥2 LGE/mL during the initial 6 months, two (33.3%) of six patients with short IFN therapy of ≤6 months showed a HBV-DNA loss, but 13 (68.4%) of 19 patients with long-term IFN therapy of >6 months lost HBV-DNA. Of 32 patients with a HBV-DNA decrease of <2 LGE/mL in the first 6 months, one (20.0%) of five patients with short IFN therapy showed HBV-DNA loss, but nine (33.3%) of 27 patients with long-term IFN administration lost HBV-DNA. Therefore, according to the early HBV-DNA response and the duration of the therapy, the rate of sustained HBV-DNA decrease to <3.7 LGE/mL varied, with a range of 20.0–68.4%.

Prognosis after IFN therapy

A total of eight patients (14.0%) died in the period of observation: six from development of HCC and the

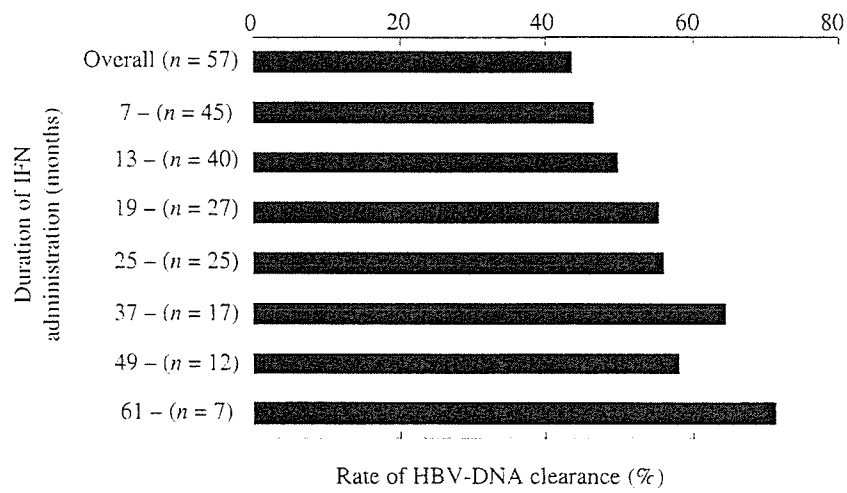

Figure 5 Influence of the length of interferon (IFN) therapy on hepatitis B virus (HBV)-DNA clearance.

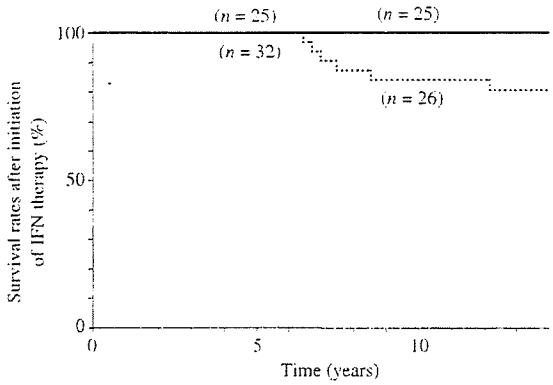

Figure 6 Cumulative survival rates after the initiation of interferon (IFN) therapy in patients (—; $n = 25$) with and (---; $n = 32$) without eventual hepatitis B virus DNA clearance.

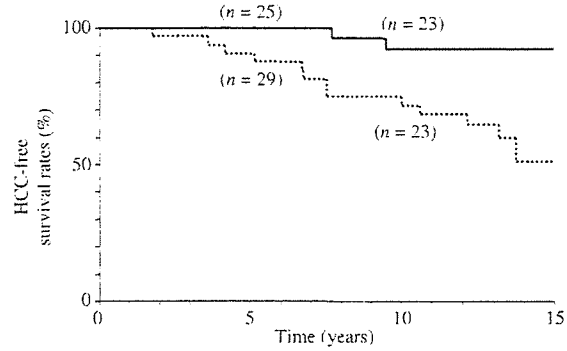

Figure 7 Hepatocellular carcinoma (HCC)-free survival rates in patients (—; $n = 25$) with and (---; $n = 32$) without eventual hepatitis B virus DNA clearance.

other two from liver failure due to aggravation of cirrhosis.

Of 13 patients with HCC development, two patients with HBV-DNA loss have not shown any tumor recurrence after surgical resection, and both patients are alive at the end of the observation. In contrast, nine (81.8%) of 11 patients with persistently high HBV-DNA developed HCC recurrence after therapy, and six (54.5%) of the patients died during the observation period. All six patients died from the development of HCC and none from aggravation of cirrhosis or extrahepatic disease.

Of 44 patients without HCC development until the end of the observation period, none of 23 patients with HBV-DNA loss died, but two (9.5%) of 21 patients with persistently positive HBV-DNA have died from liver failure.

Survival rates were compared between those patients with and without HBV-DNA loss (Fig. 6). Fifth-year survival rates in patients with and without HBV-DNA loss were 100% and 100%, seventh year rates were 100% and 90.5%, tenth year rates were 100% and 84.1%, and twelfth year rates were 100% and 80.6%, respectively. The cumulative survival rate in patients with HBV-DNA loss was significantly higher than that in patients without HBV-DNA clearance ($P = 0.0030$, log-rank test).

The HCC-free survival rates were also assessed in the two patient groups (Fig. 7). Fifth-year HCC-free survival rates in patients with and without HBV-DNA loss were 100% and 90.6%, seventh year rates were 100% and 81.3%, tenth year rates were 92% and 74.8%, and fifteenth year rates were 92% and 51.2%, respectively. The HCC-free survival rate in patients with HBV-DNA loss was significantly higher than that in patients without HBV-DNA clearance ($P = 0.0036$, log-rank test).

DISCUSSION

Until recently, several authors mentioned the anti-carcinogenic activity of IFN in patients with HBV-related cirrhosis. Oon[18] and Ikeda *et al.*[21] have shown that IFN significantly decreased carcinogenesis in patients undergoing IFN therapy with a relative risk of 0.03 and 0.39, respectively. Lin *et al.* also demonstrated an anti-tumor activity of IFN, with a relative risk of 0.11 in a randomized controlled trial for patients with chronic hepatitis and cirrhosis.[23] Mazzella *et al.*,[19] Fattovich *et al.*[20] and the International Interferon-alpha Hepatocellular Carcinoma Study Group in Europe[22] demonstrated a low relative risk for carcinogenesis in patients with IFN therapy, but none could show a statistically significant difference. Aside from the slightly inconsistent results after IFN therapy for cirrhosis, we tried to elucidate the relationship between virological response and HCC development, using a cohort of consecutive patients with cirrhosis who underwent IFN therapy more than 10 years ago. Considering that the disease activity and carcinogenic potency can change significantly in the course of HBV-related liver disease, a longitudinal analysis was carried out for the study of the clinical process and the mechanism of anti-tumor activity of IFN in HBV-positive cirrhosis patients.

In this clinical study, sequential trends of HBV concentration were significantly associated with hepatocellular carcinogenesis, as was found in natural clinical courses of patients without IFN.[27] Although only two of 25 patients who developed HCC showed a disappearance of HBV-DNA during or after IFN therapy, 11 of 32 patients who showed carcinogenesis could not eliminate HBV-DNA using treatment with IFN ($P = 0.019$). A point in common found in the two exceptional patients with HCC development after elimination of HBV-DNA was that the HCC were detected immediately after a significant decrease in the HBV-DNA level after using IFN in the clinical courses: 1.2 years and 3.6 years after in each patient. We can reasonably consider that the discovered HCC in the patients already existed at an indiscernible size at the time of HBV-DNA elimination, and that the minimal HCC automatically grew gradually for the following few years after the decrease in HBV-DNA levels occurred. Even including these two patients with HCC development, the risk of hepatocellular carcinogenesis was significantly associ-

ated with the persistence of a high HBV-DNA concentration. Hepatocellular carcinogenesis was assessed using serial HBV-DNA assay with a cut-off value of 3.7 LGE/mL (or $10^{3.7}$ copy/mL) in this study. Although a detailed analysis of HBV-DNA concentration with a more sensitive measurement may demonstrate a better correlation with the carcinogenesis rate than the present study, setting the HBV-DNA concentration at this cut-off value was significantly valuable in the prediction for HCC appearance.

The mechanism of anticarcinogenic activity of IFN was regarded as an anti-necroinflammatory process through suppression of HBV-DNA concentration from these results. This study dealt with the relationship between carcinogenesis and HBV-DNA principally, but clinical courses of aminotransferase were also significantly related to the HCC development. Aminotransferase values were less valuable than HBV-DNA levels in the prediction of HCC development in the natural clinical course of HBV-cirrhosis,[27,28] and aminotransferase values were also less associated with the future rate of carcinogenesis in patients undergoing IFN therapy.

Although the mere use of IFN does not guarantee a decrease in the rate of carcinogenesis in patients with HBV-related cirrhosis, a serial course of HBV-DNA concentration was significantly correlated with future HCC development during and after treatment. The value of cancer prediction was much higher from the assay of HBV-DNA than that of HBe antigen. Indeed the cut-off values of HBV-DNA concentration seemed to be discretionary; the advantage in clinical practice was marked and conspicuous. When more sensitive ways of measuring HBV-DNA concentration were applied to the analysis, hepatocellular carcinogenesis could be more successfully predicted.

In conclusion, persistence of a high concentration of HBV-DNA was significantly associated with hepatocellular carcinogenesis in cirrhotic patients with IFN therapy, and its sequential analysis would be useful in the early detection of HCC. IFN therapy is recommended to be continued as long as possible until HBV-DNA loss occurs in HBV-cirrhosis patients, from the viewpoint of cancer prevention. Further studies with a greater number of patients are required to confirm the relationship, and future studies should be aimed at defining the role and basic mechanisms by which the carcinogenesis rate was suppressed by IFN in the cohort.

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Hepatitis B Virus-Related Hepatocellular Carcinogenesis and Its Prevention

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Key Words
Carcinogenesis · Hepatocellular carcinoma · Liver cirrhosis · Hepatitis B virus · DNA · Interferon · Cancer prevention

Abstract
To elucidate the influence of serum hepatitis B virus (HBV) load on hepatocellular carcinogenesis in cirrhotic patients, HBV-DNA was sequentially measured. In a nested, case-control study using 96 patients without antiviral therapy, high HBV-DNA ($\geq 10^{3.7}$ copies/ml) in the last 3 years was significantly associated with carcinogenesis (a patient group without hepatocellular carcinoma (HCC) development; 0/48 vs. a patient group with eventual HCC development; 22/48, $p < 0.0001$). No patient with a continuously low HBV-DNA for the last 3 years developed HCC. Persistence of high HBV-DNA concentration suggested an increased risk of carcinogenesis. In a retrospective cohort study using 57 patients with interferon therapy, HCC developed in 2 (8.0%) of the 25 patients with HBV-DNA loss, while carcinogenesis was found in 11 (34.4%) of 32 patients without HBV-DNA loss (Fisher's exact test, $p = 0.026$). A significant decrease or loss of serum HBV-DNA stops HCC development, and its sequential analysis could be very useful both in the prediction and early detection of small HCC.

Introduction

Hepatocellular carcinoma (HCC) is a principal cause of death in many parts of sub-Saharan Africa and in Asia [1, 2]. It is also one of the most common neoplasms in Japan [3]. Abundant epidemiological and molecular biological evidence shows that hepatitis B virus (HBV) is an important factor in the development of HCC [4–6], but the precise role of HBV DNA viruses in oncogenesis is still unknown. Although increasing evidence indicates that the HBV plays an important role in the development of HCC after discovery of integrated forms of HBV [7–9], current serological and virological markers are still insufficient in establishing this relationship. Since a really curative therapy is not available for HCC at present, an accurate prediction and early detection of HBV-related HCC is essential in the current situation.

Hepatocellular carcinogenesis rates were estimated in patients with HBV-related chronic hepatitis (n = 297) and cirrhosis (n = 246), who have not received interferon (IFN), lamivudine, or steroid therapy. They were diagnosed by peritoneoscopy and/or biopsy as having chronic liver disease in the Toranomon Hospital, Tokyo, Japan, from 1974 to 1999. Cumulative carcinogenesis rates in F1 fibrosis, F2–3, and F4 were 0.5, 6.3, and 19.7% at the end of the 5th year, 2.7, 14.9, and 30.3% at the end of the 10th year, 4.1, 19.5, and 35.8% at the15th year, and 15.0, 29.6, 41.9% at the 20th year, respectively (fig. 1).

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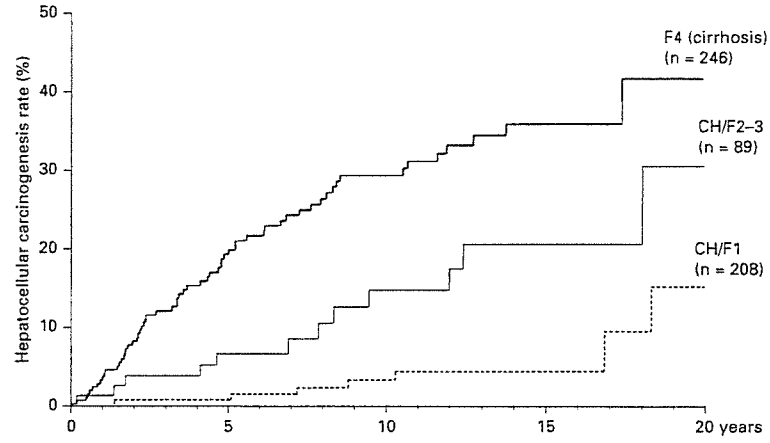

Fig. 1. Cumulative hepatocellular carcinogenesis rates in patients with chronic hepatitis or cirrhosis.

Needless to say, patients with HBV-related cirrhosis have a significantly higher risk for HCC development [10, 11], but the degree of the carcinogenesis risk in an individual patient cannot be predicted as yet. How can we recognize a super-high-risk group or a rather low-risk group in HBV-related cirrhosis? Can we predict and specify a patient who is not likely to develop HCC in the future? Hepatocellular carcinogenesis in patients with HBV infection may well be associated with persistence of aminotransferase, concentration of HBV DNA, or merely the severity of the liver disease. One of the purposes of this article is, therefore, to elucidate the relationship of hepatocellular carcinogenesis with longitudinal clinical courses of biochemical data and HBV DNA concentration in consecutive patients with cirrhosis.

IFN has been reported to be effective in patients with HBV-related chronic hepatitis, which decreases serum HBV DNA concentration and improves biochemical data on early control studies [12–14], and subsequently suppresses disease progression to cirrhosis [15, 16]. Although various effects of IFN in hepatitis B virus infection have been well investigated from virological, biochemical, and medico-economical viewpoints [17–19], the influence on a long-term outcome of liver cirrhosis or on hepatocellular carcinogenesis is still controversial [20–25]. In order to clarify the mechanism of anti-carcinogenic activity of IFN, if any, we analyzed HBV DNA concentration serially in a cohort of 60 patients with cirrhosis. The other purposes of this study are to elucidate the relationship of hepatocellular carcinogenesis with longitudinal clinical courses in consecutive cirrhotic patients with interferon therapy and to investigate an early prediction of HBV DNA elimination and the cancer preventive activity.

Factors Affecting Hepatocellular Carcinogenesis in Cirrhosis (without Anti-Viral Therapy)

Patients and Methods

Analyzable Patients without Anti-Viral Therapy

Among 217 patients who were diagnosed as having HBV-related cirrhosis by peritoneoscopy and/or liver biopsy from 1976 to 1989 in our hospital, 160 patients had not undergone interferon or other antiviral therapy. Out of the consecutive 160 patients, sequential assay of serum HBV DNA using serial sera stored at −80°C was available in 146 patients (91.3%). All 146 patients showed a positive hepatitis B surface antigen and negative anti-hepatitis C virus antibody in the assay of their initial sera. Among the 146 patients with HBV-related cirrhosis, 48 patients (32.9%) developed HCC during a median follow-up period of 7.2 years after the diagnosis of cirrhosis, and the other 98 patients (67.1%) have not developed HCC during 11.7 years.

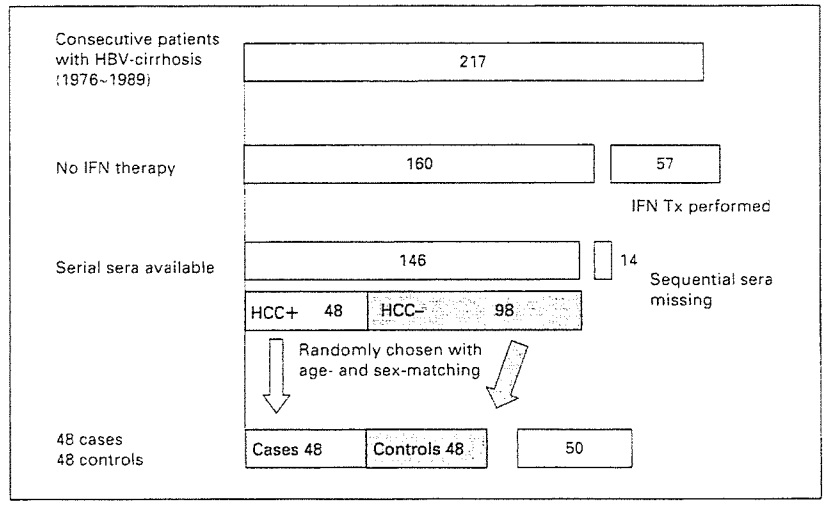

Fig. 2. Analyzed patients with HBV-related cirrhosis. Since almost all of consecutive untreated patients (146/160, 91.3%) were analyzable, a nested case-control study was established using a total of 96 patients.

Follow-Up of Patients and Diagnosis of HCC

Follow-up of the patients was made on a monthly or bi-monthly basis after diagnosis of liver cirrhosis by monitoring α-fetoprotein (AFP) and other biochemical data. Imaging diagnosis was carried out two or more times per year for each patient with computed tomography (CT), ultrasonography (US), or scintigraphy. HCC was diagnosed by typical hypervascular characteristics on angiography in addition to certain features of CT and US. A pathological confirmation of surgically resected specimens or autopsy was made in 38 (79.2%) of 48 patients with HCC development.

Nested Case-Control Study

In order to elucidate the relationship between hepatocellular carcinogenesis and longitudinal courses of clinical markers, a nested case-control study was introduced. Age- and gender-matched control patients were selected from the 98 HCC-free patients with a ratio of 1:1. The control patients were randomly selected using a computer-generated random number table, avoiding those with a short observation period of less than 3 years. Thus, a nested case-control study was made, consisting of 48 cases with cancer development (group A) and 48 demography-adjusted controls without sings of cancer (group B) (fig. 2).

Assays of HBV Markers

HBV DNA was assayed using frozen sera stored at −80°C, and quantified using transcription mediated amplification and hybridization protection assay described by Kamisango et al. [26] (TMA-HPA, Chugai Diagnostics Science, Tokyo, Japan). A lower value of HBV DNA of 3.7 LGE/ml (equivalent for $10^{3.7}$ copies/ml or 5,000 copies/ml) was considered as a low value. For annual sera from the diagnosis of cirrhosis to the end of observation period in each patient, the DNA quantification was simultaneously performed after fixation of the 48 cases and the 48 controls.

Statistical Analysis

Standard statistical measures and procedures were used. Mann-Whitney U test, χ^2 test, and Fisher's exact test were employed for examination of background characteristics of the patient groups with and without HCC development. $p < 0.05$ with the two-tailed test was considered significant. Data analysis was performed using the computer program SAS version 6.12 [27].

Results

Demography and Initial Laboratory Data of the Groups with or without HCC Development [28]

Table 1 shows the demography and initial laboratory data of the patients in groups A and B. The ratio of men was 39 of 48 (81.3%) in the both groups, and the median age was 49.5 and 49 in groups A and B, respectively. The proportion of decompensated cirrhosis, and a history of past alcohol consumption, were not significantly different

Table 1. Demography and initial laboratory data of 48 patients with HCC development and the 98 patients without HCC development during the observation period

	Group A HCC development (n = 48)	Group B no HCC (n = 48)	p
Demography			
Men:women	39:9	39:9	NS
Age, median (range)	49.5 (30–71)	49 (30–71)	NS
Decompensated cirrhosis	1 (2.1%)	7 (14.6%)	0.65
Past alcohol consumption of 500 kg or more	8 (16.6%)	9 (18.8%)	0.79
Initial laboratory data (median, range)			
Anti-HCV antibody positive	0	0	NS
HBe antigen positive	33/48 (68.8%)	17/48 (36.1%)	0.001
Bilirubin, mg/dl	1.0 (0.6–9.8)	1.0 (0.5–7.5)	0.46
Albumin, g/dl	3.95 (2.4–4.8)	4.0 (2.5–5.2)	0.23
Aspartic transaminase, IU	39.5 (15–820)	31.5 (13–376)	0.23
Alanine transaminase, IU	32 (8–740)	31 (9–313)	0.82
Platelet count, $\times 10^3/mm^3$	100 (28–225)	121 (49–255)	0.047
AFP, ng/ml	16 (3–785)	7 (3–1,520)	0.037

between the two groups. The prevalence of positive HBe antigen was, however, significantly higher in group A than that in group B. Although median platelet count was slightly lower, and alpha-fetoprotein concentration was higher in group A, there was no significant difference in bilirubin, albumin, aspartic transaminase, and ALT between the two groups.

Individual HBV DNA Concentration until the End of the Observation Period [28]

Quantitative HBV DNA assessment was sequentially performed until the diagnosis of HCC in each patient. In group A (HCC development), 9 patients showed intermittently high HBV DNA concentration and 39 patients showed a continuously high HBV DNA concentration from the diagnosis of cirrhosis to the development of HCC. All the patients experienced high HBV DNA during their clinical courses, and no patient showed low HBV DNA for a consecutive 3 years just before the detection of HCC.

Serial HBV DNA concentration of each patient was also assessed in group B (no HCC development). HBV DNA was continuously low in 9 patients, and HBV DNA concentration showed a settling down and lowering for 3 years or more until the end of observation period in 13 patients. Nine patients showed a fluctuated HBV DNA concentration, and the remaining 17 patients had a continuously high HBV DNA during the observation period. Of the 48 patients, 9 patients never experienced a high HBV DNA load, and a total of 22 patients (45.8%) showed low HBV DNA values for a successive 3 years until the end of the observation (fig. 3).

The incidences of HBV DNA patterns were significantly different between the two groups (χ^2 test, $p < 0.001$). The rates of low or a settling down trend of HBV DNA concentration was significantly lower in group A

Table 2. Demography and laboratory data of 57 patients with HBV-related cirrhosis undergoing interferon therapy

Demography	
Men:women	45:12
Age, median (range)	41 (19–60)
Decompensated cirrhosis	3 (5.3%)
Past alcohol consumption of 500 kg or more	3 (5.3%)
Laboratory data, median (range)	
Bilirubin, mg/dl	0.9 (0.4–2.6)
Albumin, g/dl	4.1 (3.0–4.9)
Aspartic transaminase, IU/l	65 (16–404)
Alanine transaminase, IU/l	74 (12–586)
Platelet count, $\times 10^3/mm^3$	125 (68–332)
Anti-HCV antibody positive	0
HBe antigen positive	41 (71.9%)
HBV-DNA, LGE/ml[1]	7.2 (3.9 to >8.7)
Observation period, years	13.6 (6.5–16.1)

[1] HBV-DNA (LGE/ml): log-genome equivalent, expressed as 10^n copies/ml.

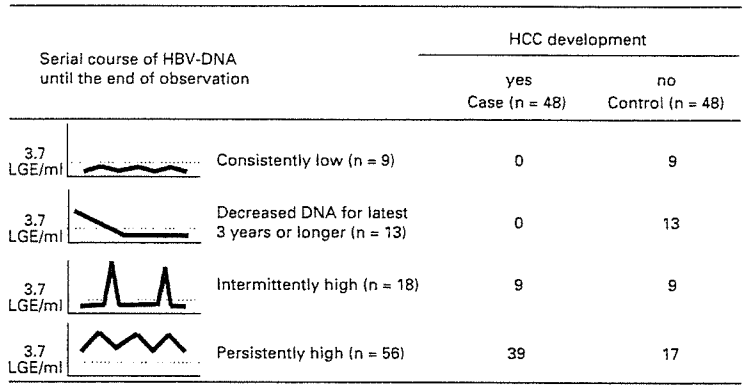

Fig. 3. Patterns of longitudinal courses of HBV-DNA in groups A and B.

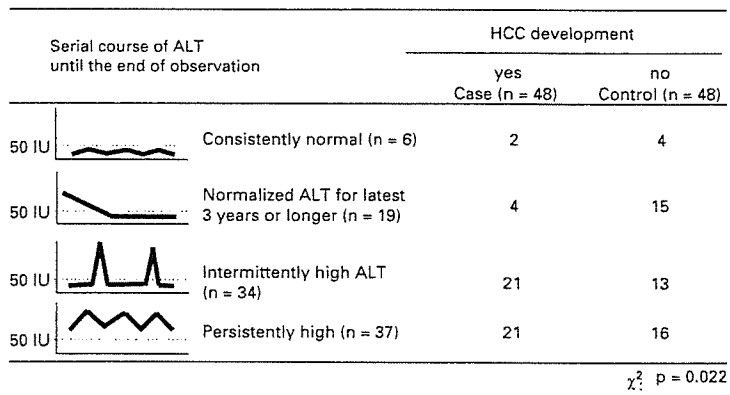

Fig. 4. Patterns of longitudinal courses of alanine transaminase in groups A and B.

than in group B (0/48 in group A vs. 22/48 in group B, Fisher's exact test, $p < 0.00001$). Any patients with a continuously low HBV DNA concentration for 3 years or longer did not develop HCC during the clinical courses.

Patterns of Longitudinal Courses of Alanine Transaminase [28]

ALT values were also assessed sequentially throughout the entire clinical courses. In group A, ALT was continuously normal in 2 patients (4.2%), ALT was high initially but normalized for the last 3 years or longer in 4 (8.3%), it showed abnormal values intermittently in 21 (43.8%), and had a continuously high value during the observation period in the remaining 21 (43.8%). In group B, 4 patients (8.3%) showed consistently normal ALT, 15 (31.3%) showed a decrease in ALT values, 13 (27.1%) intermittent elevation, and the remaining 16 (33.3%) showed continuously high ALT values during the follow-up period (fig. 4).

The incidence of HBV DNA patterns was significantly different between the two groups (χ^2 test, $p = 0.022$). While persistently or intermittently elevated ALT value slightly favored higher carcinogenesis rate (42/48 in group A vs. 29/48 in group B), statistical significance was, however, not obtained between carcinogenesis and ALT values (χ^2 test, $p = 0.077$).

Discussion

Liver cirrhosis due to hepatitis C virus usually shows a rather steady and constant clinical course, which enables us to estimate the future carcinogenesis rate from only clinical information at the time of the diagnosis of cirrhosis. Disease activity and carcinogenic potency of HBV-related liver disease, on the contrary, often change in natural clinical courses, accompanying significant fluctuation of HBe antigen system or amount of HBV DNA. When we investigate the relationship between hepatocellular carcinogenesis and its affecting and contributing factors, explanatory parameters should include not only initial demographic data but also chronological clinical data after starting the observation [29]. A longitudinal analysis is, therefore, necessary for the study of carcinogenesis in chronic liver disease caused by HBV. We, therefore, established a nested case control study using longitudinal clinical data until the end of the observation period or just before carcinogenesis, including HBV DNA quantification and ALT.

In this study, the sequential trend of serum HBV DNA concentration was significantly associated with hepatocellular carcinogenesis, and the relationship of HBV DNA to the carcinogenesis was much stronger than that of ALT. Indeed, mere initial background features and laboratory data of the patients could predict a future risk of carcinogenesis, and the chronological analysis demonstrated more discrete differentiation of a high-risk group and provided more detailed information about HBV-related carcinogenesis. Although this study illustrates that a consistence of low HBV-DNA concentration for 3 years or longer saves cirrhotic patients from carcinogenesis, the combination of 'low HBV-DNA' and '3 years' might not avoid the carcinogenesis risk sufficiently, considering the fact that hepatocellular carcinoma does develop without hepatitis, without high ALT, or without high HBV-DNA. It is, however, true that HCC scarcely develops in a patient with HBV-related cirrhosis whose HBV-DNA concentration is consistently low for the recent 3 years or longer.

Although a high load of HBV-DNA seems to promote carcinogenesis or tumor growth, the reason why a high concentration of HBV-DNA affected hepatocellular carcinogenesis remains unknown. Taking into account that hepatitis patients with positive HBe antigen and fluctuated aminotransferase values often show a high serum HBV-DNA concentration, a large amount of HBV-DNA load may be associated with a high carcinogenesis rate through an active inflammatory state and indirect cancer promotion [30]. Relationship between hosts and hepatitis virus should also be considered in future studies on carcinogenesis.

Hepatocellular Carcinogenesis in Cirrhotic Patients with Interferon Therapy

Patients and Methods

Analyzed Patients

Among 189 patients who were diagnosed as having HBV-related cirrhosis by peritoneoscopy and/or liver biopsy from 1983 to 1990 in our hospital, a total of 60 patients underwent interferon therapy from 1986 to 1990. Since 3 patients were lost to follow-up, the remaining 57 patients (95.0%) were analyzed for virological outcome, carcinogenesis, and eventual prognosis: the reason for the dropout from the observation in the 3 patients was simply house moving.

Interferon Therapy

IFN-α was administered in 35 patients (61.4%) and IFN-β in the remaining 22 patients (38.6%). The daily amount of IFN was 3 million units in 22 (38.6%) and 6 million units in 35 (61.4%), and twice a week administration was performed in 54 (94.7%) and three times a week in 3 (5.3%). All patients received an intermittent interferon therapy for a median of 18 months (range 2–132 months), but the duration of the IFN therapy was arbitrary in this pilot study. Although the amount of daily dose of IFN and the duration of the therapy varied in this study, 52 (91.2%) of the 57 patients received IFN for 6 months or longer.

Follow-Up and Diagnosis of HCC

Follow-up of the patients was made on a monthly basis after diagnosis of liver cirrhosis by monitoring virological, hematological, and biochemical data including α-fetoprotein (AFP). All these laboratory tests including HBV-markers were obtained throughout the observation period in each patient. Patients were classified into four groups according to patterns of serial concentration of HBV DNA: type A, disappearance of HBV DNA during and after IFN therapy; type B, loss of HBV DNA after cessation of IFN administration; type C, transient loss of HBV DNA only during IFN administration; type D, persistently positive HBV DNA during and after the therapy. Clinical courses of ALT fluctuation were also classified into four groups according to normalization of ALT value.

Imaging diagnosis and establishment of diagnosis of HCC were carried out as shown above.

Assay of HBV DNA

HBV-DNA was assayed using frozen sera stored at −80°C, and quantified using transcription mediated amplification and hybridization protection assay described by Kamisango et al. [26] as shown above.

Statistical Analysis

Standard statistical measures and procedures were used. Mann-Whitney's U and χ^2 tests were employed for examination of background characteristics between the groups with and without HBV DNA elimination. Fisher's exact test was also used to analyze the relationship of HBV markers with carcinogenesis. Cumulative HBV DNA disappearance rate, carcinogenesis rate, and survival rate were calculated by Kaplan-Meier technique [31], and the differences between the analyzed groups were assessed by log-rank test. $p < 0.05$ with the two-tailed test was considered to be significant. Data analysis was performed using the computer program SPSS version 11 [32].

Results

HBV DNA in Clinical Courses [33]

HBV DNA was positive in all the patients at the initiation of IFN therapy (3.9 to >8.7 LGE/ml). HBV DNA became negative (<3.7 LGE/ml) in 25 of 57 patients (43.9%) during the observation period with a median of 13.6 years. The remaining 32 patients did not show a sustained negative HBV DNA after the therapy, although 9 patients did show transient negative values for a limited period during the therapy.

Clinical courses of HBV DNA were classified into the four categories mentioned above. Nine patients (15.8%) lost HBV DNA during and after IFN therapy (type A), 16 patients (28.1%) lost HBV DNA after cessation of the therapy (type B). The other 9 patients (15.8%) showed a transient loss of HBV DNA (type C), and the remaining 23 (40.4%) retained persistently positive HBV DNA (type D).

Cumulative rate of HBV DNA disappearance was calculated using Kaplan-Meier technique. DNA became negative in 10.5% at the end of the first year after initiation of IFN, 12.3% at the third year, 21.0% at the fifth year, 43.7% at the tenth year, and 46.7% at the fifteenth year, respectively.

Hepatocellular Carcinogenesis and Serial Concentration of HBV DNA [33]

A total of 13 patients developed HCC during the observation period.

The relationship between carcinogenesis and serial concentration of HBV DNA was analyzed (fig. 5). No patients (0%) developed HCC among 9 patients in type A. Two (12.5%) of 16 patients developed HCC in type B: HCC were detected 1.2 year after disappearance of HBV DNA in one patient, and 3.6 years after disappearance of HBV DNA in the other patient. Three (33.3%) of 9 patients showed carcinogenesis in type C, and 8 (34.8%) of 23 patients developed HCC in type D during the observation. Hepatocellular carcinogenesis was significantly associated with persistent positive HBV DNA after initiation of IFN (2/25 vs. 11/32, $p = 0.019$ by χ^2 test, $p = 0.026$ by Fisher's exact test).

Cumulative carcinogenesis rates were analyzed according to the ultimate courses of serial assay of HBV DNA. Fifth-year hepatocellular carcinogenesis rate were 0% in patients with HBV DNA loss, and 9.4% in patients without HBV DNA elimination, 10-years rates were 8.0 and 22.5%, and 15-year rates were 8 and 44.0%, respectively. The carcinogenesis rate in patients with HBV DNA elimination was significantly lower than those without DNA elimination ($p = 0.011$, log-rank test).

Hepatocellular Carcinogenesis and HBe Antigen and Aminotransferase [33]

Relationship was assessed between carcinogenesis and HBeAg positivity during the clinical courses. HBeAg was positive in 41 patients (71.9%) and negative in 16 (28.1%) at the initiation of IFN therapy. Twenty-eight (68.3%) of the 41 patients showed continuous loss of HBeAg after IFN therapy. HCC developed in 4 (25.0%) of the 16 patients without HBeAg from the beginning, 4 (14.3%) of the 28 patients with HBeAg clearance, and 5 (38.5%) of 13 patients with persistent HBeAg positivity. HBeAg clearance did not significantly decrease the incidence of carcinogenesis risk ($p = 0.12$, χ^2 test with Yates' correction).

Relationship was also analyzed between carcinogenesis and a longitudinal course of ALT after IFN therapy. Four (18.2%) of 22 patients with normalization of ALT after IFN therapy developed HCC, 9 (25.8%) of 35 patients with persistent abnormal ALT developed HCC. Serial values of ALT was not significantly associated with carcinogenesis risk ($p = 0.075$, χ^2 test with Yates correction).

Cumulative HBe antigen disappearance rate, HBV-DNA disappearance rate, and ALT normalization rate

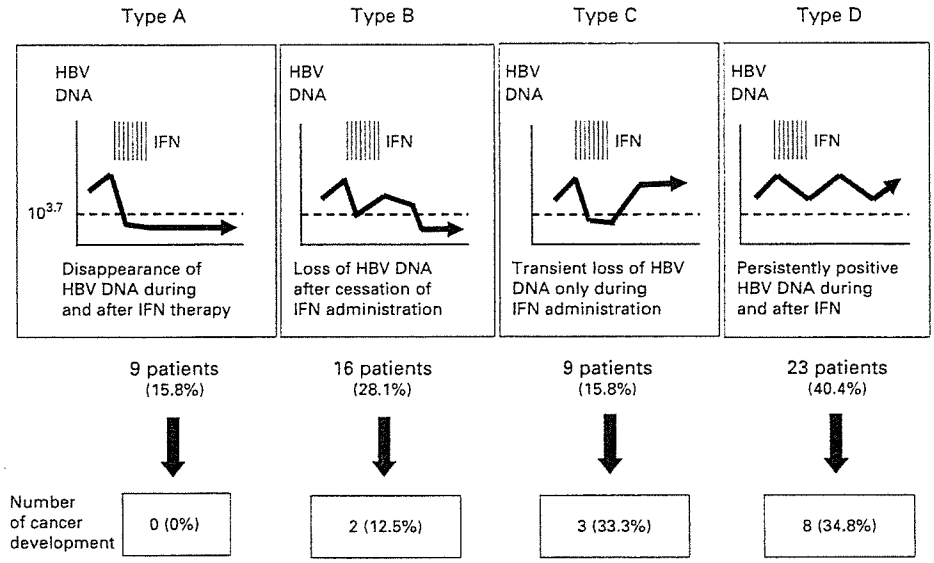

Fig. 5. Relationship between types of serial HBV-DNA concentration and carcinogenesis.

were calculated in those patients with positive HBe antigen at the beginning of IFN treatment. HBe antigen disappearance rate and DNA disappearance rate were 55.4 and 14.6% at the end of the 5th year, and 55.4 and 40.1% at the 10th year, respectively. ALT normalization rate at the 5th year was 25.4% and 10th-year rate was 41.2%. Although the incidence of virological and biochemical improvement gradually increased after the therapy, the rates evidently differed among them.

Discussion

Until recently, several authors mentioned the anti-carcinogenic activity of IFN in patients with HBV-related cirrhosis. Oon [20] and we [23] showed that IFN significantly decreased a carcinogenesis in patients with IFN therapy with a relative risk of 0.03 and 0.39, respectively. Lin et al. [25] also demonstrated an anti-tumor activity of IFN with a relative risk of 0.11 in a randomized controlled trial for patients with chronic hepatitis and cirrhosis. Mazzella et al. [21], Fattovich et al. [22], and the International Interferon-alpha Hepatocellular Carcinoma Study Group in Europe [24] demonstrated a low relative risk for carcinogenesis in patients with IFN therapy, but they could not show a statistical significance. Aside from the slightly inconsistent results after IFN therapy for cirrhosis, we tried to elucidate the relationship between virological response and HCC development, using a cohort of consecutive patients with cirrhosis who underwent IFN therapy more than 10 years ago. Considering that the disease activity and carcinogenic potency can change significantly in the course of HBV-related liver disease, a longitudinal analysis was performed for the study of clinical process and mechanism of anti-tumor activity of IFN in HBV-positive cirrhosis.

In this clinical study, sequential trends of HBV concentration were significantly associated with hepatocellular carcinogenesis, as was found in natural clinical courses of patients without IFN [28]. Although only 2 of 25 patients developed HCC who showed a disappearance of HBV-DNA during or after IFN therapy, 11 of 32 patients showed carcinogenesis who could not eliminate HBV DNA by the treatment with IFN ($p = 0.019$). Hepatocellular carcinogenesis was assessed using serial HBV DNA assay with a cut off value of 3.7 LGE/ml or $10^{3.7}$ copies/ml in this study. Although a detailed analysis of HBV-DNA concentration with more sensitive measurement may

demonstrate a better correlation with carcinogenesis rate than current one, this setting of HBV-DNA concentration as a cut-off value was significantly valuable in the prediction for HCC appearance.

Although the mere use of IFN does not guarantee the decrease of carcinogenesis in patients with HBV-related cirrhosis, a serial course of HBV DNA concentration was significantly correlated with the future HCC development during and after treatment. The value of cancer prediction was much higher in the assay of HBV DNA than that of HBe antigen. Indeed the cut-off values of HBV DNA concentration seemed to be discretionary, the advantage in clinical practice was marked and conspicuous. When more sensitive ways of HBV DNA concentration were applied to the analysis, hepatocellular carcinogenesis could be more successfully predicted.

Conclusions

Persistence of high concentration of HBV DNA was significantly associated with hepatocellular carcinogenesis in cirrhotic patients with and without IFN therapy and its sequential analysis would be useful in early detection of HCC. Further studies with a greater number of patients are required to confirm the relationship, and future studies should be aimed at defining the basic mechanism of hepatocellular carcinogenesis and the role of IFN by which the carcinogenesis rate was suppressed in the cohort.

Acknowledgment

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Cost-effectiveness of radiofrequency ablation and surgical therapy for small hepatocellular carcinoma of 3 cm or less in diameter

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Abstract

Background: Cost-effectiveness of radiofrequency ablation (RFA) was assessed in treatment of hepatocellular carcinoma (HCC).
Patients and methods: During 5 years, 153 patients with HCC of 3 cm or less received RFA, and 60 underwent surgery. Judgment after RFA therapy was classified into three grades: residual tumor (grade 1), necrotic area with a less safety margin of 5 mm (grade 2), and necrosis with a safety margin of 5 mm in all directions (grade 3).
Results: Local recurrence rates after RFA and surgery were 7.9% and 0% at the third year. The rates in patients with grades 2 and 3 after RFA were 18.7% and 1.2% at the third year, respectively ($P = 0.0005$). Among 91 patients with grades 1 and 2 necrosis after initial therapy, 52 received additional ablation. Although local recurrence rate was 24.9% in 39 patients without additional therapy, the rates after therapy repetition were 10.9% in 21 patients with eventual grade 2 necrosis, and 0% in 31 patients with grade 3 ($P = 0.038$). Median costs of single RFA, repeated RFA, and surgery were ¥849,900, ¥1,086,000, and ¥1,745,100, respectively. Additional ablation reduced local recurrence by 20.7% at the cost of ¥236,100.
Conclusion: Cost-effectiveness of RFA in the treatment of small HCC was superior to that of surgery.

Keywords: Hepatocellular carcinoma; Radiofrequency ablation; Recurrence; Cost-effectiveness; Local recurrence

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common neoplasms in Africa and Asia including Japan [1]. Since it has recently became well known that more than 80% of the patients with HCC are associated with liver cirrhosis, a routine check-up for cirrhotic patients with ultrasonography (US) can usually detect small HCCs. However, because of the association of cirrhosis and tumor multiplicity, surgical resection is performed only in 20% or less of the cases [2–5]. Percutaneous loco-regional therapy is effective and feasible in the treatment of patients with a small-sized HCC of 3 cm or less in diameter. Percutaneous ethanol injection (PEI) [6,7], microwave coagulation therapy (MCT) and radiofrequency ablation (RFA) therefore, became prevalent recently in Japan, where viral liver disease due to hepatitis B and C was often found.

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In RFA therapy, radiofrequency waves are emitted from a percutaneously inserted electrode and they bring about tumor tissue necrosis by heating to a temperature of 60 °C or more [8–12]. It is now considered as one of the most effective ways of therapy for a small-sized HCC, among varied options of percutaneous tumor ablation [13,14]. Although this choice of treatment for a small HCC produces a round and good necrotic volume at the site of tumor tissue, local recurrence still occurs during a long-term observation for various conditions [12,14,15]. Possible reasons for the local recurrence include a limitation of proper judgment by current imaging diagnosis, shortage of necrotic rim around a tumor (so-called "safety margin"), and insufficient tissue necrosis adjacent to the major portal vein or hepatic vein. Because of the relatively high rate of local recurrence, RFA therapy seemed less radical than surgical resection even in the treatment of a small HCC. Treatment repetition is also associated with a decrease in quality of life of patients and increase in medical and social expense as a whole. Surgical therapy, on the other hand, is an invasive manner of treatment with a higher cost for the procedure, but is considered to show a lower recurrence rate.

The purposes of this study were [1] to examine the efficacy of RFA from the viewpoint of local recurrence, and [2] to elucidate cost-utility of RFA therapy for a small HCC compared with that of surgical treatment. Significance of repeated percutaneous ablation against HCC was also analyzed in the study.

2. Patients and methods

2.1. Patients

A total of 290 patients were diagnosed as having a small HCC of 3 cm or less in diameter, from March 1999 to April 2003, in the Department of Gastroenterology, Toranomon Hospital, Tokyo, Japan. Of these 290 patients, 153 patients underwent percutaneous RFA therapy as a curative manner of treatment, 60 patients received surgical resection, 45 had transcatheter arterial embolization, and the remaining 32 patients were treated with ethanol injection, microwave coagulation therapy, or other palliative manners of treatment.

A total of 213 consecutive patients with a small HCC, who underwent either RFA or surgery, were analyzed in this study. These contemporary patients were analyzed for total recurrence rate, manners of recurrence, admission period, and medical cost.

The patients consisted of 144 men and 69 women, and the age ranged from 38 to 87 years old with a median age of 65 years. Before the treatment with RFA or surgical resection, all the patients underwent an evaluation consisting of a medical history inquiry, physical examination, tumor measurement, performance status, chest radiograph, liver imagings (computerized tomography, ultrasonography, and digital subtraction angiography), complete blood count, blood chemistry, alpha-fetoprotein (AFP) measurement, and urinalysis.

Demography and laboratory data were compared between the two therapy modalities (Table 1). The rate of decompensated cirrhosis was slightly higher in RFA treatment group, and indocyanine retention rate at 15 min were significantly higher and platelet count were lower in patients with RFA therapy.

2.2. Hepatocellular carcinoma

Patients were required to have HCC with a definitive diagnosis by either typical hypervascular radiological features or histology through needle biopsy. Although elevation of

Table 1
Demography and laboratory data of the patients with small liver cancer

	Radiofrequency ablation ($N = 153$)	Hepatic resection ($N = 60$)	P
Demography			
Men:women	101:52	43:17	0.52
Age (median, range)	66 (38–87)	64 (38–73)	0.25
Decompensated cirrhosis	17 (11.1%)	3 (5.0%)	0.20
HBs antigen	22 (14.4%)	15 (25.0%)	0.074
Anti-HCV antibody	119 (77.8%)	43 (71.7%)	0.37
History of alcohol intake > 500 kg	21 (13.7%)	12 (20.0%)	0.29
Observation period (years)	2.5 (0.7–5.0)	2.7 (0.7–5.0)	0.25
Laboratory data (median, range)			
ICG R15 (%)[a]	30 (8–100)	20 (3–68)	<0.001
Bilirubin (mg/dl)	1.0 (0.2–3.1)	1.0 (0.3–2.2)	0.057
Albumin (g/dl)	3.5 (2.2–4.2)	3.6 (2.6–4.4)	0.008
Aspartic transaminase (IU)	50 (17–281)	46 (20–202)	0.097
Platelet count ($\times 10^3$ mm^{-3})	88 (27–229)	140 (40–245)	<0.001
Prothrombin time (%)	84 (46–121)	91 (68–122)	0.002
Alpha-fetoprotein (ng/ml)	19 (2–4290)	12 (1–1460)	0.142
PIVKA-II (AU/l)[b]	16 (5–888)	20 (5–768)	0.152

[a] ICG R15: indocyanine green retention rate at 15 min.
[b] PIVKA-II: protein induced by Vitamin K antagonist-II.

Table 2
Characteristics of hepatocellular carcinoma in the both patient groups

	Radiofrequency ablation ($N = 153$)	Hepatic resection ($N = 60$)
Initial tumor:recurrent tumor	103:50	57:3
Median tumor size (range) (mm)	18 (6–30)	20 (7–30)
Tumor multiplicity		
Solitary	114 (74.5%)	50 (83.3%)
Multiple, localized to one segment	9 (5.9%)	0
Multiple, localized to one lobe	13 (8.5%)	7 (11.7%)
Multiple, extended to both lobes	17 (11.1%)	3 (5.0%)
Number of tumor nodules		
Solitary	114 (74.5%)	50 (83.3%)
Two	22 (14.4%)	8 (13.3%)
Three	12 (7.8%)	1 (1.7%)
Four or more	5 (3.4%)	1 (1.7%)
Portal vein invasion		
No	151	56
Yes	2	4

AFP without aminotransferase fluctuation was also taken into account in the diagnosis of HCC, imaging and pathology took precedence in the establishment of diagnosis. Disease had to be measurable by US, computerized tomography (CT), and digital subtraction angiography. In order to elucidate the detailed characteristic of the HCC, CT during arterial portography (CT-AP) and computerized tomographic hepatic arteriography (CT-HA) were performed in all the patients.

The median size of the largest tumor was 18 mm in diameter (range, 6–30 mm). The numbers of the tumor were one in 164 patients (74.5%), two in 30 patients, three in 13 patients, and four or more in 6. Details of HCC were compared between the RFA and surgery group in Table 2. Patients with a recurrent tumor tended to receive RFA therapy more frequently. Although tumor size was slightly larger in the surgery group, multiple tumors were more found in the RFA group.

2.3. Method of treatment

RFA was performed using three different apparatus: radiofrequency interstitial tumor ablation system (RITA, RITA Medical Systems Inc., Mountain View, USA), cool-tip system (Tyco Healthcare Group LP, Burlington, USA) and radiofrequency tumor coagulation system (RTC system, Boston-Scientific Japan Co., Tokyo, Japan). In all three systems, treatment procedures were performed according to their company's recommendation as to generator power and process time. In the treatment with the RTC system, we adopted a "stepwise hook extension technique" [16] instead of a standard method shown by the manufacturer.

Hepatic resection was performed under intra-operative ultrasonographic monitoring and guiding. In the case of small and superficial HCC, arterial, and portal vein clumping at hepatic hilum was not usually performed for maintenance of liver perfusion.

Physicians and surgeons usually held a conference about the choice of therapy in individual patients. The choice of treatment for the small HCCs principally depended on liver function and the site of the tumor in the liver: a tumor situated deeply in the liver was usually treated with RFA, and a superficial tumor was more often treated with surgical resection.

2.4. Evaluation of therapeutic effect and follow-up

Effect of RFA was evaluated with dynamic CT in a week after each RFA therapy. Judgment of necrotic area was classified into three categories: grade 1, necrotic area smaller than original tumor size; grade 2, necrotic area of the same size or larger than original tumor, but no safety margin of 5 mm around tumor; and grade 3, necrotic area larger than original tumor size with a safety margin of 5 mm or more in all directions. At least two physicians or radiologists confirmed the judgment of the treatment effect.

Physicians observed the patients every 4 weeks after the first treatment. Liver function test, hematology, and tumor markers were measured every month. After completion of eradication of HCC, recurrence was surveyed with CT imaging (helical CT or multi-detector CT) every 3 months.

During a median observation period of 2.6 years, two patients (0.9%) were lost to follow-up.

2.5. Evaluation of cost-effectiveness

The cost-utility of RFA was analyzed in comparison with that of surgery, from the viewpoint of society expense. Direct medical costs were only calculated in this study, excluding other social costs. Evaluation of cost-effectiveness balance was based on incidence of local recurrence as an intermediate endpoint. Discount rate was set at 0%. Sensitivity analysis was performed using [1] local recurrence rate after RFA therapy, and [2] net cost of surgical therapy.

2.6. Statistical analysis

Standard statistical measures and procedures were used. The chi-square test, Fisher's exact test, and Mann–Whitney's *U*-test were used to analyze the differences of demography, laboratory findings, and tumor characteristics between RFA group and surgery group. Recurrence and survival rate were analyzed using the Kaplan–Meier technique [17] with log-rank test. A *P*-value of less than 0.05 in two-tailed test was considered to be significant. Data analysis was performed using the computer program SPSS version 11 [18].

3. Results

3.1. Judgment of necrotic area after RFA therapy

Judgment of necrotic area as grades 1, 2, and 3 after first RFA therapy was 2 (1.3%), 89 (58.2%), and 62 (40.5%), respectively. Among 91 patients with grades 1 and 2, additional ablation therapy was performed in 52 patients (57.1%): 37 patients received therapy twice, 11 patients three times, and 4 patients four times or more as an initial session of loco-regional therapy. Although RFA was carried out in 36 patients as an additional loco-regional therapy, PEI or MCT was performed in the other 14 patients with the principal reason of tumor location in the liver.

Of the 52 patients with additional therapy, 31 patients (59.6%) accomplished grade 3 necrosis, and the other 21 (40.4%) showed grade 2 necrosis. At the end of the initial session of RFA, 60 (39.2%) attained grade 2 necrosis, 93 (60.8%) grade 3, and none remained at grade 1 (Fig. 1). The principal reason why therapy repetition did not bring about grade 3 necrosis in 21 patients was because tumor was adjacent to a large vessel of portal vein or hepatic vein. The other reasons included patient's disagreement, a problem in location of tumor, and transient aggravation of liver function.

3.2. Judgment of resected area and pathology after surgery

All the tumors in 60 patients with hepatic resection were completely removed on dynamic CT films after surgery. When the length between tumor margin and cut-surface of surgical resection was evaluated on resected pathology specimens, the "surgical margin" of HCC varied from 0 to 15 mm with a median of 6 mm.

3.3. Incidence and manners of recurrence

During the median follow-up period of 2.6 years, 70 patients showed tumor recurrence after therapy. Cumulative recurrence rates in patients with RFA and surgical resection were 16.6% and 14.9% at the end of the first year, 40.3% and 27.1% at the second year, and 50.2% and 30.3% at the third year, respectively (Fig. 2). The recurrence rate in patients with RFA therapy was higher than that of surgical resection (log-rank test, $P = 0.069$).

Recurrence rate was considered to be higher in patients with a "recurrent" tumor than in those with an "initial" tumor, recurrence rates were compared in a subgroup of patients with an initially developed HCC between the two groups. Cumulative recurrence rates in patients with RFA ($N = 103$) and surgical resection ($N = 57$) were 11.1% and 13.8% at the end of the first year, 31.6% and 26.5% at the second year, and 38.6% and 29.7% at the third year, respectively (Fig. 3). The recurrence rate in patients with RFA therapy was higher than that of surgical resection by 8.9% at the end of the third year (log-rank test, $P = 0.54$).

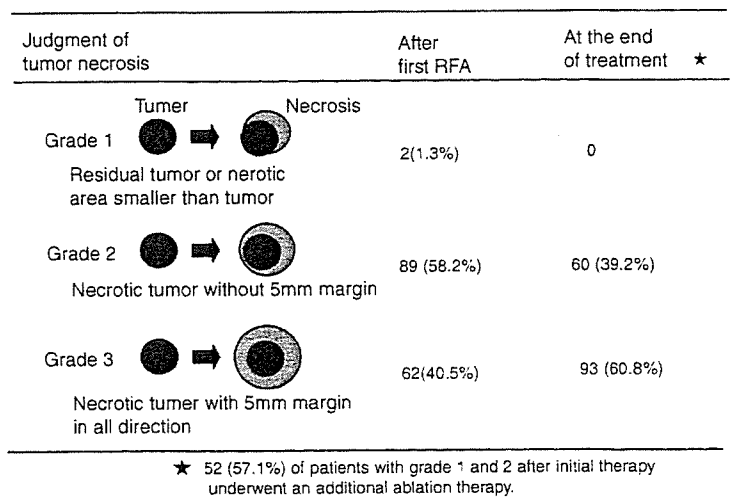

Fig. 1. Judgment of tumor necrosis after radiofrequency ablation therapy, according to three grades of treatment completeness.