Table 4. Clinical features at the time of diagnosis of HCC in 29 patients who developed hepatocellular carcinoma after sustained response to interferon therapy given for chronic hepatitis C

Age (years)	Sex	HCV RNA	HBs Ag	Histological fibrosis stage	Histological activity grade	AFP (ng/ml)	PIVKA II (AU/ml)	Number of tumors
64	Male	Negative	Negative	NA	NA	2	0	4
60	Male	Negative	Negative	NA	NA	51	0.211	1
38	Male	Negative	Negative	NA	NA	4.3	NA	>5
67	Male	Negative	Negative	F4	A0	4.2	0.033	1
75	Male	Negative	Negative	F1	A1	5	0.054	1
65	Female	Negative	Negative	NA	NA	5	0.029	1
62	Male	Negative	Negative	NA	NA	3	NA	1
61	Male	Negative	Negative	F2	A0	3	0.001	1
64	Male	Negative	Negative	F2	A0	4	0.426	1
70	Male	Negative	Negative	NA	NA	46 000	NA	1
64	Male	Negative	Negative	NA	NA	146	0.049	1
54	Female	Negative	Negative	F3	A1	2165	6690	1
65	Male	Negative	Negative	F4	A2	25.9	0.015	>5
61	Male	Negative	Negative	F2	A1	4	1.79	1
64	Male	Negative	Negative	F2	A0	NA	NA	1
63	Male	Negative	Negative	NA	NA	135.3	0.06	1
67	Male	Negative	Negative	NA	NA	3.5	0.013	1
75	Male	Negative	Negative	NA	NA	2	NA	1
62	Male	Negative	Negative	F2	A1	1026	13.32	1
62	Male	Negative	Negative	F2	A1	2.3	1.79	1
68	Female	Negative	Negative	F3	A2	9.1	0.016	1
59	Male	Negative	Negative	F0	A0	29	0.029	1
70	Male	Negative	Negative	NA	NA	488.3	601 371	1
54	Male	Negative	Negative	NA	NA	258	2.1	1
68	Female	Negative	Negative	NA	NA	2.8	0.023	1
60	Male	Negative	Negative	F2	A1	3.2	0.023	1
70	Male	Negative	Negative	NA	NA	5463	6.566	2
70	Female	Negative	Negative	NA	NA	464.2	NA	1
77	Male	Negative	Negative	NA	NA	72	0.136	2

NA, not available; HBs Ag, hepatitis B surface antigen; AFP, alpha-fetoprotein; PIVKA II, protein induced by vitamin K absence or antagonist-II; Vp, portal vein invasion; Vv, hepatic vein invasion; B, bile duct invasion; US, ultrasonography; CT, computed tomography

in sustained responders in whom HCC developed after successful IFN therapy, but data could be obtained for only two patients, who were negative for hepatitis B virus DNA. We cannot rule out the presence of occult hepatitis B virus in the other patients, although all patients were negative for hepatitis B antigen. In spite of these uncertainties, this study represents a comprehensive analysis of HCC developing after sustained response to IFN therapy, because we were able to collect clinical data for a large number of sustained responders at 16 major hospitals.

In this study, we encountered 29 patients in whom HCC developed after successful IFN therapy, but the reason why HCC developed in these sustained responders is unclear. The existence of a small undetected HCC at the time of IFN therapy may have been responsible for the appearance of HCC after the sustained response to IFN therapy. However, in 11 patients (38%), HCC was detected more than 5 years after IFN therapy, and the incidence of HCC gradually increased for at least 9 years after IFN therapy. Considering the late onset of HCC in these patients, we cannot neglect the possibility of the de-novo development of HCC after the eradication of HCV. HCV is a single-stranded RNA virus without a DNA intermediate in its replicative cycle, so that the integration of HCV nucleic acid sequences into the host genome seems unlikely. Therefore, it is difficult to believe that HCV itself is a causative factor of HCC in the absence of chronic inflammation, liver cell necrosis and regeneration, and extensive fibrosis. It is probable that carcinogenesis is not a single-step event, but a complex multistep process. Future studies should aim to define the basic oncogenic mechanisms by which sustained responders to IFN develop HCC. Exploration of these mechanisms may point the way toward new strategies for the prevention of HCC.

In conclusion, some patients showing a sustained response to IFN therapy given for chronic hepatitis C demonstrated potential for the development of HCC for up to 9 years following cessation of the treatment. This suggests that the risk of HCC in sustained responders is not completely eliminated. The establishment of risk factors and an index for the development of HCC may be useful in determining follow-up strategy in patients after a sustained response to IFN therapy given for chronic hepatitis.

Table 4. *Continued*

Maximum tumor size (mm)	Vp	Vv	B	Differentiation of HCC	Period to development Of HCC (years)	Medical follow-up period (months)	Diagnostic modality
18	0	0	0	Moderately	1.43	3	US
16	0	0	0	NA	1.51	1	US
>20	3	0	0	NA	1.79	None	US
15	0	0	0	Moderately	2.52	1	US
25	0	0	0	Moderately	3.32	2	CT
20	0	0	0	Well	3.39	3	US
34	2	1	2	Well	3.54	2	US
20	0	0	0	Well	3.59	3	Laparoscopy
40	0	0	0	NA	3.70	None	US
50	2	2	2	NA	3.89	None	US
30	0	0	0	Well	4.35	1	US
110	0	0	0	Poorly	4.38	6	US
15	0	0	0	Well	4.48	6	US
50	1	0	1	Moderately	4.58	12	US
80	0	0	0	Moderately	4.60	None	CT
NA	0	0	0	NA	4.70	6	US
44	0	0	0	NA	4.88	6	US
28	0	0	0	NA	4.97	3	US
60	1	1	1	Moderately	5.52	None	US
50	1	0	1	Moderately	5.58	6	US
51	0	0	0	Combined type	5.80	3	US
40	0	0	0	Moderately	5.86	None	US
>20	2	0	0	NA	6.61	3	US
150	3	0	0	Poorly	6.86	None	US
15	0	0	0	NA	8.05	3	US
15	0	0	0	Well	8.39	6	US
60	0	0	0	Well	8.78	None	US
16	0	0	0	NA	8.79	3	US
42	0	0	0	NA	8.98	1	CT

Appendix

In addition to the study authors' hospitals (the four institutions listed on the title page), data were supplied by the following hospitals and clinics in the Kyushu Division of the Japanese Society of Gastroenterology: Shinnittetsu Yahata Memorial Hospital; Yame General Hospital; First Department of Internal Medicine, Ryukyu University School of Medicine; Second Department of Internal Medicine, Kagoshima University School of Medicine; Hayato Town Medical Association Medical Center; Department of Internal Medicine, Saga Medical School; Department of Medicine and Biosystemic Science, Kyushu University School of Medicine; Nishinihon Hospital; Kagoshima Kouseiren Hospital; Miyata Memorial Hospital; Second Department of Internal Medicine, Nagasaki University School of Medicine; and Yonabaru Central Hospital.

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EVALUATION OF A HEPATITIS B VACCINATION PROGRAM IN CHIANG MAI, THAILAND

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Abstract. Chiang Mai is a province in northern Thailand that started a vaccination program for hepatitis B virus (HBV) infection in 1989. In this paper, we report the long-term efficacy of this program. Of children aged 4-9 years, 65.7% had a complete course and 3.8% had an incomplete vaccination course. Urban schoolchildren had higher percentage of HB vaccination than rural schoolchildren (89.1% *vs* 46.9% for the complete course, $p < 0.001$). The overall prevalence rate of HBsAg in Chiang Mai schoolchildren was 1.2%, with no significant differences between gender ($p = 0.496$) and school areas ($p = 0.477$). Anti-HBc antibodies were detected in 6.9% of children. Overall, 26.2% of children had protective levels of anti-HBs antibodies (≥10.0 mIU/ml), and 11.2% had low levels of these antibodies (1.0-9.9 mIU/ml). Compared to previous reports, our results show a lower percentage of anti-HBs antibodies, 33.8% of children age 4 years had protective anti-HBs antibodies, dropping to 18.4% by age 9 years. Among those anti-HBs seropositive, 9.1% were anti-HBc positive, indicating a natural infection with HBV. We found a small number of children, despite adequate immunization, developed HBV infection.

INTRODUCTION

Hepatitis B virus (HBV) infection is endemic in Southeast Asia and Africa, and is transmitted by parenteral routes, maternal-infant exposure, and horizontal spread between children (Merican *et al*, 2000). Almost all HBV-infected infants become carriers of HBV, and act as sources of the infection to their family and community. Such individuals are at significant risk for developing chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Thus, control of HBV infection and interruption of its spread are important public health goals. The most important method of prevention of HBV infection is the vaccination of newborns and children with hepatitis B (HB) vaccine. Studies showed that before immunization, about 20% of children under 5 had serologic evidence of HBV infection, rising to 70% by age 15 (Grossman *et al*, 1975). About 4% of non-immunized Thai children were carriers of hepatitis B (Luksamijarulkul *et al*, 1995), reaching 8% by adulthood (Grossman *et al*, 1975; Merican *et al*, 2000). Population-based studies have shown that the use of the HB vaccine in infants can reduce the HBV chronic carrier prevalence from high (>8%) to low (<2%) in immunized cohorts of children (Kane, 1998).

Thailand has undertaken a systematic approach toward control of HBV infection. Before systematic immunization, one study found that 5.7% of children acquired the infection in a one-year period (Kozik *et al*, 2000). The carrier rate in Thai children age 2-16 years was found to be 13% of those who were infected with HBV (Kozik *et al*, 2000). In 1989, the Thailand Ministry of Public Health (MOPH) established a pilot project of HB immunization in Chiang Mai and Chon Buri Provinces demonstrating that HB vaccine can be effectively administered along with other Expanded Program of Immunization (EPI) vaccines. In 1992, the Thai Government integrated the HB vaccine into the national EPI. Children receive 0.5 ml of HB vaccine intramuscularly within 7 days of birth, at 2 months and at 6 months of age. The HB vaccines given to older children varied with the year of inoculation. In 1989, a

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plasma-derived vaccine (the Cheil Suger, Korea), with a 3 µg per dose, was used. From 1990 to 1994, the plasma-derived vaccine produced by Korean Green Cross Corporation (Korea) was administered at 10 µg per dose. Since 1995, the 10 µg per dose of recombinant Engerix B® vaccine (SmithKline Biologicals, Belgium) has been used (Poovorawan *et al*, 2001). Long-term evaluation of this project in Chiang Mai, where the project began, has not been done. In this paper, we report the results of hepatitis B vaccination in Chiang Mai, Thailand, and estimate the efficacy of this program.

MATERIALS AND METHODS

Study population

From July 1998 to August 2000, children aged 4-9 years were randomly selected from 7 rural schools (377 children, including 185 males and 192 females) and 3 urban schools (303 children, including 147 males and 156 females) in Chiang Mai Province, northern Thailand. The purpose of the study was discussed with the parents or guardians, and written consent was obtained in all cases. A questionnaire was completed and personal vaccination records were reviewed. A total of 680 blood samples were obtained from the schoolchildren (332 males and 348 females). Serum samples were prepared on the day they were obtained, stored at 4°C for not more than 3 days, or stored at -20°C until tested.

Serologic studies

A qualified technician tested for the presence of HBsAg using the Monolisa Ag HBs second generation ELISA kit (Sanofi Diagnostic Pasteur, Manes la Coquette, France). Testing for anti-HBs antibodies was done using the Monolisa Anti-HBs 3.0 kit together with the 5 points calibration Monolisa Anti-HBs Standard (negative control, and 10, 50, 100, and 150 mIU/ml) (Sanofi Diagnostic Pasteur). All positive samples were re-tested, and all remained positive. The anti-HBs antibody titers were reported as the average value between the initial positive test and the repeated test. Levels above 10.0 mIU/ml were considered to be protective; anti-HBs and levels between 1.0-9.9 mIU/ml were considered to be low.

Data analysis

Data were analysed by determining the percentages of hepatitis B vaccine coverage and each viral marker obtained per population group. Prevalence among the different groups was compared using χ^2 or Fisher's exact test as appropriate. Results were considered statistically significant when $p < 0.05$.

RESULTS

Coverage of the HB vaccination program

Children aged 4-9 years should reflect the status of subjects born after the HB immunization program in Chiang Mai started in 1989.

Older age schoolchildren represented children at the start of the program. Younger age schoolchildren represent children under the current national EPI program. The children were divided into three groups: those who received a complete course of HB vaccination (*ie*, 3-5 doses of HB vaccine); those who received an incomplete course of HB vaccination (1-2 doses of HB vaccine) and those who did not received HB vaccination. This information was obtained by questionnaire along with review of the personal vaccination records. The results by age group and children school areas are presented in Fig 1. Of the 680 children, 447 (65.7%) had a history of complete HB vaccination, including 221 males (66.6%) and 226 females (64.9%); 26 (3.8%) had

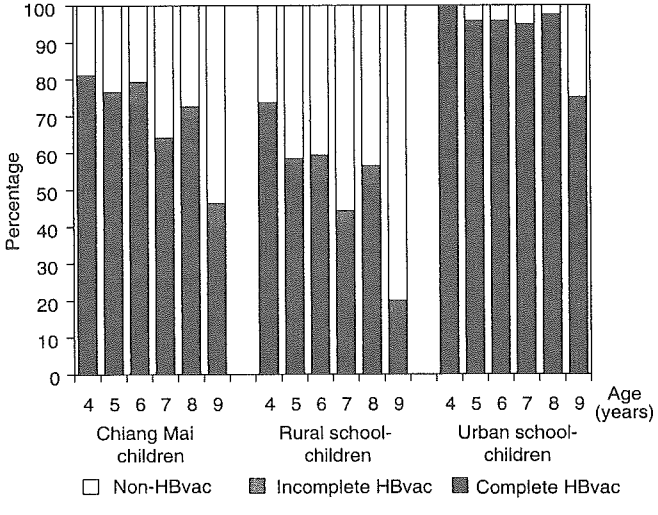

Fig 1–Coverage of hepatitis B vaccination in Chiang Mai children.

Table 1
HBsAg and anti-HBc positivity among Chiang Mai children by age and comparison between school areas.

Age (years)	HBsAg positive (%)			Anti-HBc positive (%)		
	Chiang Mai children	Rural school-children	Urban school-children	Chiang Mai children	Rural school-children	Urban school-children
4-5	1/223 (0.4%)	0/130 (0.0%)	1/93 (1.1%)	18/223 (8.1%)	16/130 (12.3%)	2/93 (2.2%)
6-7	2/230 (0.9%)	1/120 (0.8%)	1/110 (0.9%)	13/230 (5.7%)	5/120 (4.2%)	8/110 (7.3%)
8-9	5/227 (2.2%)	2/127 (1.6%)	3/100 (3.0%)	16/227 (7.0%)	10/127 (7.9%)	6/100 (6.0%)
Total	8/680 (1.2%)	3/377 (0.8%)	5/303 (1.7%)	47/680 (6.9%)	31/377 (8.2%)	16/303 (5.3%)

a history of incomplete HB vaccination, including 14 males (4.2%) and 12 females (3.4%). Children in urban schools showed a much higher prevalence in terms of HB vaccination than children in rural schools (89.1% *vs* 46.9% for a complete course, $p < 0.001$). This difference was not related to the sex of the children, $p = 0.722$. In urban schools, 87.8% of males and 90.4% of females received a complete course, and in rural schools, 49.7% of males and 44.3% of females did. The coverage of HB vaccination increased over time. For the oldest age group (9 years), 49/125 (39.2%) and the youngest age group (4 years), 59/74 (79.7%) had received a complete course of vaccination. Most of these children were from urban schools, 68.3% of the oldest age group (9 years) and 100% in the youngest age group (4 years) had undergone a complete course of vaccination, compared to 12.3% in the oldest age group and 71.7% in the youngest age group of children from rural schools.

Prevalence of HBV infection in Chiang Mai children

The results of testing for HBsAg and anti-HBc are presented in Table 1. The average prevalence rate of HBsAg in Chiang Mai schoolchildren age 4-9 years was 1.2% (8/680). There was a general increase in the prevalence rate with age, reaching 2.2% in the 8-9 years age group. The positive rate in males was 1.5% (5/332) and in females was 0.9% (3/348), which was not statistically different ($p = 0.496$). HBsAg positive rates in urban schoolchildren (1.7%) and rural schoolchildren (0.8%) were not significantly different, $p = 0.477$.

Another indicator of true infection by HBV is the development of anti-HBc antibodies. The overall prevalence of anti-HBc antibodies was 6.9% (47/680), with no significant difference between the sexes (6.9% in males and females, $p = 0.999$). When rural and urban children were considered separately, the prevalence in the rural children (8.2%) was not significantly different from urban children (5.3%), $p = 0.133$.

Surprisingly, all 5 HBsAg positive children from urban schools had a history of HB vaccination (two at 9 years of age, one at 7 years of age and one at 4 years of age had complete HB vaccination and one at 9 years of age had an incomplete HB vaccination). Most of them were anti-HBc positive, except the one 9 years of age who was anti-HBc negative (who had a history of complete HB vaccination). All 3 HBsAg positive children from rural schools were non-vaccinated and anti-HBc positive, two at 9 years of age and one at 6 years of age.

Efficacy of the vaccination program

A simple indicator of efficacy is the seroprevalence of anti-HBs antibodies. These values are presented in Fig 2. In our study group, we found that of those age 4-9 years, 178 (26.2%) had protective anti-HBs and 76 (11.2%)

Table 2
Anti-HBc positivity among Chiang Mai children with anti-HBs negative and positive by age groups and comparison between school areas.

Age (years)	Anti-HBc positive/Anti-HBs negative			Anti-HBc positive/Anti-HBs positive		
	Chiang Mai children	Rural school-children	Urban school-children	Chiang Mai children	Rural school-children	Urban school-children
4-5	8/119 (6.7%)	7/73 (9.6%)	1/46 (2.2%)	10/104 (9.6%)	9/57 (15.8%)	1/47 (2.1%)
6-7	5/152 (3.3%)	1/82 (1.2%)	4/70 (5.7%)	8/78 (10.3%)	4/38 (10.5%)	4/40 (10.0%)
8-9	11/155 (7.1%)	7/93 (7.5%)	4/62 (6.5%)	5/72 (6.9%	3/34 (8.8%)	2/38 (5.3%)
Total	24/426 (5.6%)	15/248 (6.0%)	9/178 (5.1%)	23/254 (9.1%)	16/129 (12.4%)	7/125 (5.6%)

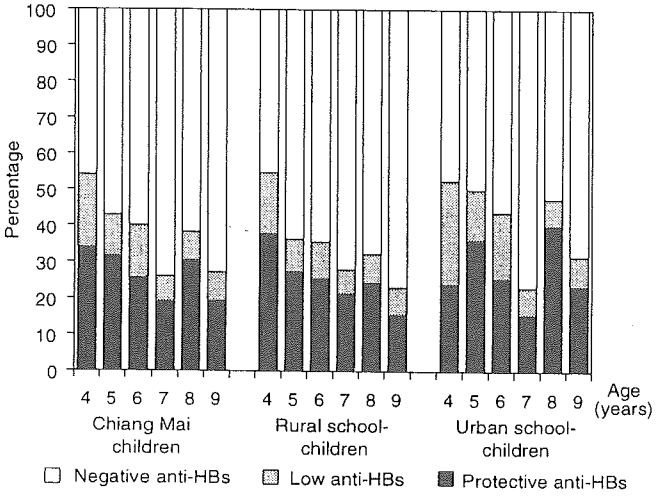

Fig 2–Seroprevalence of anti-HBs levels in Chiang Mai schoolchildren, by age and areas (rural *vs* urban).

had low levels of anti-HBs. The prevalence decreased with age, from 33.8% with protective anti-HBs and 20.3% with low levels of anti-HBs at age 4 years to 18.4% with protective anti-HBs and 8.0% with low levels of anti-HBs by age 9 years. No significant difference was observed between male and female children, p = 0.096. When separated into rural and urban groups, the seroprevalence of anti-HBs antibodies in urban children (28.1% with protective anti-HBs) was not significantly different from rural schoolchildren (24.7% with protective anti-HBs), p = 0.131.

Efficacy may be more accurately judged by the development of protective levels of anti-HBs antibodies (≥10 mIU/ml). Compared to the coverage with HB vaccination, the proportion of protective anti-HBs antibodies is quite low, demonstrated by Figs 1 and 2. The coverage of complete HB vaccination was 65.7%. The presence of protective anti-HBs in our study group was 26.2%. In the youngest age group (4 years) with 79.7% with complete HB vaccination, 33.8% had protective levels of antibodies. Even in the youngest age group (4 years) of urban schoolchildren with 100% complete HB vaccination, 23.8% had protective levels of antibodies.

The presence of anti-HBc antibodies indicates natural infection with HBV. The prevalence of anti-HBc in anti-HBs seronegative and anti-HBs seropositive individuals is presented in Table 2. The prevalence of anti-HBc among the anti-HBs seropositive group (9.1%) was not significantly different from the anti-HBs seronegative group (5.6%), p = 0.089. In the anti-HBs seropositive group, the prevalence of anti-HBc in rural schoolchildren was 12.4%, while in the urban schoolchildren it was 5.6%, which was not significantly different (p = 0.059).

DISCUSSION

From 1989 to 1992, the Thailand's HB immunization model program was conducted in Chiang Mai and Chon Buri Provinces and demonstrated that HB vaccine could be effectively administered along with other EPI vaccines. By the end of the project, overall coverage for complete HB immunization in Chiang Mai had

reached 93.1%. A minority of children received incomplete HB immunization or no immunization. HB vaccination was shown to provide over 80% protective efficacy against HBV infection (Chunsuttiwat *et al*, 1997). Stepwise expansion of HB vaccination evolved into a nationwide program in 1992. The HB vaccination coverage rate has been rapidly catching up with its EPI counterparts, with the coverage rate of the third dose ranging from 71.2-94.3% (Chongsrisawat *et al*, 2000; Poovorawan *et al*, 2001). We found that the coverage rates of Chiang Mai children age 4-9 years old, who should have received 3 doses of HB vaccine, was 65.7%. Some children received incomplete HB immunization (3.8%). In Chon Buri, after its integration into the EPI program, the complete HB vaccination rate was 71.2% and the incomplete HB vaccination rate was 12.9% (Poovorawan *et al*, 2001). There was a marked difference between the urban and rural environments. City children had a coverage rate of 89.1% compared to only 46.9% for rural children. These results are slightly less than those reported in other study. These numbers may be an underestimate, since the data were obtained by questionnaire and from health record booklets, and some of which had been lost, especially in older children and in rural schools.

The prevalence of HBsAg can be used as an indicator of true HBV infection, as opposed to anti-HBs antibodies, which develop following infection and immunization. Other studies have shown that the prevalence of HBsAg in non-immunized children is 3.64% (Luksamijarulkul *et al*, 1995), but this figure has dropped by 85% after the immunization program was instituted (Chongsrisawat *et al*, 2000), with a prevalence of 0.67% reported (Poovorawan *et al*, 2000). The current carrier rate in Thai children has been determined to be 0.55-7% (Chub-uppakarn *et al*, 1998; Poovorawan *et al*, 2000; 2001). In Chon Buri, after the integration of the HB vaccine into the EPI, the HBsAg positive rate was 0.7% (Poovorawan *et al*, 2001). In Chiang Mai, 1.2% of all children had circulating HBsAg in serum, a figure comparable to other studies. The presence of anti-HBc antibodies can also serve as an indicator of infection by HBV. Other studies have found that 5.5% of children 1-10 years old have anti-HBc antibodies (Poovorawan *et al*, 2000), compared to 6% of non-immunized children (Luksamijarulkul *et al*, 1995). In our study group, we found the prevalence rate among children 4-9 years old was 6.9%. While in Chon Buri, after the integration of HB vaccine into the EPI, the anti-HBc positive rate was 6.3% (Poovorawan *et al*, 2001).

The efficacy of immunization can be evaluated by measuring the levels of protective anti-HBs antibodies. By age 10, about 56% of immunized children have antibodies to HBsAg (Poovorawan *et al*, 2000), compared to 15% of non-immunized children (Luksamijarulkul *et al*, 1995). The prevalence rate is higher in younger children, with values of 94% at 0-2 years of age, dropping to 76% by 3-5 years of age (Chub-uppakarn *et al*, 1998). In our study, 33.8% of children 4 years old had protective anti-HBs antibodies, dropping to 18.4% by age 9 years. The prevalence of samples with anti-HBs antibodies declined as children got older, except for an increase in the 8-year old age group (30.4%). The pattern of anti-HBs present is similar to the pattern of the coverage of HB vaccination, by age and area (rural *vs* urban). Compared to previous reports, our results show a lower percentage of anti-HBs antibodies. Anti-HBs antibodies may be from natural infection with HBV. This was demonstrated by the fact that 9.1% of children with anti-HBs were anti-HBc positive.

In our study group, there were some apparently completely immunized children who had evidence of true HBV infection with circulating HBsAg and/or anti-HBc antibodies. Children in Thailand are not screened for previous HBV infection prior to immunization. The children with anti-HBc antibodies may well have acquired the natural infection before they were immunized. The three HBsAg positive children from rural schools were non-vaccinated and had anti-HBc as a result of natural infection. While the five HBsAg positive children from urban schools had a history of HB vaccination (four had complete HB vaccination and one had incomplete HB vaccination). Most of them were anti-HBc positive, except one who was anti-HBc negative (had a history of complete HB vaccination). The children with circulating HBsAg despite immuniza-

tion were true failures of vaccination. These children have the potential to become chronic carriers of HBV. These results imply failures or gaps in the immunization program. Our study did not uncover any specific source of the problem. Several factors, such as the commercial source of the vaccines, inadequate transportation and storage systems, and inefficient methods of administration might reduce the effectiveness of the vaccine. More recently, new variants of HBV have been reported that occur more frequently in vaccinated individuals (Theamboonlers *et al*, 2001). These viruses have critical amino acid differences that allow them to escape the host immune system and the protective effects of the vaccine.

Previous studies have reported seroprevalence based on any positive value for anti-HBs antibodies. In our study, we distinguished between positive serology for anti-HBs antibodies as protective levels (≥10.0 mIU/ml) and low levels (1.0-9.9 mIU/ml) ≥ of these antibodies. We found that, overall 26.2% of children had protective levels of anti-HBs antibodies, and 11.2% had low levels of these antibodies. After vaccination, the strongest antibody response was detected within the first year, and after approximately 5 years it decreased to low or undetectable levels in some individuals. Most studies in Thailand have suggested that a booster dose after the initial three doses is not necessary, and that immunologic memory provides adequate protection, even if levels of anti-HBs antibodies are below 'protective' levels (Chongsrisawat *et al*, 2000; Poovorawan *et al*, 2000). The presence of HBsAg/anti-HBc in vaccinated children from urban schools with high coverage under the HB vaccination program call for evaluation HBV infection after HB vaccination. Some children can become chronic carriers, despite adequate vaccination.

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Body surface area is an independent factor contributing to the effects of lamivudine treatment

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Abstract

Background: It has been suggested that lamivudine therapy may be even more effective if administered at higher doses than is dictated by the current standard regimen. We analyzed the correlation between the effects of lamivudine and body surface area (BSA).
Method: We evaluated 134 patients with chronic hepatitis B who had been treated with lamivudine for more than 12 months. The effect of the treatment was evaluated from the levels of serum alanine aminotransferase (ALT) and HBV–DNA. Several variables that could influence the response to treatment, including ALT, albumin, and bilirubin levels, platelet counts, BSA, HBV–DNA, and HBeAg were analyzed.
Results: Univariate logistic analysis selected platelet counts, BSA, HBV–DNA and HBeAg in the biological evaluation, and bilirubin, BSA, HBV–DNA and HBeAg in the virological evaluation ($\chi^2 > 1.0$). Using these factors, multivariate analysis revealed that BSA ($\chi^2 = 12.8$, $p = 0.0004$) was the only factor that could contribute significantly to the improvement of ALT levels, and that BSA ($\chi^2 = 4.4$, $p = 0.0354$) and HBeAg ($\chi^2 = 8.1$, $p = 0.0044$) were independent factors that could influence the suppression of HBV–DNA.

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Conclusion: We revealed that BSA is a significantly predictor of the effect of lamivudine therapy, suggesting that lamivudine dosage should be based on the individual BSA.

Keywords: Lamivudine; Hepatitis B virus (HBV); Body surface area (BSA); Dose

1. Introduction

Chronic hepatitis B is an important cause of morbidity and mortality resulting from cirrhosis-related liver failure and hepatocelluar carcinoma (HCC) [1,2]. Lamivudine is a nucleoside analogue with activity against hepatitis B virus (HBV) replication. A daily dosage of lamivudine of 100 mg has been accepted worldwide for the treatment for chronic hepatitis B, since early studies showed that there was no significant difference in the effect of lamivudine at doses of 100 mg and 300 mg [3,4]. However, to establish ideal dosages in those studies, efficacy was mainly evaluated by measuring HBV–DNA, and the assay used was not as sensitive as the PCR assay [5,6]. Studies in which HBV–DNA was measured by PCR assay reported an additional viral suppressive activity with high doses (300 mg) of lamivudine for 24 weeks [7]. In addition to limits imposed by the assay methods used, the observation periods in the studies on lamivudine doses of 100 mg to 300 mg were limited to a period of 12 [3] or 24 weeks [4], because 1 year treatment with lamivudine often resulted in the relapse of HBV viremia [8]. Furthermore, the major drawback of lamivudine monotherapy is the emergence of resistant HBV with mutations of the tyrosine–methionine–aspartate–aspartate (YMDD) motif. The incidence of these mutants rises from 15 to 20% in the first year of therapy to 40% by the second year, and to 67% by the fourth year [9]. Although the effect of doses greater than 100 mg on the emergence of YMDD mutants has not been evaluated, base line body mass index has been reported to be significantly related to the emergence of mutation of HBV during lamivudine treatment in patients with co-infection of HBV and human immunodeficiency virus-1 HIV-1 [10]. Therefore, we evaluated the relationship between lamivudine effects and body surface are (BSA).

2. Patients and methods

2.1. Patients

A total of 134 patients with chronic hepatitis B were evaluated. Patients with fatty liver, patients with viral hepatitis C, patients with alcoholic abuser and patients with autoimmune disorder such as autoimmune hepatitis and primary biliary cirrhosis were excluded. They had been treated with 100 mg of lamivudine for more than 12 months at Kyushu University Hospital and its related hospitals (Table 1). For all patients, the existence of serum HBV–DNA was confirmed by TMA assay ($10^{3.7}$–$10^{8.7}$ genome equivalents/mL; 3.7–8.7 log genome equivalents [LGE]/mL) (Chugai Diagnostic Science, Tokyo, Japan) or by a Roche Monitor kit ($10^{2.6}$–$10^{7.6}$ copies/ml; 2.6–7.6 log copies/mL) (Roche Diagnostics, Tokyo, Japan) before treatment. None of the patients dropped out and all were treated with 100 mg/day lamivudine until the end of the observation period. After the start of medication, basic hepatic function and serum levels of HBV–DNA were measured at least every 3 months for all patients. The efficacy of lamivudine was evaluated from the serum levels of alanine aminotransferase (ALT) and HBV–DNA, as biological and virological effects, respectively. The evaluation using serum ALT was done as follows: (1) sustained responder- (SR-) ALT: the serum levels of ALT decreased, and remained at less than 30 U/L continuously during the observation; (2) transient responder- (TR-) ALT: the serum ALT decreased to less than 30 U/L, but increased to more than 30 U/L again during the subsequent observation; (3) non-responder- (NR-) ALT: the serum ALT always remained at more than 30 U/L throughout the observation. Similarly, the virological evaluation by HBV–DNA was done as follows, using a Roche Monitor kit: (1) SR-HBV: serum HBV–DNA decreased to levels undetectable by PCR (<2.6 log copies/mL), and remained negative continuously during the observation; (2) TR-HBV: the serum HBV–DNA decreased to undetectable levels once (<2.6 log copies/mL), but become positive again during the subsequent observation; (3) NR-HBV: the serum HBV–DNA was never negative throughout the observation (>2.6 log copies/mL). BSA was calculated using the method of DuBois.

Table 1
Characteristics of the 134 patients at base line[a]

	Total
Age	48.9 ± 11.4 (21–70)
Male/Female	98/36
CH/LC (Child A/B/C)	83/51 (35/9/7)
Observation period (month)	23.1 ± 7.9 (12–40)
ALT (U/L)	184.6 ± 243.4 (17–1722)
Albumin (g/dl)	3.76 ± 0.57 (2.3–4.9)
Bilirubin (mg/dl)	1.41 ± 1.79 (0.4–12.9)
Platelet ($10^4/\mu l$)	13.62 ± 6.19 (2.6–29.9)
BSA (m^2)[a]	1.702 ± 0.189 (1.28–2.24)
Height (m)	1.644 ± 0.084 (1.44–1.85)
Weight (kg)	64.2 ± 11.7 (38.0–105.0)
<5.0 (LEG/ml)	24
5.0 < ≤ 6.0	15
6.0 < ≤ 7.0	35
>7.0	48
Positive	77
Negative	57

[a] Plus-minus values are means ± S.D.

2.2. Statistical analysis

The backgrounds of the patients at the beginning of lamivudine treatment are shown as mean ± S.D. for the quantitative variables. Differences in the backgrounds of the SR, TR and NR-patients were examined by one-way ANOVA or χ^2-test. In order to confirm the contribution of the variables toward the effect of the treatment, univariate and multivariate logistic analysis were performed. For multivariate logistic analysis, we analyzed BSA as an independent factor contributing to effects of lamivudine treatment variables that showed χ^2 values of more than 1.0 in the univariate logistic model.

3. Results

Differences in background at the beginning of lamivudine treatment among the variables were evaluated by one-way ANOVA or χ^2-test. In the studies evaluating ALT (the biological response), the mean values of BSA and body weight were significantly lower in the SR-ALT group than in the other groups, and there was no significant difference in sex, observation period, height, ALT, albumin, bilirubin, platelet counts, HBV–DNA, or HBeAg among the groups (Table 2). Fig. 1 shows the distribution of BSA in each group, and demonstrated that there was an inverse relationship between BSA and the effects of lamivudine ($p = 0.0394$ at SR versus TR and $p = 0.0004$ at SR versus NR by Fisher's PLSD test). Similarly, the evaluation of HBV–DNA (the virological response) showed that BSA and body weight were also significantly lower in the SR-HBV group than in NR-HBV group (Table 3 and Fig. 1 [BSA: $p = 0.0087$ at SR versus NR by Fisher's PLSD test]). The prevalence of HBeAg differed significantly among the groups ($p < 0.005$ by χ^2-test), and in the NR-DNA group, most of the cases were positive for HBeAg (Table 3). The observation period also differed significantly among the groups ($p < 0.05$), and the SR-DNA group had longer observation period than the others. There was no difference in sex, height, ALT, alubumin, bilirubin, platelet counts, or HBV–DNA among the groups.

Table 2
Evaluation using the serum levels of ALT

	SR	TR	NR
Number	60	43	31
Male/Female	39/21	31/12	28/3
Observation period (month)	22.3 ± 8.8	23.9 ± 7.1	23.5 ± 7.3
ALT (U/L)	196.7 ± 195.2	181.2 ± 263.9	165.6 ± 299.6
Albumin (g/dl)	3.82 ± 0.51	3.66 ± 0.67	3.80 ± 0.54
Bilirubin (mg/dl)	1.42 ± 2.18	1.58 ± 1.75	1.16 ± 0.70
Platelet ($\times 10^4$ μl^{-1})	14.49 ± 6.25	13.36 ± 6.31	12.30 ± 5.83
BSA (m^2)*	1.644 ± 0.184	1.719 ± 0.184	1.790 ± 0.172
Height (m)	1.629 ± 0.087	1.650 ± 0.091	1.667 ± 0.060
Weight (kg)*	60.45 ± 10.41	65.24 ± 10.53	70.10 ± 13.27
HBV–DNA			
<5.0 (LEG/ml)	11	8	5
5.0 < ≤ 6.0	6	1	8
6.0 < ≤ 7.0	17	11	7
>7.0	26	23	11
HBeAg			
Positive	32	25	20
Negative	28	18	11

* $p < 0.005$.

In order to confirm the contribution of the variables toward the treatment, univariate and multivariate logistic analysis were performed. In univariate logistic analysis, platelet counts, BSA, body weight, height, HBV–DNA and HBeAg in the biological evaluation, and bilirubin, BSA, body weight, height, HBV–DNA and HBeAg in the virological evaluation, had χ^2 values of more than 1.0 (Table 4). Therefore, we used these factors except body weight and height as variables for multivariate logistic analysis, because there was a strong correlation between BSA and body weight ($R^2 = 0.89072$, $p < 0.0001$), or BSA and height ($R^2 = 0.67708$, $p < 0.0001$). The results of multivariate analysis revealed that BSA was the only significant factor that could contribute to the improvement of ALT levels ($\chi^2 = 12.8$, $p = 0.0004$), and BSA and HBeAg were independent factors that could influence the disappearance of serum HBV–DNA (BSA: $\chi^2 = 4.4$, $p = 0.0354$ and HBeAg: $\chi^2 = 8.1$, $p = 0.0044$) (Table 5).

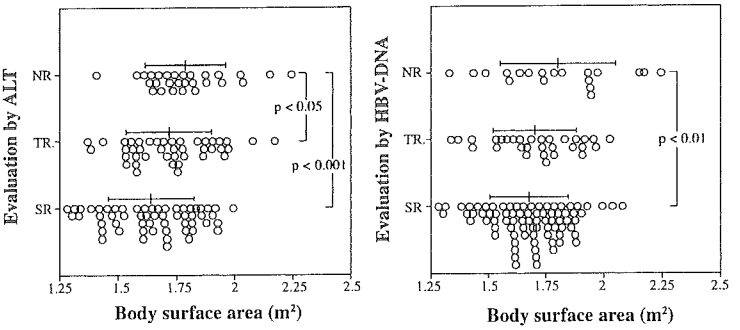

Fig. 1. The distribution of BSA according to the effect evaluated by ALT (left panel) and by HBV–DNA (right panel) was shown (open circle). The bars represent mean ± S.D. Non responders had significantly larger BSA than sustained responders in both evaluations.

Table 3
Evaluation using the serum levels of HBV-DNA

	SR	TR	NR
Number	83	31	20
Sex	60/23	22/9	16/4
Observation period (month)*	22.6 ± 7.5	26.7 ± 7.7	19.6 ± 7.7
ALT (U/L)	193.7 ± 246.6	188.5 ± 296.3	138.2 ± 91.4
Albumin (g/dl)	3.74 ± 0.58	3.78 ± 0.52	3.83 ± 0.61
Bilirubin (mg/dl)	1.56 ± 2.16	1.30 ± 0.98	0.92 ± 0.43
Platelet ($\times 10^4\ \mu l^{-1}$)	13.51 ± 6.05	13.04 ± 6.32	15.04 ± 6.70
BSA (m^2)*	1.676 ± 0.170	1.702 ± 0.181	1.801 ± 0.248
Height (m)	1.629 ± 0.087	1.650 ± 0.091	1.667 ± 0.060
Weight (kg)*	62.40 ± 10.03	64.33 ± 10.14	71.59 ± 17.26
HBV-DNA			
<5.0 (LEG/ml)	18	6	0
5.0 < ≤ 6.0	10	4	1
6.0 < ≤ 7.0	20	8	7
>7.0	35	13	12
HBeAg**			
Positive	40	19	18
Negative	43	12	2

* $p < 0.05$.
** $p < 0.005$.

Table 4
Univariate analysis on the lamivudine effects

Variable	χ^2	p-value
ALT evaluation		
ALT	0.35543766	0.5511
Albumin	0.31157046	0.5767
Bilirubin	0.19658636	0.6575
Platelet	2.68462623	0.1013
BSA	13.060725	0.0003
Height	4.09176033	0.0431
Weight	12.734481	0.0004
HBV-DNA	3.05562744	0.3831
HBeAg	1.02785409	0.3107
HBV-DNA evaluation		
ALT	0.4754853	0.4905
Albumin	0.39014806	0.5322
Bilirubin	2.42720044	0.1192
Platelet	0.25597276	0.6129
BSA	5.54675909	0.0185
Height	1.24367306	0.2648
Weight	5.45864661	0.0380
HBV-DNA	4.18232253	0.2424
HBeAg	10.2577192	0.0014

4. Discussion

In this present study, we found that BSA was a significant factor that could contribute to both the improvement of ALT levels (biological response) and the disappearance of serum HBV-DNA (virological response). Because the pharmacokinetics of lamivudine correlate with body weight, as is the case with many other drugs [11], it is reasonable to conclude that patients with lower BSA would have the higher blood concentration of lamivudine, although we did not actually monitor the lamivudine blood concentration. Recent reports suggest

Table 5
Multivariate analysis of the effects of lamivudine using potential univariate predictors

Variable	χ^2	p-value
ALT evaluation		
Platelet	3.2287548	0.0724
BSA	12.7808491	0.0004
HBV-DNA	2.42555739	0.4889
HBeAg	0.93377434	0.3339
HBV-DNA evaluation		
Bilirubin	3.76147767	0.0524
BSA	4.42516561	0.0354
HBV-DNA	2.23355431	0.5254
HBeAg	8.10893404	0.0044

that baseline body mass index is significantly related to the emergence of HBV mutation during lamivudine treatment (300 mg/day, >6 months) in patients coinfected with HBV and HIV-1 [10]. Therefore, the results of our study question whether a lamivudine dosage of 100 mg/day is adequate, particularly for long-term treatment.

The standard lamivudine dose of 100 mg daily was based on early studies in which doses of 25, 100, 300 mg were compared for 12 [3] or 24 weeks [4]. Dienstag et al. [3] reported that levels of HBV-DNA became undetectable in 70% of the patients who received the 25 mg dose of lamivudine ($n = 10$) and 100% of those treated with doses of 100 mg ($n = 11$) or 300 mg ($n = 11$). Nevens et al. [4] reported that HBV-DNA was undetectable at the end of 24 weeks of lamivudine treatment in 58% (25 mg), 93% (100 mg), and 88% (300 mg) of patients ($n = 16$, 19 and 19, respectively), and that abnormal ALT levels at baseline were normalized in 64% (25 mg), 45% (100 mg), and 36% (300 mg) of the patients at treatment completion. Because there were no significant differences reported in the rates of non-detection of HBV-DNA and nomalization of ALT levels between the 100 and 300 mg dose, the dose of 100 mg has become accepted as a therapeutic standard. However, several issues should be considered when evaluating the results of these studies, including: (1) number of patients; (2) period of treatment; (3) emergence of lamivudine-resistant mutants in long-term treatment; and (4) detection limit of HBV-DNA.

As we showed in the present study of 134 cases, there was a significant difference in the effect of BSA on both the biological and virological response at the end of observation period (mean = 2 years), although the differences in the mean values of BSA in each group were relatively small. Therefore, it is possible that previous studies failed to detect a significant contribution of BSA to the effects of lamivudine because of the smaller numbers of patients examined.

Observation periods of 24 weeks are not adequate for detecting the emergence of lamivudine-resistant mutants, since the incidence of the mutants rises from 15 to 20% in the first year to 40% by the second year and to 67% by the fourth year of treatment [9]. In our present study of HBV-DNA evaluation, the rate of TR-DNA and NR-DNA was 38% in patients

who were positive for HBV–DNA (>2.6 log copies/mL) at the end of observation (average of about 2 years). We did not confirm the YMDD mutation in all cases of TR-DNA and NR-DNA, and further study will be needed to confirm whether BSA affects the incidence of YMDD mutants.

It is important to consider the sensitivity of measurement of HBV–DNA in evaluating the effects of lamivudine, because a decrease in HBV–DNA is reported to be associated with a lower incidence of YMDD mutants [12]. In previous studies, which showed no difference in the effects of 100 and 300 mg lamivudine on HBV–DNA reduction (as described above) [3,4], HBV–DNA was measured quantitatively by liquid hybridization assay (Abbott Laboratories), which has a detection limit of 10^7 geq/mL [13]. Honkoop et al. studied the efficacy of 100 and 300 mg lamivudine in viral suppression for 24 weeks using a semi-quantitative PCR method with a detection limit of 10^2–10^3 geq/mL [7]. They showed that 29 and 37% patients were HBV–DNA negative after 24 week treatment with 100 and 300 mg lamivudine, respectively, indicating the possibility of additional viral suppressive activity with higher doses and longer-term therapy with lamivudine. Furthermore, using a more sensitive real-time quantitative PCR method with a detection limit of 1.7 log copies/mL, Ide et al. [12] reported that neither emergence of YMDD mutants nor a virological breakthrough of serum HBV DNA was observed in patients with <1.7 copies/mL. In our study, we measured HBV–DNA with a Roche Monitor kit, which has detection limit of 2.6 log copies/mL; 23% patients (TR-DNA) were HBV–DNA negative once, but became positive again during the subsequent observation. The TR-DNA group had a longer observation period than the other groups, this difference might affect the result that there was no significant difference in BSA between TR-DNA and SR-DNA groups, or TR-DNA and NR-DNA groups in the evaluation of HBV–DNA (Fig. 1). More precise analysis with longer observation period and/or using the real-time PCR method might show a clearer relationship between BSA and virological response with lamivudine.

In conclusion, we have revealed that BSA is a statistically significant and potentially important factor that can predict the effect of lamivudine therapy for chronic hepatitis B. A noteworthy finding in our study was that small differences in BSA could significantly influence the effect of the lamivudine treatment, suggesting that a small dose increase might increase the efficacy of lamivudine therapy. We believe that a long-term clinical trial with higher dose lamivudine treatment and a large number of cases is warranted, since lamivudine will continue to be a first-line treatment for HBV.

Appendix A

In addition to the authors, the Kyushu University Liver Disease Study Group includes the following individuals: R. Sugimoto (Harasanshin Hospital, Fukuoka), H. Amagase and S. Tominaga (Mihagino Hospital, Kitakyushu), K. Yanagita (Saiseikai Karatus Hospital, Karatsu), K. Ogiwara (Kyushu Rosai Hospital, Kitakyushu), M. Tokumatsu (Saiseikai Fukuoka Hospital, Fukuoka), S. Tabata (Hayashi Hospital, Fukuoka), M. Yokota (National Kyushu Cancer Center, Fukuoka), H. Tanaka (Chihaya Hospital, Fukuoka), S. Nagase (Fukuoka Teishin Hospital, Fukuoka), S. Tsuruta (Nakabaru Hospital, Fukuoka), S. Tada (Moji Rosai Hospital, Kitakyushu), M. Nagano (Kyushu Koseinenkin Hospital, Kitakyushu), M. Honda (Nishi-Fukuoka Hospital, Fukuoka), T. Umeno (Sawara Hospital, Fukuoka), T. Sugimura (National Hospital Organization Fukuoka Higashi Hospital, Fukuoka), S. Ueno (Kitakyushu Municipal Wakamatsu Hospital, Kitakyushu), K. Miki (Kitakyushu Municipal Moji Hospital, Kitakyushu), H. Okubo (Shineikai Hospital, Kitakyushu), H. Fujimoto (Mitusbishikagaku Hospital, Kitakyushu), N. Higuchi (Shin-Nakama Hospital, Kitakyushu), S. Shigematsu (Kouseikan Hospital, Saga), N. Higashi (National Hospital Organization Beppu Hospital, Ohita).

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• *CLINICAL RESEARCH* •

High relative fat-free mass is important for maintaining serum albumin levels in patients with compensated liver cirrhosis

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Abstract

AIM: In patients with liver cirrhosis, hypoalbuminemia causes edema and ascites, and a reduction in the quality of life. Since musculature is catabolized to supply amino acids for albumin synthesis in malnutritional cirrhotic patients, muscular volume is hypothesized to play an important role in albumin production. Therefore, we investigated the correlation between serum albumin levels and the fat-free mass (FFM) in cirrhotic patients.

METHODS: Fifty-seven patients (26 males and 31 females) with compensated liver cirrhosis were evaluated. Patients with edema or ascites were excluded from the study. Healthy volunteers (n = 104; 48 males and 56 females) were also evaluated as controls. FFM was measured using 5-500 kHz multifrequency bioelectric impedance analysis. To minimize the difference in FFM distribution between males and females, we introduced a new marker, relative FFM (rFFM), which represents the ratio of FFM in a patient relative to that in a volunteer of the same height. Following FFM measurement, the serum albumin levels of patients were assayed monthly.

RESULTS: In patients with active cirrhosis (alanine aminotransaminase [ALT] >50 U/L), both albumin (the difference between maximum and minimum levels) and the standard deviation of albumin levels (SD-albumin) during the observation period showed a significant correlation with rFFM. Multiple linear regression analysis using variables such as rFFM, platelet number, and serum cholesterol levels, choline esterase, albumin, bilirubin, and ALT revealed that rFFM and ALT were significant and independent factors that influenced albumin or SD-albumin in cirrhotic patients.

CONCLUSION: Our results indicate that cirrhotic patients with high rFFM showed less of a decrease in albumin levels, and that the muscle volume is one of the most important factors for maintaining serum albumins level in active cirrhosis. Exercise and protein-rich nutrition at the early stage of liver cirrhosis may be advisable for maintaining or increasing muscular volume.

Key words: Muscular volume; Fat-free mass (FFM); Multifrequency bioelectric impedance analysis; Albumin; Liver cirrhosis

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INTRODUCTION

In earlier reports, several factors were established for predicting the prognosis of patients with liver cirrhosis, such as ascites, liver volume, encephalopathy, esophageal varices, serum albumin, serum bilirubin and clotting factors[1-5]. Among these factors, serum albumin level was commonly regarded as the most reliable. The usefulness of utilizing hepatic protein synthesis as a parameter for predicting the prognosis of cirrhotic patients is supported by a series of reports, which showed that a medication with branched amino acid elevated the serum levels of albumin and improved the prognosis[6-8]. Besides predicting survival, maintaining high albumin levels is clinically important for patients with liver cirrhosis, because decreased serum albumin levels cause ascites and edema, which lower the quality of life.

With respect to protein metabolism in cirrhotic patients, it should be emphasized that musculature plays an important role as an amino-acid pool. In healthy controls, the amino acid pool is distributed in the musculature (80%), liver (15%) and plasma (5%)[9]. In cirrhotic patients, starvation readily induces muscle protein catabolism because there is relatively little glycogen stored in the cirrhotic liver. Therefore, in the assessment of nutritional status in cirrhotic patients, the evaluation of muscle protein stores is important.

Bedrest is commonly prescribed for the management of patients with liver cirrhosis. However, it is obvious that a bedrest results in muscle volume reduction, which might reduce the serum levels of albumin. Therefore, it seems reasonable to postulate that cirrhotic patients with high muscle volume would be more resistant to reductions in

serum albumin associated with the progression of liver damage. In order to confirm this hypothesis, we examined the relationship between serum albumin levels and body composition in cirrhotic patients.

A number of techniques are available for measuring body compartments, such as dual energy X-ray absorptiometry (DEXA), total body potassium measured by whole-body potassium-40 counting, total body water measured by isotope dilution, *in vivo* neutron activation analysis (IVNAA), and bioelectric impedance analysis (BIA). Those techniques, except BIA, associated with high cost and labor-intensive methods. Although BIA has been used to estimate fat-free mass (FFM) of cirrhotic patients at bedside, the validity of the measurements obtained with the single-frequency method has been questioned. Borghi (1996) showed that multifrequency BIA might yield valid body composition data for cirrhotic patients without ascites[10]. In the present study, we used multifrequency BIA for measuring FFM and we limited the study population to cirrhotic patients without ascites or edema.

MATERIALS AND METHODS

Patients

Fifty-seven patients with compensated liver cirrhosis, who were outpatients of our department from April 2000 to March 2002, were evaluated (Table 1). They consisted of 26 males and 31 females, and ranged in age from 27 to 83 years. Fifty-two (92%) and five (8%) patients were classified as Child A and Child B without edema and ascites respectively. Fifty (88%) and 7 (12%) cases were caused by hepatitis C virus and hepatitis B virus, respectively. There was no significant difference in the basic characteristics between males and females (Table 1). Patients with edema were excluded from this study. Ultrasonography (US) was done to confirm that the evaluated patients did not have ascites. The patients were diagnosed with liver cirrhosis based on the results of liver biopsy and/or imaging studies (computed tomography and USA). One hundred and four volunteers (48 males and 56 females) were also evaluated as control.

For all patients, 5-500 kHz multifrequency BIA was performed using InBody 3.0 (Exercise Physiology USA and Biospace Co., Ltd.)[11]. On the same day, they underwent general laboratory examination for albumin, bilirubin, cholesterol, aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), and blood platelet count. The patients and control volunteers fasted overnight prior to the laboratory examinations. FFM was calculated as described previously[10,11]. After measurement of FFM, serum biochemistry assays were performed every month on an outpatient basis. The average observation period was 15.4 mo, ranging from 12 to 24 mo.

Table 1 Characteristics of the 57 patients at base line (mean±SD)

Variables	Male	Female	All
n	26	31	57
Age (yr)	62.0±11.4	66.0±10.0	64.2±10.8
Etiology (HCV/HBV/Alcohol/PBC)	23/2/1/0	27/2/1/1	50/4/2/1
Child-Pugh (A/B/C)	23/3/0	29/2/0	52/5/0
BMI	23.7±2.2	22.0±2.7	22.8±2.6
Albumin (g/dL)	3.38±0.49	3.57±0.47	3.48±0.48
Bilirubin (mg/dL)	1.31±0.64	1.09±0.49	1.19±0.57
AST (U/L)	87.2±71.7	76.0±55.1	81.1±62.9
ALT (U/L)	77.7±72.3	64.9±70.1	70.7±70.8
Cholesterol (mg/dL)	155.3±38.2	156.3±27.3	155.9±32.3
ChE (mg/dL)	83.0±32.2	92.0±38.9	88.0±36.0
Platelet ($\times 10^4$/L)	9.1±3.8	10.9±6.5	10.1±5.5

Statistical analysis

The results of laboratory data are shown as mean±SD. Differences in the average and dispersion of FFM and rFFM between males and females were confirmed by *t*-test and f-test. Stepwise multivariate linear regression analysis was performed to confirm the significant predictive factors for albumin stability using variables such as rFFM, albumin, bilirubin, cholesterol, ALT, and blood platelet count.

RESULTS

Standard FFM of volunteers

In order to evaluate the relationship between FFM and height, 48 male and 56 female volunteers, whose body mass index (BMI) ranged from 20 to 24, underwent multifrequency BMI. As shown in Figure 1, there was a good correlation between FFM and height for males and females, and the standard FFM could be calculated as below. Male: standard FFM (kg) = 0.565×height (cm) - 43.261; Female: standard FFM (kg) = 0.677×height (cm) - 66.084.

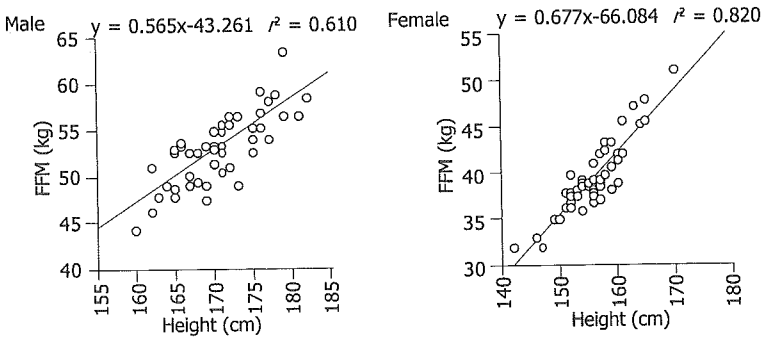

Figure 1 Correlation between fat-free mass (FFM) and height in volunteer males (right panel) and females (left panel). There was a significant positive correlation between FFM and height in each sex (male: $r = 0.781$, $P<0.0001$; female: $r = 0.906$, $P<0.0001$).

Calculation of relative FFM

According to the regression formula above, expected FFM was calculated using the height for each patient. Then relative FFM (rFFM) was then calculated as follows. rFFM (%) = (measured FFM/standard FFM) ×100. In short, rFFM represents the ratio between the FFM of a patient and that of a normal control with an average structure and the same height as the patient.

The left columns in Figure 2 show the FFM distributions of cirrhotic patients, and there was a significant difference in dispersion between males and females ($P<0.001$). In calculating rFFM for the patients (right columns of Figure 2), the dispersion did not differ significantly between males and females. Therefore, it was possible to compare rFFM of patients regardless of their sex differences.

SD-albumin and -albumin

Serum albumin levels were measured for all patients every month. Using the monthly results, standard deviation was calculated for all patients (SD-albumin). The difference between the maximum and minimum results during the entire observation period was also calculated (albumin). Both SD-albumin and -albumin were considered to be indications of the stability of hepatic synthetic function.

Comparison of rFFM and SD-albumin, or rFFM and -albumin for all patients, showed no significant correlations. However, when the analysis was limited to patients with average ALT levels over 50 U/L, we found significant correlations between rFFM and SD-albumin, and between rFFM and -albumin (Figure 3).

Multiple regression analysis

It is reasonable that rFFM correlates significantly with SD-albumin and -albumin in patients with high ALT levels, since the demand for amino acids would be increased under conditions in which hepatocytes are regenerating. However,

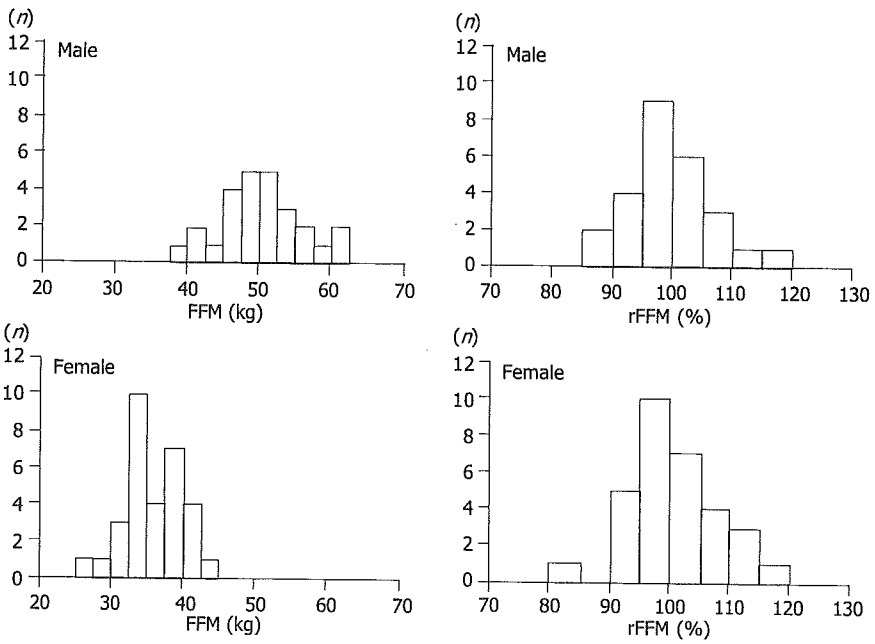

Figure 2 Distribution of fat-free mass (FFM) and relative FFM (rFFM) in cirrhotic patients. In FFM, the distributions among the sexes differed significantly (left two columns) ($P<0.001$). In contrast, when rFFM was applied for the same patients (right two columns); the dispersion did not differ significantly.

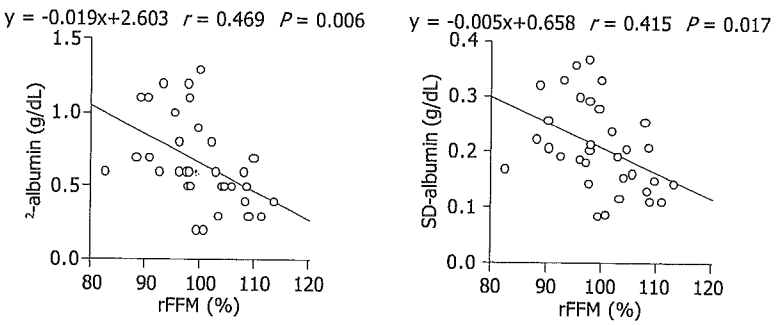

Figure 3 Correlation between rFFM with -albumin (right panel) and SD-albumin (left panel). In patients with average ALT levels >50 U/L, there was a significant correlation of rFFM with SD-albumin ($r = 0.469$, $P = 0.006$) and with -albumin ($r = 0.415$, $P = 0.017$).

other factors, such as the hepatic functional reserve or nutritional status at the time of rFFM measurement, might also influence SD-albumin and -albumin.

To investigate this possibility, we performed stepwise regression analysis using variables such as rFFM, platelet number, and the serum levels of cholesterol, chorine esterase, albumin, bilirubin and ALT. This analysis revealed that rFFM and ALT were independent predictors for both -albumin and SD-albumin (Table 2).

Table 2 Univariate analysis of -albumin and SD-albumin, and multivariate analysis using potential univariate predictors

	vs -Albumin				
	Univariate analysis			Multivariate analysis	
	R2	F	*P*	F	*P*
rFFM	0.094267	5.6202	0.0214	6.0402	0.0173
Albumin	0.016847	0.9254	0.3404	-	-
Bilirubin	0.017005	0.9342	0.3381	-	-
ALT	0.08064	4.7365	0.0339	5.0355	0.0290
Cholesterol	0.009282	0.4965	0.4841	-	-
Platelet	0.021799	1.1811	0.2821	-	-
	vs SD-Albumin				
	Univariate analysis			Multivariate analysis	
	R2	F	*P*	F	*P*
rFFM	0.06456	3.7268	0.0488	3.8602	0.0447
Albumin	0.02243	1.2390	0.2706	-	-
Bilirubin	0.003762	0.2039	0.6534	-	-
ALT	0.072818	4.2410	0.0443	4.3666	0.0415
Cholesterol	0.005361	0.2857	0.5952	-	-
Platelet	0.02186	1.1845	0.2814	-	-

DISCUSSION

It has been widely accepted that the determination of body composition is useful for evaluating nutritional status. However, traditional methods, such as skinfold measurement, are easy to perform but lack accuracy and reproducibility. On the other hand, other methods such as tracer dilution, neutron activation analysis and DEXA require sophisticated equipments and skilled technique. In recent years, BIA has emerged as a simple and reproducible method that can be used for the evaluation of FFM. Several reports have shown that the results of BMI correlate well with those of other methods[12,13].

Regardless of the method used for the analysis of body composition, sex differences have been a substantial obstacle. The results of measurements in males and females cannot be readily compared since their average body composition values differ significantly. To solve the problem, we applied a new factor, rFFM (= [measured FFM/standard FFM] ×100%). Using this new variable, we could compare the FFM results of all patients, regardless of their sex. Although we used the formula derived from the correlation between the FFM and height of the controls in calculating rFFM, the average body compositions are known to differ by race[14,15]. Therefore, the controls for standard curves should be prepared for each individual study.

Our results revealed that rFFM correlated with -albumin and SD-albumin in patients with high ALT levels, which indicated that muscle volumes would be one of the most important factors for maintaining the serum albumin level in active cirrhosis. This result seems reasonable since muscle protein serves as an amino acid pool. It should be noted that the stability of serum albumin levels is important not only for predicting the prognosis of patients with liver cirrhosis, but also to ensure a high quality of life. Serum albumin accounts for the colloid osmotic pressure of plasma; therefore hypoalbuminemia causes ascites, edema, and a reduction of circulating plasma volume, which would exacerbate hepatic failure.

In order to maintain or improve muscle volume, two interventions should be considered namely, amino acid supplementation and exercise. Cirrhotic patients often suffer from negative energy balance even at an early stage of the disease, characterized predominantly by protein deficiency and it is well known that they also have reduced plasma branched-chain amino acid (BCAA). Recent studies demonstrated the efficacy of branched-chain amino acid supplementation in improving the hypoalbuminemia of cirrhotic patients[7,8]. In the present study, none of the patients had received BCAA-supplementation because decompensated patients were excluded. Theoretically, BCAA-supplementation could inhibit muscle catabolism and contribute to the maintenance of muscle volume.

Regarding the usefulness of exercise for cirrhotic patients, most reports have focused on decompensated patients with ascites. Salo *et al.*[16], noted that moderate physical exercise caused a marked impairment in the renal function of patients with ascites as well as marked stimulation of vasoconstrictor systems, whereas Garcia-Pagan *et al.*[17], indicated that moderate exercise increased portal pressure and might therefore increase the risk of variceal bleeding in patients with esophageal varices. Although the contribution of exercise to the prognosis of cirrhotic patients remains unclear, we believe that exercise at the compensated stage would be useful for maintaining muscle volume which could delay the emergence of symptoms such as ascites and edema that accompany the progression of cirrhosis. Once ascites emerge in a cirrhotic patient, abdominal fullness causes appetite loss, which induces further hypoalbuminemia and reduction of hepatic blood flow. To avoid such an injurious cycle, it is important to prevent hypoalbuminemia at the compensated stage. Taken together, the evidence suggests that the periodic evaluation of body composition using BAI, in addition to the physician's recommendation for exercise, would be useful for improving the prognosis of compensated cirrhotic patients.

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Clinical Studies

A multi-step, incremental expansion method for radio frequency ablation: optimization of the procedure to prevent increases in intra-tumor pressure and to reduce the ablation time

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Abstract: *Background/Aims:* Radio frequency ablation (RFA) has been accepted clinically as a useful local treatment for hepatocellular carcinoma (HCC). However, intra-hepatic recurrence after RFA has been reported. We initially hypothesized that recurrence was attributable to increases in intra-tumor pressure during RFA, and we subsequently measured the pressure and optimized the procedure. *Methods:* A block of pig liver sealed in a rigid plastic case was used as a model of an HCC tumor with a capsule. We compared the pressure between a single-step full expansion of the needle (single-step method) and incremental, stepwise expansion (multi-step method), and evaluated the effect of varying the electrical power. Finally, we performed a preliminary comparison of the ablation times for these methods in HCC cases. *Results:* The multi-step method resulted in a significantly lower pressure and shorter total ablation time than the single-step method. Furthermore, incremental expansion in 10 steps resulted in a lower pressure and shorter ablation time than four steps. Seventy W-ablation resulted in a lower pressure and shorter time than 30- or 50 W-ablation. In HCC cases, the multiple-step method had a significantly shorter ablation time than the single-step method. *Conclusion:* The multi-step method can be recommended to reduce the ablation time, and suppress the increase in pressure.

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Key words: ablation time – hepatocellular carcinoma (HCC) – intra-tumor pressure – multi-step expansion – radio frequency ablation (RFA) – single-step expansion

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Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and usually is associated with hepatitis B virus (HBV) or hepatitis C virus (HCV) infection. In Japan, over 80% of HCC patients suffer from HCV-induced liver injury and most have underlying liver cirrhosis (1). Consequently, surgical resection is impossible in many cases of HCC because of a poor hepatic functional reserve. Under such conditions, percutaneous ethanol injection therapy (PEIT) has been used widely for the treatment of unresectable HCC (2). Many reports showed that the efficacy of PEIT for small HCC tumors was comparable with that of hepatic resection. As we demonstrated previously, however, PEIT demands multiple sessions to achieve complete necrosis, resulting in protracted hospitalization (3). Furthermore, many patients suffer from local recurrence after PEIT (4, 5). Such recurrence is considered to be attributable to intra-tumor septa that prevent the injected ethanol from infiltrating the entire tumor. We believe that local recurrence after PEIT should be prevented as much as possible because it is one of the most important negative prognostic factors for HCC patients (unpublished data).

Over the last several years, radio frequency ablation (RFA) has become a popular alternative

Abbreviations: RFA, radio frequency ablation; HCC, hepatocellular carcinoma; PEIT, percutaneous ethanol injection therapy.